Concurrent studies of the flow of digesta in the duodenum and of exocrine pancreatic secretion of calves

2.* The effects of addition of fat to skim milk and of 'severe' preheating treatment of spray-dried skim-milk powder

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1. The duodenal flow of digesta and the concurrent pancreatic secretion were compared when six Ayrshire calves, with duodenal re-entrant and pancreatic sac cannulas, were fed on three reconstituted milks. The diets were: reconstituted, 'mildly' preheated, spray-dried skim-milk powder (SK); the same skim milk containing 20 g fat/l (SKF); and reconstituted, 'severely' preheated skim-milk powder containing 20 g fat/l (HSKF). The calves were fed *ad lib.* from teats twice daily from 9 to 21 d of age, each diet being offered for 4 d. Collections of duodenal digesta and pancreatic secretions were made for 12 h after the fourth and eighth meals on each diet.

2. The calves tended to have the highest liquid intakes when diet SK was given. After adjustment for differences in intake, diet SK resulted in the appearance of more hydrogen, chloride and potassium ions but less undigested protein nitrogen in the duodenal digesta than with either diet SKF or HSKF.

3. Compared with diets SK and SKF, the whey fluids from diet HSKF took significantly longer to leave the abomasum, less H^+ passed through the duodenum during the first 6 h after feeding and less Cl^- during the whole postprandial period. More undigested protein N and fat from diet HSKF passed through the duodenum during the first 6 h after feeding, although this difference was significant only for protein N during the 1st hour after feeding.

4. Over the 12 h postprandial period, the duodenal digesta contained almost exactly the same quantities of polyethylene glycol (PEG), N and fat as those in the meal. The total volume of digesta was 2·25 l greater than the quantity of milk ingested. When the hourly duodenal flows of PEG and fluid were expressed as the square root of the hourly quantities recovered, the pattern of abomasal emptying was rectilinear. The flows of N and fat were curvilinear, when expressed on the same basis.

5. The concentration of 'sodium-free' chloride in the duodenal digesta, in excess of that ingested in the milk, was used as an indicator of the quantity of acid secreted by the abomasum. The relative quantity of acid secreted was greatest with diet SK and least with diet HSKF.

6. The pancreatic secretion of fluid was highest during the period 5–9 h after feeding but the secretion of enzyme activity was highest during the first 2 h after feeding.

7. Considerable variability in the secretion of enzyme activity was observed and the rate of secretion did not appear to be related to any component of the duodenal digesta.

8. Diet SKF was associated with a greater volume of pancreatic secretion and more pancreatic protease secretion than either diet SK or HSKF, but most amylase activity was secreted when diet HSKF was given. Evidence is presented which suggests that pancreatic enzyme activity adaptation occurred when diet HSKF was offered in succession to diet SK or SKF. The secretion of trypsin activity did not differ between diets.

Diarrhoea in newborn calves has been associated with many dietary factors; in particular the use of skim milk (Owen, Jacobson, Allen & Homeyer, 1958; Bush, Schuh, Tennille & Waller, 1963; Mathieu & Barré, 1964; Roy, Stobo & Gaston, 1970) or of a 'severely' heat-treated milk (Roy, 1962; Shillam & Roy, 1963).

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Changes in the rate of emptying of the abomasum of calves given milk are closely related to the volume ingested (Ash, 1964; Mylrea, 1966*a*). In contrast, changes due to differences in milk composition have been inconsistent, although Espe & Cannon (1935) found that the clot formed from skim milk was slower to leave the abomasum than that from whole milk. However, the absence of fat did not affect the digestibility of dry matter, protein and lactose (Raven & Robinson, 1960; Roy *et al.* 1970). The diarrhoea associated with skim milk may be due to the greater quantity of lactose ingested, which is likely to exceed the recommended 'hexose equivalent' for calves (Walker & Faichney, 1964). However, it appears unlikely that the ameliorating effect of the addition of fat to skim milk upon the incidence of diarrhoea in calves (Roy, 1969) can be completely explained by a reduction in dietary lactose content.

The use of 'severely' heat-treated milks, in which a large proportion of the whey proteins has been denatured, has been shown to reduce the live-weight gain and the apparent digestibility of nitrogen, fat and dry matter during the first 3 weeks of life (Roy, 1962; Shillam & Roy, 1963). These milks do not clot adequately with rennet in vitro, and Tagari & Roy (1969) showed that, compared with a 'mild' heat treatment, 'severely' heat-treated milk resulted in a higher pH in the abomasal effluent and a reduced total volume of effluent, consistent with less secretion of HCl from the abomasum. An increased outflow of undigested protein and a decreased outflow of non-protein N were also observed, especially during the 1st hour after feeding.

The pancreas is the major source of intestinal proteases. An increase in the quantity of undigested protein entering the duodenum in calves given a 'severely' heat-treated milk might, therefore, result in a compensatory increase in proteolytic activity within the duodenum.

The present experiment was designed to investigate the secretion of pancreatic enzymes in relation to the flow of duodenal effluent during the first 3 weeks of life of calves given 'mildly' heat-treated skim milk with or without fat, or 'severely' heated skim milk with fat.

EXPERIMENTAL

Animals and experimental design

Six Ayrshire bull calves were cannulated with duodenal re-entrant and pancreatic sac cannulas by means of technique 1 (Ternouth & Buttle, 1973); the experiment was conducted from the 9th to the 21st day after birth. The calves were subjected to the experimental collection periods in pairs, calves 1 and 2 during November 1968, calves 3 and 4 during March 1969 and calves 5 and 6 during October 1969.

The calves were fed *ad lib.* by teat twice daily at 09.00 and 21.00 hours. Three milks, reconstituted to contain 100–120 g dry matter/l, were used. They were based on 'mildly' preheated, spray-dried skim-milk powder (SK), 'mildly' preheated, spray-dried skim-milk powder containing 20 g fat/l (SKF), or 'severely' preheated, spray-dried skim-milk powder containing 20 g fat/l (HSKF). The composition of the milks is shown in Table 1. Analyses indicated that non-casein N represented 187 and 123 g/kg total N in the 'mildly' and 'severely' heat-treated, spray-dried skim-milk powders respectively (Roy, 1970). Milks SKF and HSKF were identical with milks B and A

Table 1. *Composition of three reconstituted milks* (*SK*, *SKF* and *HSKF**) *fed to six bull calves*

	SK	SKF	HSKF
Spray-dried skim milk (g/kg)†:			
'Mild' preheating treatment (77° for 15 s)	100	98	—
'Severe' preheating treatment (74° for 30 min)	—	—	98
Non-vitaminized margarine (g/kg)†	—	20	20
Water (g/kg)	900	882	882
Retinol (μg/l)	2102	2102	2102
Cholecalciferol (μg/l)	43·8	43·8	43·8
Measured mean concentration of electrolytes (mmol/l):			
Na^+	22·3	19·9	20·0
K^+	26·2	24·2	25·4
Cl^-	34·0	34·0	33·2

* SK, skim milk; SKF, skim milk + fat; HSKF, 'severely' heated skim milk + fat.
† On an air-dry basis.

used by Tagari & Roy (1969) respectively. The milks were prepared as described by Roy, Shillam, Thompson & Dawson (1961) and Shillam, Roy & Ingram (1962).

The experiment was of randomized block design: each calf was offered each milk at eight consecutive meals, and duodenal and pancreatic collections were made for 0·5 h before and for 12 h after the fourth and eighth meals had been given. The terms 'penultimate' and 'experimental' meals refer to the third and seventh, and the fourth and eighth meals respectively.

The intake of milk at each meal was recorded, and the calves were weighed at birth and at the end of the experiment. The milk given at the beginning of each collection period contained about 1 g polyethylene glycol (PEG)/l. On three occasions on which collections of duodenal effluent and pancreatic secretions were to be made, the milk intake of a calf was abnormally low and this was found to be due to blockage of the duodenal re-entrant cannula by a milk clot. The results from these three collection periods (two on diet SK and one on diet SKF) have been omitted from all the results.

Duodenal effluent and pancreatic juice were collected during 12 h after feeding, samples were retained for analysis and the remainder was returned to the calves as previously described (Ternouth & Buttle, 1973).

Analyses of milk and duodenal digesta

Since no enzyme inhibitor was placed in the samples, the protein concentration of the duodenal digesta was measured before any of the other components.

Protein N was precipitated by mixing 1 ml digesta with 2 ml trichloroacetic acid (TCA) (30 g/l). After centrifugation at 1500 *g* for 10 min, the supernatant liquid was discarded and the precipitate mixed with a further 10 ml TCA (30 g/l). After further centrifugation at 1500 *g* for 10 min, the precipitate was retained and dissolved in 2 ml 0·5 M-NaOH, and 10 ml biuret reagent were added. The biuret reagent was prepared by dissolving 6 g $CuSO_4.5H_2O$ and 24 g sodium potassium tartrate in 300 ml

2·5 M-NaOH, adding 1 g KI and making the solution up to 1 l with distilled water. The protein–biuret solution was allowed to stand overnight, then filtered through glass-fibre filter paper (Whatman GF/A) and the optical density of the solutions was measured at 540 nm in a Unicam 600 Spectrophotometer. Crystalline bovine serum albumin (Armour Pharmaceutical Company) was used as a reference standard.

Total N and fat were measured by the Kjeldahl and Gerber (British Standards Institution, 1969) methods respectively.

Polyethylene glycol (PEG) concentration was measured by the method of Smith (1958) as modified by the same author (Smith, 1962; R. H. Smith, personal communication).

The chloride concentration was measured by the Van Slyke modification of the Volhard method (Varley, 1967). The concentrations of sodium and potassium were measured by flame photometry in samples prepared as described by King & Wooton (1956). H^+ concentration was calculated from pH measurements.

Analyses of pancreatic activity

The amylase activity of pancreatic fluid appropriately diluted with ice-cold 0·15 M-NaCl was measured by the method described by Dahlqvist (1961) with the following modifications: a substrate solution containing 1 g soluble starch and 40 mg NaCl in 100 ml 0·05 M-sodium phosphate buffer (pH 6·9) was used and the incubation was carried out for 30 min at 37°. The increase in reducing power of the starch solution was measured with dinitrosalicylate reagent (Siddons, 1968). Porcine pancreatic α-amylase was used as a standard, and the amylase activity is expressed in terms of mg pancreatic amylase. Under these conditions, 1 mg porcine pancreatic amylase releases reducing groups equivalent to 2·8 mg maltose/h.

The tryptic activity of pancreatic fluid was measured using the synthetic substrate *N*-α-benzoyl-L-arginine ethyl ester hydrochloride (BAEE) (Schwert & Takenaka, 1955). Maximal activation of the trypsin was achieved by incubating the pancreatic fluid with an equal volume of 0·01 M-tris (hydroxymethyl) methylamine buffer (pH 7·8) containing 0·1 M-$CaCl_2$ for 4 h at 4°. After incubation, 0·1 ml of the activated pancreatic fluid was added to 3 ml 0·001 M-BAEE in 0·01 M-tris (hydroxymethyl) methylamine buffer (pH 7·8) containing 0·05 M-$CaCl_2$. Hydrolysis of the substrate was measured by the change in extinction at 253 nm in a continuous recording spectrophotometer (Optica, model CF4DR; Baird & Tatlock, London). Bovine pancreatic trypsin (Koch-Light Laboratories Ltd) was used as a reference standard and the results are expressed as mg trypsin. Under these conditions 1 mg bovine trypsin caused a change in extinction of 0·926 units/min.

Maximal activation of the proteolytic enzymes was achieved by incubating for 2 h at 4° 1 ml pancreatic fluid which had been diluted with 100–300 ml 0·01 M-tris (hydroxymethyl) methylamine buffer (pH 6·8) containing 0·01 M-$CaCl_2$. Activated pancreatic fluid (1 ml) was mixed with 1 ml 0·15 M-NaCl and incubated for 5 min at 37°. To this were added 3 ml casein substrate (10 g freeze-dried casein, 6·05 g tris (hydroxymethyl) methylamine, 20 g sodium ethylenediaminetetra-acetic acid and 25 ml methiolate mixed in 500 ml distilled water in a Waring Blender, pH adjusted

Table 2. *Mean milk intakes of six bull calves each given eight consecutive meals of each of three reconstituted milks, SK, SKF and HSKF**

	Milk intake (l)			Pooled standard error of mean (25 df)
	SK	SKF	HSKF	
All meals	3·95	3·57	3·44	—
Penultimate meal (meals 3 and 7)	4·16	3·42	3·52	0·22
Experimental meal (meals 4 and 8)	4·25	3·80	3·78	0·29

* SK, skim milk; SKF, skim milk + fat; HSKF, 'severely' heated skim milk + fat.

to 6·8 and made up to 1 l) and the incubation was continued for a further 30 min. The reaction was stopped by the addition of 5 ml trichloroacetic acid (100 g/l). After thorough mixing the solutions were allowed to stand overnight at 4° before being filtered (Whatman no. 40). Proteolytic activity was estimated by measuring the extinction of the filtrate at 277 nm. Bovine pancreatic trypsin (Koch-Light Laboratories Ltd) was used as a standard and the results are expressed as mg trypsin. Under these conditions 1 mg total proteases (trypsin equivalent) caused a change in extinction of 0·465 units/min.

Statistical analysis

Analysis of variance and covariance adjustment techniques were applied and values for the three missing collection periods were calculated by least squares technique.

RESULTS

The mean birth weight of the calves was 36·9 kg and they gained an average of 6·7 kg in the first 24 d, which included the surgical and postsurgical recovery periods as well as the experimental periods. The mean milk intakes of the calves, which tended to be higher when diet SK was given, are shown in Table 2. The milk intakes of the calves on the days when duodenal and pancreatic collections were made did not differ significantly between diets.

The patterns of duodenal flow with these diets have been recorded elsewhere (Ternouth, 1971). The general pattern was similar to that previously recorded when calves were fed on raw milk (Ternouth & Buttle, 1973).

As the milk intakes of the calves at each meal differed, the mean volume and quantities of various constituents in the duodenal digesta were adjusted, whenever possible, for a mean milk intake of 3·96 l (Table 3).

The volume of duodenal effluent was similar for all diets except during the period 0–3 h after feeding when the volume was less ($P < 0·05$) for diet HSKF than for the mean of the other two diets.

The outflow of PEG was poorly related to the volume of diet consumed and was fastest for diet SK and slowest for diet HSKF. When expressed on a square-root basis (Hopkins, 1966), the pattern of abomasal emptying of PEG was linearly related to time for the first 6–7 h after feeding, 80–90% of the ingested PEG was recovered from the duodenum. Linear regression lines were therefore fitted to the results

Table 3. *Cumulative outflow of duodenal digesta, polyethylene glycol (PEG), hydrogen, potassium, sodium and chloride ions of six bull calves each given eight consecutive meals of each of three reconstituted milks, SK, SKF and HSKF*†

Variable measured	Significance of covariance regression‡	Value			Pooled SE of a mean	Significance of difference between milks
		SK	SKF	HSKF		
0–1 h after feeding:						
Volume (l)	**	1·09	1·07	0·98	0·09	NS
PEG (ratio, recovered: intake)	NS	0·206	0·216	0·181	0·023	NS
H^+ (mmol)	*	0·0339	0·0062	0·0004	NV	*
K^+ (mmol)	**	22·4	20·0	14·9	2·1	*
Na^+ (mmol)	**	34·1	35·8	33·0	2·7	NS
Cl^- (mmol)	**	66·7	63·4	59·2	5·0	NS
0–3 h after feeding:						
Volume (l)	***	2·60	2·54	2·29	0·10	NS
PEG (ratio, recovered: intake)	NS	0·550	0·546	0·465	0·031	NS
H^+ (mmol)	**	0·0843	0·0563	0·0125	NV	**
K^+ (mmol)	***	49·6	45·3	34·6	2·5	***
Na^+ (mmol)	***	77·6	80·5	71·1	3·8	NS
Cl^- (mmol)	*	170·8	163·9	137·0	6·8	**
0–6 h after feeding:						
Volume (l)	***	4·39	4·29	4·10	0·09	NS
PEG (ratio, recovered: intake)	*	0·895	0·857	0·817	0·023	NS
H^+ (mmol)	NS	0·4720	0·3190	0·2052	0·0662	*
K^+ (mmol)	***	75·9	69·9	59·3	3·4	**
Na^+ (mmol)	***	131·6	138·3	123·8	5·0	NS
Cl^- (mmol)	**	329·3	312·9	273·8	9·2	***
0–12 h after feeding:						
Volume (l)	***	6·17	6·22	6·06	0·13	NS
H^+ (mmol)	NS	1·2942	1·1109	1·1695	0·1700	NS
K^+ (mmol)	***	90·0	84·1	73·0	3·8	*
Na^+ (mmol)	**	207·5	217·2	213·9	8·4	NS
Cl^- (mmol)	***	533·6	519·3	483·5	13·9	*

NS, not significant; * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.
NV, no SE given as variability differed between means.
† SK, skim milk; SKF, skim milk + fat; HSKF, 'severely' heated skim milk + fat.
‡ Whenever covariance regressions were significant, the mean values were adjusted for a milk intake of 3·96 l.

obtained during the first 6 h after feeding for each of the thirty-three completed experiments, and the lines were extrapolated to estimate the theoretical time when all the PEG would have been recovered from the abomasum. After covariance adjustment of the values for differences in milk intake ($b = 0·864 \pm 0·328$ h/l) the mean time taken for all the PEG to leave the abomasum was 8·8, 9·6 and 10·8 h (SE = 0·50 h) for diets SK, SKF and HSKF respectively, the value for diet HSKF differing ($P < 0·05$) from the mean for the other diets. When the total volume of fluid recovered was expressed on a square-root basis, a similar linear relationship with time after feeding was found (Ternouth, 1971).

In comparison with the mean for the other two diets, the total duodenal flow of ionized acid (H^+) was significantly less during the first 6 h after diet HSKF was given.

Table 4. *Total outflow of fluid and of sodium and chloride ions, in excess of that ingested, in the duodenal digesta of six bull calves each given eight consecutive meals of each of three reconstituted milks, SK, SKF and HSKF*†

Variable measured	Value			Pooled SE of mean	Significance of difference between milks
	SK	SKF	HSKF		
	Total flow				
Volume (l)	2·37	2·20	2·17	0·14	NS
Na^+ (mmol)	125·8	134·0	135·7	9·3	NS
Cl^- (mmol)	414·9	372·9	352·6	15·4	*
'Cl^- minus Na^+' (mmol)	286·9	238·9	209·6	11·6	***
	Concentration				
Na^+ (mmol/l)	50·6	64·8	64·7	3·8	*
Na^+ (mmol/l) adjusted for milk intake	48·7	65·3	66·1	3·3	**
Cl^- (mmol/l)	179·9	172·5	163·8	7·5	NS
'Cl^- minus Na^+' (mmol/l)	128·2	107·6	96·0	5·9	***

NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
† SK, skim milk; SKF, skim milk + fat; HSKF, 'severely' heated skim milk + fat.

However, over the whole 12 h period after feeding the quantity of H^+ passing through the duodenum was similar for all diets.

The concentration and total quantity of K^+, Na^+ and Cl^- passing through the duodenum each hour were generally similar to that recorded by Mylrea (1966*b*); the detailed results for the present experiment are recorded elsewhere (Ternouth, 1971). The total outflow of K^+ (Table 3) in the duodenal digesta was less than the quantity ingested and differed between diets during the first 3 h after feeding. In contrast, there were no differences between diets in the quantities of Na^+ passing through the duodenum. During the period 0–6 h after feeding, the quantity of Cl^- passing through the duodenum was greater for diets SK and SKF than for diet HSKF ($P < 0.01$). Over the 12 h postprandial period, the total volume of digesta passing through the duodenum in excess of that ingested was 2·25 l, with no significant differences between diets (Table 4). Differences between diets in the total outflow of Cl^- and the concentration of Na^+ in the digesta during this period were reflected in the differences in the total outflow and concentration of Cl^- minus Na^+ in the digesta.

The cumulative quantities of protein N (PN) and total N (TN) in the duodenal digesta were related to milk intake at both the penultimate and the experimental meal (Table 5). After adjustment of the mean values to the same milk intake at both meals, the quantities of PN, but not of TN, passing through the duodenum differed between diets. During the 1st hour after feeding, the least amount of PN was passed through the duodenum when diet SK was given and most when the diet was HSKF, PN:TN ratios were 0·286, 0·418 and 0·471 for diets SK, SKF and HSKF respectively. During 0–6 h and 0–12 h of the postprandial period, the quantities of PN in the digesta also differed between treatments; the PN:TN ratios for the period 0–12 h after feeding were 0·315, 0·407 and 0·401 for diets SK, SKF and HSKF respectively. Thus the PN:TN ratio differed ($P < 0.01$) between diet SK and the other two diets.

Table 5. *Cumulative outflow (g) of protein nitrogen (PN), total nitrogen (TN) and fat in the duodenal digesta of six bull calves each given eight consecutive meals of each of three reconstituted milks, SK, SKF and HSKF*†

Variable measured	Significance of covariance adjustments‡ PMI	MI	Value SK	SKF	HSKF	Pooled SE of mean	Significance of difference between milks
0–1 h after feeding:							
PN	*	**	0·97	1·42	1·78	0·20	*
TN	*	***	3·39	3·40	3·78	0·36	NS
Fat	NS	NS	—	9·13	12·24	1·76	NS
0–3 h after feeding:							
PN	*	*	2·18	2·80	3·03	0·28	NS
TN	*	**	7·20	7·01	7·06	0·45	NS
Fat	NS	NS	—	16·61	20·66	2·22	NS
0–6 h after feeding:							
PN	**	*	3·73	4·90	5·10	0·32	*
TN	*	*	12·57	12·53	12·87	0·61	NS
Fat	NS	NS	—	32·70	37·99	3·21	NS
0–12 h after feeding:							
PN	**	**	7·19	9·29	8·72	0·45	*
TN	*	*	22·85	22·79	21·72	1·07	NS
Fat	NS	NS	—	75·52	70·26	8·21	NS

NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† SK, skim milk; SKF, skim milk+fat; HSKF, 'severely' heated skim milk+fat.

‡ Whenever covariance adjustments were significant, mean values for PN and TN were adjusted to a penultimate milk intake (PMI) of 3·71 l and milk intake (MI) of 3·96 l.

No significant relationship was found between the cumulative outflow of fat in the duodenal digesta and milk intake at either the penultimate or experimental meal (Table 5). There was, however, some tendency for more fat to appear during the 1st hour after feeding in the digesta of calves fed on diet HSKF than in those fed on diet SKF.

When the cumulative recovery of TN and fat was expressed on a square-root basis no linear relationship with time after feeding was observed.

The rate of secretion of pancreatic fluid was highest when the calves were given diet SKF and considerably lower for diet SK (Table 6). Smaller differences were observed between diets SKF and HSKF. No correlations between the pancreatic volume and the concurrent duodenal volume, H^+ flow, Cl^- minus Na^+ flow or TN flow were found. The pattern of secretion of pancreatic fluid over the 12 h period was similar to that previously described (Ternouth & Buttle, 1973), the rate of secretion being highest 5–9 h after feeding.

The enzyme activity in the pancreatic secretions was highest in the first 3 h after feeding and generally declined thereafter. Thus the largest total hourly secretion of enzyme activity occurred during the 1st or 2nd hour after feeding even though the volume of pancreatic fluid secreted was greatest during the 5–9 h period. Over the 12 h period of collection more protease activity was present in the pancreatic secretions when the calves were fed on diet SKF than with the other two diets. On the other

Table 6. *Cumulative secretion of pancreatic fluids and enzymes by six bull calves each given eight consecutive meals of each of three reconstituted milks, SK, SKF and HSKF*†

Variable measured	Value			Pooled SE of mean	Significance of difference between milks
	SK	SKF	HSKF		
0–1 h after feeding:					
Volume (ml)	24·8	29·5	30·0	1·8	NS
Trypsin (mg)	15·2	18·0	19·1	2·3	NS
Protease: g	0·268	0·285	0·195	0·057	NS
mg/g N digesta	78·2	89·6	57·3	16·1	NS
mg/g PN digesta	227·6	215·1	137·4	50·0	NS
Amylase (mg)	4·54	3·91	4·76	1·33	NS
0–3 h after feeding:					
Volume (ml)	60·3	74·4	71·3	3·6	*
Trypsin (mg)	41·1	43·7	45·3	5·7	NS
Protease: g	0·605	0·821	0·494	0·153	NS
mg/g N digesta	78·5	116·5	69·5	19·8	NS
mg/g PN digesta	248·8	312·3	174·6	66·5	NS
Amylase (mg)	10·79	10·52	15·35	2·86	NS
0–6 h after feeding:					
Volume (ml)	131·0	165·2	130·4	7·2	**
Trypsin (mg)	84·3	88·0	78·9	10·9	NS
Protease: g	1·100	1·444	0·916	0·230	NS
mg/g N digesta	81·9	112·6	69·6	15·9	NS
mg/g PN digesta	283·4	301·8	181·1	52·5	NS
Amylase (mg)	22·74	23·35	28·96	5·31	NS
0–12 h after feeding:					
Volume (ml)	273·5	339·9	299·6	15·7	*
Trypsin (mg)	148·8	156·8	148·1	19·2	NS
Protease: g	1·769	2·331	1·690	0·327	NS
mg/g N digesta	73·6	101·6	78·1	14·0	NS
mg/g PN digesta	226·0	251·8	194·0	40·1	NS
Amylase (mg)	39·6	38·7	50·8	8·29	NS

NS, not significant; * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.
† SK, skim milk; SKF, skim milk + fat; HSKF, 'severely' heated skim milk + fat.

hand, the total amylase activity secreted over the 12 h period was greater for diet HSKF than for the other two diets. However, owing to the high variability associated with the mean values, these differences were not statistically significant. Adjustments for differences in the volume of duodenal fluid, total H^+, total N or total Cl^- minus Na^+ outflow from the cranial duodenal cannula did not reduce the variability of the enzyme activity during any period.

Comparisons of sets of 3 h values for the total outflow of proteases, instead of cumulative secretion, showed that diet SKF was associated with significantly more protease secretion than either diet SK or HSKF ($P < 0{\cdot}05$). When total protease secretion was adjusted for differences between diets in the Cl^- minus Na^+ flow in the duodenal digesta, significantly less protease ($P < 0{\cdot}01$) was secreted with diet SK than with diet SKF. Similarly, covariance adjustment of the mean amylase secretion for differences between diets in TN in the duodenal effluent resulted in diet HSKF being associated with significantly more amylase secretion ($P < 0{\cdot}05$) than the mean of the values for the other two diets.

DISCUSSION

In an attempt to maximize any physiological effects induced by the absence of fat or the use of a 'severely' heat-treated milk, the calves were fed *ad lib.*, a technique which has been shown by Mylrea (1966*c*) not to normally result in any gross digestive abnormalities. Based on a mean live weight of 41 kg, the mean daily dry-matter intakes of the calves were equivalent to 51·8, 57·8 and 56·0 g/kg live weight$^{0.75}$ for diets SK, SKF and HSKF respectively. These high food intakes, the absence of diarrhoea under the isolated conditions in which the calves were kept, and the satisfactory live-weight gains suggest that cannulation did not affect the normal functioning of the digestive tract.

The general pattern of duodenal flow of digesta was similar to that recorded by Mylrea (1966*a*, *b*), except that more extreme fluctuations in the concentration of some dietary components were observed because of the higher milk intakes of our calves.

The pattern of duodenal emptying of fluid did not vary greatly between the three diets. Between 82 and 87 % of the ingested fluids had passed from the abomasum in the first 6 h after feeding, whilst in the same period less than 50 % of the total fat recovered had left the abomasum. Similar results have been recorded by Mylrea (1966*a*, *b*) and Mathieu (1968).

The slower rate of complete passage of PEG when diet HSKF was given, probably resulted from the slurry formed in the abomasum with this diet, which, in contrast to the firm clot produced with diets SK and SKF, is unlikely to separate into a liquid PEG-containing 'phase' and a coagulated protein–fat 'phase'. Thus, the longer time required for the PEG to leave the abomasum was probably the result of the failure of the milk to clot as well as of the slower rate of emptying during the first 3–4 h after feeding.

The linearity of the rate of gastric emptying when expressed on a square-root basis has been explained by Hopkins (1966) in terms of the Law of Laplace for elastic cylinders. This strongly suggests that the major factor regulating abomasal emptying is the tension of the abomasal wall and that the enteric inhibition of abomasal emptying is relatively unimportant over the 12 h postprandial period. The lack of a linear relationship when TN or fat recovery was expressed on a square-root basis was, with diet SKF, probably due to the N having to be enzymatically lysed from the milk clot before either the N or the enmeshed fat (Hill, Noakes & Lowe, 1970) could pass through the pylorus.

In their recent review, Hunt & Wan (1968) state that in man and dogs the non-parietal secretion contains 140–150 mmol Na^+/l, the parietal secretion contains 160 mmol H^+/l and the only anion present in both secretions is Cl^- at a concentration of 170 mmol/l. Ash (1959) found similar concentrations of Na^+, H^+ and Cl^- in the mixed secretions collected from the abomasal pouches of sheep. Thus parietal and non-parietal secretions of the abomasal mucosa are almost completely distinguishable by the cation in the secretion, and the expression 'Cl^- minus Na^+' concentration is a measure of the relative amount of acid secreted by the gastric glands.

In the present experiment, other non-abomasal secretions were present in the duodenal digesta. These include bile, salivary secretions and a quantity of duodenal secretions; all except saliva appear to contain bicarbonate and have a negative 'Cl^- minus Na^+' concentration varying from as high as $-123{\cdot}0$ to 0·0 mmol/l (Florey & Harding, 1934; Ash, 1959; Harrison, 1962; Sasaki, 1968; Ternouth & Buttle, 1973); in saliva (Sasaki, 1968) the Cl^- and bicarbonate concentrations become approximately equal by the time the calves are 3 weeks of age. In the present experiment, the 'Cl^- minus Na^+' concentration of non-abomasal secretion is therefore assumed to be similar to the concentration of non-parietal abomasal secretions.

From the mean duodenal concentrations of 'Cl^- minus Na^+' in excess of the quantity ingested by the calves, given in Table 4, it appears that the parietal secretion must represent a higher proportion of the total secretion for a skim-milk diet (SK) than for one containing fat (SKF and HSKF). In this experiment it is not possible to quantify the parietal and non-parietal components of the abomasal secretions of the calf as Hunt (1951) has done for the gastric secretions of man, particularly as absorption from the abomasum and proximal duodenum cannot be dismissed. However, it can be concluded that there is appreciably more acid secreted with diet SK than with either SKF or HSKF. It is of note that, although calves given diet SK consumed more milk, the total duodenal outflow of H^+ was greater than for diets SKF or HSKF, although the difference was not significant.

The volume of endogenous fluids produced in 12 h was 2·2 l for diet SKF. When the volume of bile added within the duodenum is considered, 35–40 ml/h for sheep of similar live weight (Harrison, 1962), this value agrees with that of 1·8 l recorded in calves cannulated just distal to the pylorus (Mylrea, 1966*c*).

As the ingested milk proteins and fat had no markers associated with them, it is impossible to differentiate the source of the TN and fat recovered in the duodenal effluent between the milk given at the beginning of the 12 h collection period, the milk given at previous meals, and TN and fat of endogenous origin. Of the residual variation in duodenal outflow of N, 54% was explicable by the multiple regression equation

$$\underset{}{\text{TN (g)} = 15{\cdot}80 + 1{\cdot}017^{*}\ \text{PM} + 0{\cdot}778^{*}\ \text{M}, \quad (^{*}P < 0{\cdot}05),} \qquad (1)$$
$$(\pm 0{\cdot}416) \quad (\pm 0{\cdot}369)$$

where PM is the intake (l) at the penultimate meal and M is the intake (l) at the experimental meal. This result compares favourably with the observation of Hill *et al.* (1970), who have shown that the abomasum commonly contains aggregates of old clots embedded in the new clot formed from the most recent meal. When over-all multiple regression equations were calculated separately the results were:

(*a*) for diets SK and SKF,

$$\underset{}{\text{TN} = 4{\cdot}14 + 3{\cdot}49^{***}\ \text{PM} + 1{\cdot}46\ \text{M (SD} = 2{\cdot}89, \text{ df} = 18), \quad (^{***}P < 0{\cdot}001);} \qquad (2)$$
$$(\pm 0{\cdot}78) \quad (\pm 0{\cdot}70)$$

(*b*) for diet HSKF,

$$TN = 10{\cdot}0 - 1{\cdot}68\,PM + 4{\cdot}47^{***}\,M \quad (\pm 1{\cdot}08) \quad (\pm 0{\cdot}91)$$

(SD = 2·54, df = 9), (***P < 0·001). (3)

These results strongly suggest that less milk clot remained within the abomasum at the end of a 12 h postprandial period when the diet was HSKF than when it was SK or SKF.

From a comparison of PEG recoveries, the fat did not appear to inhibit the rate of emptying of the ingested fluids, and similar amounts of N were recovered in the duodenal effluent throughout the postprandial period. At least for the period when most of the PEG was passing from the abomasum (7–8 h), it can be concluded that margarine fat had no effect upon the rate of abomasal emptying.

The greater acid secretion with diet SK, together with a tendency for the milk clot to remain longer in the abomasum (Espe & Cannon, 1935; Ternouth, 1971), would account for the lower proportion of protein in the duodenal digesta of calves given diet SK, since both these factors favour more extensive proteolysis.

More pancreatic fluid was secreted with diet SKF than with either of the other diets, but the quantity of trypsin secreted did not differ significantly between diets. With diet SK, lower pancreatic volumes may have been associated with relatively less undigested protein present in the duodenal effluent or with the lack of dietary fat. Similar differences were noted for pancreatic volume and trypsin when diets SKF and HSKF were compared. This tends to suggest that the quantity of trypsin secreted is relatively constant but the volume of secretion is subject to intestinal control (possibly via secretin). Increased H^+ and N outflow from the duodenal cannula 4–12 h after feeding was associated with some increase in the secretion of pancreatic fluid. However, when diets were compared over the whole 12 h period, the highest quantity of free H^+, and 'Cl^- minus Na^+', passed through the duodenum of the calves when they were given diet SK, this diet being associated with the smallest pancreatic volume. No ready explanation for these apparently conflicting results is available, although Wang & Grossman (1951) have found that sodium oleate markedly increases the volume of pancreatic secretion in dogs, as well as increasing the secretion of pancreatic enzymes.

An increase in the amount of undigested protein entering the duodenum from the abomasum might be expected to result in a compensatory increase in the quantity of proteolytic enzymes secreted by the pancreas. However, the results obtained show that the secretion of pancreatic enzymes is poorly correlated with the duodenal flow of PN. Thus, although in the 0–12 h period the duodenal flows of PN and of protease activity were highest for diet SKF, during the 0–6 h period the duodenal flow of PN was highest for diet HSKF and the protease activity was lowest. This suggests that the emptying of the zymogen granules within the pancreas is mainly under neural rather than entero-pancreatic hormonal control, i.e. the secretion of enzymes does not appear to be related to the digestive requirements of the intestine.

Calves given diet HSKF secreted more amylase but less protease than those given

diet SKF, suggesting that some chemical moiety in the heated skim milk was stimulating amylase synthesis or depressing protease synthesis, or both, within the pancreas, in a manner reminiscent of starch-rich diets in adult cows and sheep (Clary, Mitchell, Little & Bradley, 1969), and glucose-rich diets in rats (Grossman, Greengard & Ivy, 1944; Howard & Yudkin, 1963; Bucko, Simko & Kopec, 1969). When diet HSKF was given, the quantity of proteases secreted fell to 73 % of that secreted when diet SKF was given and the quantity of amylases increased to 134 %. Diet HSKF is known to be less efficiently digested than diet SKF by the young calf (Shillam & Roy, 1963); therefore less amino acids are being absorbed into the blood, but the efficiency of carbohydrate (lactose) digestion is not impaired. Ben Abdeljlil, Visani & Desnuelle (1963) have found that adaptation of the rate of secretion of pancreatic enzymes commences almost immediately after a sudden change in the composition of the diet and is completed within 8 d. Adaptation to diet HSKF in the present experiment appeared to be occurring in a similar manner. In comparison with diet SKF, the difference in the total secretion of proteases was $-6{\cdot}5$ and $-33{\cdot}2$ % and of amylase $+15{\cdot}3$ and $+58{\cdot}6$ % for diet HSKF, in the experimental periods 1·5–2 and 3·5–4 d respectively after the calves had been transferred to the new diet.

The method used for measuring pancreatic total protease activity was based on the release of acid-soluble tyrosine residues from casein (Braude, Newport & Porter, 1970). Chymotrypsin is known to have a high specificity for amino acid bonds involving tyrosine (Keller, 1968) so that changes in total protease activity, not associated with changes in trypsin activity, are likely to be associated with changes in chymotrypsin activity. When diets are changed, Ben Abdeljlil *et al.* (1963) have shown that the chymotrypsin activity in the pancreatic secretions or homogenates from rats changes by a far greater proportional amount than the quantity of trypsin activity. In the experiments of Tagari & Roy (1969), the quantity of protein passing from the abomasum was higher during the early postprandial period after feeding with diet HSKF than after feeding with SKF. During this period, the pancreatic secretion of chymotrypsin, which has a supplementary action to pepsin and rennin, may also have been secreted at a considerably reduced rate.

In this experiment, in which three diets were offered within 12 d, long-term effects of the diets could not be investigated. The experiments of Ben Abdeljlil *et al.* (1963) have shown that changes in the rate of enzyme synthesis with a change in diet take twice as long to occur (8 d) than was allowed in the present experiment. Thus, when calves are given diet HSKF continually the difference in total quantity of proteases secreted, compared with that secreted on diet SKF, may be considerably greater than the 33 % measured in the present experiment. In rats, Pelot & Grossman (1962) have shown that the concentration of enzymes is greater in the caudal regions of the small intestine. Thus the quantity of enzyme secreted/g N in the duodenum (Table 6) is likely to be the most valid indicator of pancreatic function since enzyme activity is likely to be limiting proteolysis in the duodenum. If this is so, the reason for the inferior digestion of diet HSKF may well result from the lower rate of secretion of pancreatic protease, associated with more undigested protein leaving the abomasum during the first 6 h of the postprandial period.

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