Digestion, absorption and utilization of single-cell protein by the preruminant calf

Abomasal outflow and its composition from calves given milk-substitute diets containing varying amounts of either bacterial or yeast protein

BY CYNTHIA A. SEDGMAN*, J. H. B. ROY† AND JOANNE THOMAS

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

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1. Two experiments of Latin square design, with four Friesian bull calves fitted with re-entrant duodenal cannulas at 4–10 d of age, were made to study the effect of giving varying levels of single-cell protein on the abomasal outflow and composition of digesta.

2. In Expt 1, diets in which 0, 220, 440, and 660 g/kg milk protein were replaced by the bacterial protein Pruteen were compared from 14 d of age. In Expt 2, which began at about 61 d of age, a comparison was made of diets in which 0, 220, 440 and 660 g/kg milk protein were replaced by the yeast protein Toprina.

3. Collection of abomasal outflow was made for 8 h after feeding for 2 d within each 6 d period of the Latin square design experiment. The amount of diet offered daily was 50 g dry matter/kg body-weight$^{0.75}$. Polyethylene glycol (PEG), which was used as an indigestible marker, total nitrogen (TN), protein-N (PN), fat, and potassium, sodium and chloride ion outflows were measured together with pepsin (*EC* 3.4.23.1) and chymosin (*EC* 3.4.23.4) activities, pH and titratable acidity.

4. In Expt 1 there was little difference in the outflow of liquid digesta between diets and about 0·9 of the dietary PEG was recovered within the 8 h collection period. With increasing amounts of Pruteen in the diet, outflows of TN, PN, fat and Na^+ increased, and the pH of digesta increased. However, the volume of 'apparent secretion' into the abomasum (outflow – intake), pepsin activity, chymosin activity, titratable acidity, (outflow of Cl^-–outflow of Na^+) as a measure of outflow of HCl, and outflows of K^+ and of Cl^- were reduced. All outflows decreased with the time interval after feeding, except (Cl^-–Na^+) outflow.

5. In Expt 2, the same trends as in Expt 1 were apparent, but since one calf had to be slaughtered and the experiment had to be analysed as a randomized block, only PN and K^+ outflows and pH were significantly affected by dietary treatment, with K^+ outflow increasing, rather than decreasing, with increasing concentration of single-cell protein in the diet.

6. Reduced proteolysis in the abomasum, associated with a faster and greater outflow of protein as a result of poor or no coagulation of protein in the abomasum, and a reduction in secretion of enzymes and in acidity may partly explain the poor protein digestibility and growth rate obtained in other experiments when diets containing more than 100 g single-cell protein/kg diet (about 200 g protein/kg total protein) were given to young calves.

Growth trials with calves given 'Toprina' (the yeast *Candida lipolytica* grown on *n*-alkanes; BP Ltd) led Shacklady & Gatumel (1972) to conclude that 75 g Toprina/kg air-dry powder could be used to replace dried skim-milk with no adverse effects on growth rate, feed conversion efficiency or carcass quality. Kirchgessner & Roth (1973) also found that 50–75 g Toprina/kg air-dry powder could be included without detrimental effect, but 100 g/kg inclusion caused a reduction in weight gain and feed conversion efficiency. Other workers have also shown that the inclusion of 100 g Toprina/kg air-dry powder (approximately 250 g/kg replacement of total protein) or more in a diet for young calves resulted in reduced growth rate and protein digestibility (Paruelle *et al.* 1975; Stobo & Roy, 1977; Toullec *et al.* 1979). Stobo & Roy (1977) also noted thickening of the walls of the small intestine when Toprina was given. However, Paruelle *et al.* (1972) gave a diet in which 700 g/kg protein were derived from Toprina supplemented with methionine and the remainder of the protein was from whey; they reported that when the yeast had a particle size of 50 μm, live-weight

* Present address: Lilly Research Centre Ltd, Erl Wood Manor, Windlesham, Surrey.
† For reprints.

gain and protein digestibility were satisfactory from 1 month of age but carcass quality was mediocre.

Inclusion of 200 g 'Pruteen' (the bacterium *Methylophilus methylotropus* grown on methanol; ICI Ltd)/kg air-dry powder in milk-substitute diets for young calves has resulted in reduced nutrient digestibilities and growth rates (Stobo & Roy, 1977; Roth & Kirchgessner, 1978; Roth *et al.* 1979). Hinks (1977) also reported severe diarrhoea and growth retardation when 200 g/kg were included, but this was probably associated with the use of poorly-utilized uncooked maize starch in the diets. In contrast, van Weerden & Huisman (1977) obtained crude protein (nitrogen × 6·25) and individual amino acid digestibilities comparable with milk protein when 200 g Pruteen/kg were included in diets for veal calves. However, the calves used in that experiment were either 8 or 15 weeks old and this could account for the more favourable results since it is well known that the digestibility of single-cell protein (SCP) increases with the age of the calf (Paruelle *et al.* 1972; Stobo & Roy, 1977). With only 100 g Pruteen/kg inclusion, Hinks (1977, 1978) reported good N digestibility and growth rates, although a slight reduction in energy digestibility was observed. Stobo & Roy (1977) also found satisfactory growth rates with 100 g Pruteen/kg air-dry powder in the diet. Since the inclusion of more than 100 g yeast or bacterial protein/kg in the diet of young calves or more than 200 g/kg in the diet of 2- to 3-month-old veal calves had detrimental effects, an investigation was made of the digestive function of calves given these protein sources. Observations were made on the changes in abomasal outflow and composition of the digesta that occurred, when increasing amounts of either bacterial protein (Expt 1) or yeast protein (Expt 2) were included in milk-substitute diets. The detailed results of these experiments have previously been published (Sedgman, 1980).

METHODS

Calves

Four Friesian bull calves, obtained within 24 h of birth from the Institute's herds, were used. Each calf was given 7 kg colostrum, obtained from the first two milkings after parturition from Friesian cows, in the first four feeds of life. All calves were given liquid diets from a bucket, the diets being reconstituted at 143 g powder/kg liquid.

The calves were prepared with duodenal re-entrant cannulas between 4 and 10 d of age. The cannulas were made of polyacetal (Kematel; ICI Plastics Division) and placed between the pyloric sphincter and the bile duct. The cannulas were connected by 'U'-shaped plastic tubing held in place by Unex NH 22 clips (Portland Plastics, Hythe, Kent). The calves were kept in individual metabolism cages (1·5 × 0·75 m) in a heated, purpose-built room throughout the experiment. During collections only, the calves were tethered by means of a leather collar attached by a chain to the front of the cage.

Diets

After the colostrum-feeding period, the calves were given Diet L, a milk-substitute diet based on skim-milk powder (Table 1) until they were allocated their first experimental diet at about 1 week after surgery. The amount of diet offered daily to each calf was 50 g dry matter/kg body-weight$^{0·75}$, which was calculated to give a gain in weight of approximately 0·75 kg/d. The compositions of the ingredients used in the experimental diets are given in Table 1.

In Expt 1, the calves were given the following four diets (Table 2) in a Latin square design with 6-d periods: diet MW, the control diet, composed of spray-dried skim-milk powder (DSM) and spray-dried whey powder (DW) in which fat had been incorporated by mechanical homogenization, together with a mineral and vitamin supplement; diet IL in which 220 g/kg protein were derived from bacterial protein (Pruteen); diet IH, in which

Table 1. *Composition of the ingredients of the diets (g/kg powder)*

	Diet L*	Ultra-high-fat milk powder†	Spray-dried skim-milk powder	Spray-dried whey powder	'Pruteen' bacterial protein	'Toprina' yeast protein
Dry matter (DM)	969	985	968	925	916	970
Fat‡	220	491	3·2	19·4	126	137
Lactose and other carbohydrates	418	290	519	605	58§	214§
Ash	66·8	49·8	79·9	86·3	76·0	61·5
Crude protein‖ (CP)	269	131	357	132	740	588
Amino acids	285	126	357	109	600	538
Calcium	10·8	6·9	12·2	6·0	0·6	0·1
Phosphorus	8·6	5·3	9·9	6·6	20·6	15·5

* 800 g spray-dried skim-milk powder/kg; 200 g fat/kg. Vitamin and mineral mixture (/kg DM): 300 mg Mg as $MgCl_2 . 6H_2O$, 100 mg Fe as $FeC_6H_5O_7 . 5H_2O$, 40 mg Mn as $MnSO_4 . 4H_2O$, 20 mg Zn as $ZnSO_4 . 7H_2O$, 10 mg Cu as $CuSO_4 . 5H_2O$, 100 μg Co as $CoSO_4 . 5H_2O$, 120 μg I as KI, 9·01 mg retinol and 1·05 μg cholecalciferol as Rovimix Type A 500 (31·5 mg) and Rovimix A+D (4:1) Type 500 (27·5 mg), 20 mg α-tocopherol as Rovimix E25 (80 mg), 30 μg cyanocobalamin, 50 mg butylated hydroxytoluene.

† 250 g spray-dried skim-milk powder/kg, 250 g spray-dried whey powder/kg, 500 g fat/kg. Vitamin and mineral mixture, 2·5 times those in diet L.

‡ Fat in ultra-high-fat milk powder and spray-dried high-fat milk powder consists of tallow–palm oil–soya lecithin (13:6:1, by wt).

§ Carbohydrate by difference.

‖ Nitrogen × 6·38 for milk and milk by-products, N × 6·25 for non-milk products.

440 g/kg protein were derived from Pruteen; diet IVH, in which 660 g/kg protein were derived from Pruteen.

Calcium carbonate was added to diets IL, IH and IVH (Table 2) to raise the Ca level to that of the control diet MW. After the completion of Expt 1 the calves were given diet L (Table 1) for approximately 2 weeks before the start of Expt 2 at a mean age of 61 d.

In Expt 2, the calves were given the following four diets (Table 2) in the same manner as for Expt 1: diet MW, the control diet as for Expt 1; diet TL, in which 220 g/kg protein were derived from yeast protein (Toprina); diet TM, in which 440 g/kg protein were derived from Toprina; diet TH, in which 660 g/kg protein were derived from Toprina.

$CaCO_3$ and DL-methionine were added to diets TL, TM and TH (Table 2) to raise the values to those of the control diet MW. In both experiments, 1 g polyethylene glycol (PEG)/kg liquid diet was added before each experimental meal as an indigestible marker. The calves were given each diet at 08.30 and 16.30 hours daily. Collection of digesta was made for 8 h after the morning feed on the 3rd and 6th day of each period of the Latin square design. A 'pre-feed' sample was taken before each collection began.

The compositions of the diets, as fed, that were used in the two experiments are given in Table 3.

Collection and sampling of digesta

The abomasal outflow was collected in a 500 ml polyethylene bottle attached to a harness on the calf. The bottle was emptied when it was approximately half-full and also at hourly intervals after feeding. The digesta were homogenized, the pH and volume measured and a 100 ml/l sample taken and kept on ice. The remaining 900 ml/l of the digesta were returned to a stainless-steel container attached to the side of the cage. The digesta were mixed with an electric stirrer and maintained at 37° by circulating water at 42° in a jacket around the stainless-steel container. Digesta were returned to the calf at a rate approximately equal to the outflow by means of a plastic tube attached to the distal duodenal cannula.

Table 2. *Proportion of ingredients and composition of the diets used in the experiments*

	Expts 1 and 2	Expt 1			Expt 2		
Diet...	MW	IL	IH	IVH	TL	TM	TH
Ingredient (g/kg):							
Ultra-high-fat milk powder	400	380	360	340	370	340	310
Spray-dried skim-milk powder	600	420	240	60	430	260	90
Spray-dried whey powder	—	100	200	300	100	200	300
Pruteen	—	100	200	300	—	—	—
Toprina	—	—	—	—	100	200	300
DL-Methionine	—	—	—	—	0·57	1·14	1·72
Calcium carbonate	—	4·3	8·6	12·9	4·2	8·3	12·5
Composition (g/kg powder):							
Dry matter	975	965	956	946	971	966	945
Fat	198	202	206	211	199	199	199
Carbohydrate	428	389	351	312	392	355	319
Ash	67·9	68·7	69·6	70·4	67·6	67·3	67·0
Crude protein*	267	287	307	328	274	282	289
Amino acids	265	269	273	277	265	265	266
Calcium	10·1	8·4	6·8	5·1	8·4	6·7	5·1
Phosphorus	8·1	8·9	9·7	10·6	8·4	8·9	9·2

* Nitrogen × 6·38 for milk and milk products, N × 6·25 for non-milk products.

Table 3. *Composition of the experimental diets (as fed)**

	Expt 1				Expt 2			
Diet...	MW	IL	IH	IVH	MW	TL	TM	TH
PEG (g/kg)	1·0	1·0	1·0	1·0	1·0	1·0	1·0	1·0
TN (g/l)	6·0	6·4	6·9	7·5	6·0	6·2	6·1	6·2
PN (g/l)	4·6	4·7	4·7	5·1	4·6	4·3	3·9	3·3
Fat (g/l)	26	27	27	24	26	26	25	24
K^+ (mM)	52	50	47	41	52	60	64	68
Na^+ (mM)	43	64	65	81	43	40	43	41
Cl^- (mM)	46	42	22	7	49	47	46	46
(Cl^-–Na^+) (mM)	3	−22	−43	−74	7	7	2	5
pH	6·4	6·3	6·3	6·2	6·4	6·3	6·1	6·1
Titratable acidity (mM)	26	23	26	26	26	32	33	32

PEG, polyethylene glycol; TN, total nitrogen; PN, protein nitrogen.
* For details of diets, see Tables 1 and 2.

The samples obtained were bulked for the periods 0–1 h, 1–2 h, 2–4 h, 4–6 h and 6–8 h after giving the experimental meal. At the end of each period, the samples were deep frozen at −20° and stored until needed for analysis.

Chemical analyses

Diets. Fat, protein, lactose, ash, phosphorus and chloride were determined by the methods given in Rowland *et al.* (1953), Ca by atomic absorption spectrometry and amino acids by a modification of the method of Spackman *et al.* (1958).

Digesta. Protein-N (PN) and non-protein-N (NPN) were measured as soon as each 1 or 2 h collection sub-period was completed to avoid further enzymic action. It was not feasible to assay for enzyme activity on the same day as the samples were obtained. To avoid loss of activity, about 10 ml of each bulked sample were frozen separately from the main sample and assayed for enzyme activity as soon as possible.

Total nitrogen (TN) was measured using a microKjeldahl technique with a potassium sulphate–mercuric oxide catalyst (Fleck & Munro, 1965). For measurement of NPN, digesta or liquid diet (5 ml) were mixed with 5 ml trichloroacetic acid (TCA; 100 g/l), left overnight at 4° and centrifuged at 2365 ***g*** for 20 min. The cap of fat–solid digesta was removed by suction and 2 ml supernatant fraction were analysed for N by the method used for TN. For determination of PN, digesta or liquid diet (1 ml) were mixed with 1 ml TCA (100 g/l), left at 4° overnight and centrifuged at 2365 ***g*** for 20 min. The supernatant fraction was discarded and the precipitate resuspended in 10 ml TCA (100 g/l) and centrifuged for 10 min. The precipitate was dissolved in 2 ml 0·5 M-sodium hydroxide; 10 ml Biuret reagent (6 g $CuSO_4 . 5H_20$ and 24 g sodium potassium tartrate dissolved in 300 ml 2·5 M-NaOH with 1 g potassium iodide added and made up to 1 litre with water) were added and the mixture left overnight for the colour to develop. It was then filtered (glassfibre paper GF/A, Whatman) and the optical density of the filtrate measured at 540 nm. Crystalline bovine albumin (Armour Pharmaceutical Co.) was used as a reference standard.

Proteolytic activity was determined in the duodenal digesta after a 1 in 20 dilution by the method of Williams *et al.* (1976), titratable acidity was determined in the duodenal digesta and liquid diets by the method of Williams *et al.* (1976), and pH was measured immediately after sampling and homogenization.

PEG was measured in the duodenal digesta by the method described by Smith (1958, 1962) except that 20 min were allowed for the development of turbidity. Sodium and potassium were determined by flame emission spectrometry after samples were prepared by the method described in Ministry of Agriculture, Fisheries and Food (1973). Cl^- was determined by the method of Whitehorn (1921) and fat by the Gerber method (British Standards Institution, 1962).

The acidity of the abomasal outflow was measured in three ways: (1) pH, to give a measure of the free hydrogen ions present in the digesta; (2) (Cl^-–Na^+), to give a measure of the hydrochloric acid present, on the assumption that any Cl^- present in excess of Na^+ must be as HCl (Ternouth *et al.* 1974); (3) titratable acidity (TA), titration of the digesta against 0·1 M-NaOH using phenolphthalein as an indicator to give a measure of the total acid present.

Measurement of digesta flow

Outflows of digesta were directly measured and no corrections were made to measured outflows for recovery of PEG.

Statistical methods

For the purposes of statistical analysis, the bulked time periods of all the experiments were adjusted to give time periods of equal length, namely 2 h.

In Expt 1, the results were analysed as a Latin square and the significance of the effect

of treatment, calf, age and time interval after feeding on duodenal outflow was determined by analysis of variance.

In Expt 2, one calf suffered from pyrexia, diarrhoea and severe dehydration and had to be killed after completion of only one period of the Latin square. Thus only three calves completed the experiment and the results were analysed as randomized blocks to give the significance of the effect of treatment and time after feeding on abomasal outflow.

Analyses were also made to show whether the treatment and time effects had significant linear or quadratic functions. The regression equations on time interval after feeding were obtained by using orthogonal polynomials where y was the mean value for each time-period and x was the mid-point of the time-period.

RESULTS

Expt 1

Changes with time interval after feeding (*Table 4*). The rate of digesta flow was most rapid immediately after feeding and then decreased during the following 8 h period. The outflow of the water-soluble marker PEG reflected the pattern of total abomasal flow. Between 0·87 and 0·94 of the PEG and thus of the liquid portion of the diet was recovered over an 8 h period after feeding.

As can be seen from the regression coefficients given in Table 4, all variables with the exception of TA showed a significant difference in total outflow with time after feeding. The patterns of outflow of TN, PN and fat were very similar with the amount of outflow decreasing with time after feeding.

The total activities of pepsin (*EC* 3.4.23.1) and chymosin (*EC* 3.4.23.4) in the digesta and total outflows of K^+, Na^+ and Cl^- decreased with time after feeding. Whereas the concentration of K^+ and Na^+ in the digesta decreased with time after feeding, that of Cl^- showed a considerable increase. Hence the reduction in the total amount of Cl^- in the digesta with time after feeding was only due to the decreasing volume.

By all three methods of determination of acidity, there was an increase in concentration of acid with time after feeding. However, the increase in total amount of (Cl^-–Na^+) was gradual and that of total TA varied little because of the decreasing volume of digesta with time. The mean pH showed a marked linear decrease with time.

Effect of concentration of Pruteen in the diet (*Table 5*). There was little effect of concentration of Pruteen in the diet on the pattern of abomasal emptying and the total volume outflow in 8 h after a meal for the four treatments was similar. However, there was a reduction in the volume of apparent secretion, i.e. volume of outflow minus volume of intake, as the amount of Pruteen in the diet was increased.

As shown in Table 5, with the exception of PEG and TA, the total outflow of all the variables measured showed a significant linear response to increasing amounts of Pruteen in the diet. The amount of outflow of TN, PN and fat increased markedly with increasing amounts of Pruteen in the diet. At the highest level of inclusion of Pruteen (IVH), recovery of the TN intake in the abomasal outflow was 1·2 of that recovered from the control diet; for PN this effect was more pronounced with recovery of 1·3 of that recovered from the control diet.

The total activities of pepsin and chymosin in the digesta decreased with increasing amounts of Pruteen in the diet. For all calves, there was approximately twice the amount of total chymosin as pepsin activity during the 8 h collection period. Although the overall treatment effects for chymosin were significant ($P < 0·01$) and those for pepsin, due to greater variability, were not significant, there was a significant linear reduction in pepsin with increasing inclusion of Pruteen in the diet.

Table 4. *Expt* 1. *Mean abomasal outflow over an 8 h period after feeding of preruminant calves given milk-substitute diets containing varying proportions of bacterial protein (Pruteen)*

(Regression equations relating abomasal outflow (y) to time after feeding (x): $y = a+bx$ and $y = a_1+b_1x+b_2x^2$)

	Time interval after feeding (h)					Linear coefficients			Quadratic coefficients				
	0–2	2–4	4–6	6–8	SEM (36 df)	a	b	SE b	a_1	b_1	SE b_1	b_2	SE b_2
Outflow (g):													
PEG	1·41	0·85	0·54	0·22	0·057	—	—	—	1·69	−0·31***	0·058	0·02*	0·007
TN	7·2	3·7	3·6	2·6	0·33	—	—	—	8·69	−1·92***	0·342	0·15***	0·042
PN	4·2	1·5	1·7	1·5	0·25	—	—	—	5·43	−1·58***	0·259	0·15***	0·032
Fat	24·3	12·8	14·8	11·7	1·61	—	—	—	28·9	−6·0**	1·65	0·5*	0·20
Enzyme activity (μmol tyrosine/min):													
Pepsin (*EC* 3.4.23.1)	389	268	260	182	25·5	400·6	−31·5***	5·70	—	—	—	—	—
Chymosin (*EC* 3.4.23.4)	821	459	448	379	37·3	—	—	—	995·3	−213·3***	38·26	18·3***	4·67
Outflow (mmol):													
K^+	59	34	24	13	2·3	—	—	—	72·2	−14·8***	2·31	0·9**	0·28
Na^+	106	63	48	28	3·3	—	—	—	126·6	−24·0***	3·36	1·5**	0·41
Cl^-	90	79	82	70	3·9	91·3	−2·8**	0·87	—	—	—	—	—
Outflow of acid:													
pH	5·6	4·4	3·5	2·5	0·08	5·99	−0·5***	0·02	—	—	—	—	—
Titratable acidity (mmol)	60	53	57	50	3·7	—	—	—	—	—	—	—	—
(Cl^-–Na^+) outflow (mmol)	−16	16	34	42	3·0	—	—	—	36·0	21·6***	3·11	−1·5***	0·38

PEG, polyethylene glycol; TN, total nitrogen; PN, protein N.
*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

Table 5. *Expt 1. Mean intake and abomasal outflow over an 8 h period after feeding of preruminant calves given milk substitute diets based on spray-dried skim-milk and whey powder (MW) or containing 220 g (IL), 440 g (IH) or 660 g (IVH) bacterial protein (Pruteen)/kg total protein*

(Regression equations relating abomasal outflow (y) to the proportion of bacterial protein in the milk substitute diets (x): $y = a + bx$)

	Diets					Overall significance of treatment effects	Linear coefficients†		
	MW	IL	IH	IVH	SEM (6 df)		*a*	*b*	SE *b*
Intake (l)	3·31	3·31	3·31	3·31	—	—	—	—	—
Outflow (l):									
0–1 h	1·04	1·01	0·97	1·11	—	—	—	—	—
1–2 h	0·54	0·48	0·37	0·52	—	—	—	—	—
2–4 h	1·11	1·01	1·19	0·65	—	—	—	—	—
4–6 h	0·75	0·89	0·76	0·90	—	—	—	—	—
6–8 h	0·59	0·48	0·58	0·63	—	—	—	—	—
Total	4·03	3·86	3·86	3·81	—	—	—	—	—
Apparent secretion (l):	0·72	0·55	0·55	0·49	—	—	—	—	—
Outflow (g):									
PEG	2·95	3·11	2·90	3·12	0·104	—	—	—	—
TN	13·8	15·9	17·9	20·6	0·96	*	13·7	10·2**	1·95
PN	5·2	7·4	11·4	11·4	1·06	*	5·5	10·2**	2·16
Fat	55·6	59·8	68·4	70·7	2·67	*	55·6	24·4**	5·44
Enzyme activity (μmol tyrosine/min)									
Pepsin (*EC* 3.4.23.1)	1818	1099	639	838	275·4	—	1608	−1544*	560·0
Chymosin (*EC* 3.4.23.4)	2355	2551	1588	1930	137·8	**	2441	−1016*	280·4
Outflow (mmol):									
K^+	143	131	124	120	6·0	—	140·5	−34·0*	12·24
Na^+	229	227	242	282	10·6	*	218·7	79·6*	21·60
Cl^-	365	314	306	296	16·6	—	352·2	−97·2*	33·68

Proportion of intake recovered in 8 h:									
K^+	0·83	0·79	0·80	0·88	—	—	—	—	—
Na^+	1·63	1·06	1·12	1·05	—	—	—	—	—
Cl^-	2·40	2·24	4·16	12·59	—	—	—	—	—
Apparent secretion (mmol):									
Na^+	88	13	26	13	—	—	—	—	—
Cl^-	213	174	232	273	—	—	—	—	—
Outflow of acid:									
pH					0·21	—	3·7	0·8***	0·09
0–2 h	5·3	5·5	5·5	5·9	—	—	—	—	—
2–4 h	3·9	4·2	4·6	4·8	—	—	—	—	—
4–6 h	2·8	3·5	3·7	3·9	—	—	—	—	—
6–8 h	2·7	2·8	2·3	2·4	—	—	—	—	—
Titratable acidity (mmol)	228	214	221	218	22·9	—	—	—	—
(Cl^-–Na^+) outflow (mmol)	136	88	64	14	11·7	**	133·6	−176·8***	23·72

PEG, Polyethylene glycol; TN, total nitrogen; PN, protein N.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Regression coefficients and their standard errors are actual values × 10^3.

The total outflow of K^+ and Cl^- decreased and that of Na^+ increased as the amount of Pruteen in the diet was increased. The concentration of Na^+ in the digesta after giving diet IVH was considerably higher than that after giving the other three diets and showed a completely different pattern of outflow from that of the total amount of Na^+ in the digesta. In contrast to the decrease in total outflow of K^+, the proportion of K^+ recovered after 8 h showed no clear pattern as the amount of Pruteen in the diet was increased.

The apparent secretion of Na^+ decreased and that of Cl^- increased with greater amounts of Pruteen in the diet. This was opposite to the effect that was observed when differences in intake were ignored. With all other variables measured, the differences in amount of intake had no effect on the overall pattern of outflow.

Although the pH of the diets decreased very slightly with increasing Pruteen concentration, the pH of the digesta was generally higher when greater amounts of Pruteen were included in the diet. The TA concentrations in the four diets were very similar. However, because the Cl^- intake decreased and the Na^+ intake increased as the amount of Pruteen in the diet was increased, there was a very considerable decrease in the (Cl^-–Na^+) intake. Thus the large decrease observed in the total amounts of (Cl^-–Na^+) in the digesta with increased inclusion of Pruteen in the diet was reversed when the results were expressed as apparent secretion of Cl^- minus apparent secretion of Na^+. When the (Cl^-–Na^+) intake varies so much between diets, it is possibly not valid to use (Cl^-–Na^+) levels in the digesta as a measure of the HCl present.

Interaction between pattern of outflow and concentration of Pruteen in the diet. There were significant diet × time after feeding interactions for PEG, TN, Na^+, Cl^- and (Cl^-–Na^+) outflows, pH and chymosin activity.

For diets MW, IL and IH, the pattern of outflow of K^+, Na^+ and Cl^- was similar and regular, but the pattern of outflow of these three ions after giving diet IVH was irregular.

Expt 2

Changes with time after feeding (*Table 6*). The patterns of outflow of digesta were similar to those for Expt 1. For all variables measured, there was a relation between outflow and time after feeding, the effects being linear for PEG, Cl^-, pH and TA.

As in Expt 1, the patterns of outflow of TN, PN and fat were similar with a gradual decrease in total outflow with time after feeding. A reduction in concentration of TN, PN and fat in the digesta occurred from 1 to 3 h after feeding followed by a steady increase from 3 to 8 h. This effect was masked when results were expressed as total amounts because of the decrease in volume with time after feeding.

The total pepsin and chymosin activities decreased from 1 to 5 h after feeding and then gradually increased to 8 h. The specific pepsin and chymosin activities also decreased initially and started to increase again about 3 h after feeding.

The total outflow and concentration of K^+ and Na^+ decreased with time after feeding. Although Cl^- concentration increased with time after feeding, total outflow of Cl^- decreased slightly due to masking by the decreasing volume of digesta.

As in Expt 1, all three methods of measuring acidity indicated an increase in concentration of acid with time after feeding. The amount of TA in the digesta decreased with time after feeding because of the decrease in volume of outflow with time. However, the total amount of (Cl^-–Na^+) still increased with time despite the volume reduction.

Effect of concentration of Toprina in the diet (*Table 7*). The pattern and amount of outflow in 8 h were similar for all four diets but, as in Expt 1, there was a reduction in apparent secretion as the amount of SCP in the diet was increased. The volume of digesta outflow was greater in Expt 2 since the calves were older and heavier and thus had a higher intake.

Table 6. *Expt 2. Mean abomasal outflow over an 8 h period after feeding of preruminant calves given milk substitute diets containing varying proportions of yeast protein (Toprina)*

(Regression equations relating abomasal outflow (y) to time after feeding (x): $y = a+bx$ and $y = a_1+b_1x+b_2x^2$)

	Time interval after feeding (h)					Linear coefficients			Quadratic coefficients				
	0–2	2–4	4–6	6–8	SEM (24 df)	a	b	SE b	a_1	b_1	SE b_1	b_2	SE b_2
Outflow (g):													
PEG	1·79	1·13	0·73	0·33	0·068	1·95	−0·24***	0·015	—	—	—	—	—
TN	7·4	4·5	5·3	4·1	0·38	—	—	—	8·38	−1·35**	0·387	0·11*	0·047
PN	3·1	1·4	2·2	1·9	0·22	—	—	—	3·68	−0·84***	0·223	0·09**	0·027
Fat	28·1	15·6	25·1	20·6	2·10	—	—	—	—	—	—	—	—
Enzyme activity (μmol tyrosine/min):													
Pepsin (*EC* 3.4.23.1)	487	287	250	282	28·7	—	—	—	615·9	−148·5***	29·37	14·5***	3·58
Chymosin (*EC* 3.4.23.4)	1080	492	448	475	46·6	—	—	—	1418	−400***	47·8	38***	5·8
Outflow (mmol):													
K^+	78	45	25	15	2·8	—	—	—	96·3	−20·0***	2·90	1·2**	0·35
Na^+	111	70	51	33	3·7	—	—	—	132·8	−24·1***	3·81	1·4**	0·46
Cl^-	142	133	123	106	7·9	149·6	−5·9**	1·77	—	—	—	—	—
Outflow of acid:													
pH	5·0	3·9	3·5	2·7	0·13	5·24	−0·36***	0·030	—	—	—	—	—
Titratable acidity (mmol)	113	87	83	78	8·8	111·7	−5·4*	1·96	—	—	—	—	—
(Cl^-–Na^+) outflow (mmol)	31	63	72	73	5·4	—	—	—	11·1	22·4***	5·58	−2·0**	0·68

PEG, polyethylene glycol; TN, total nitrogen; PN, protein N.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

Table 7. *Expt 2. Mean intake and abomasal outflow over an 8 h period after feeding of preruminant calves given milk substitute diets based on spray-dried skim-milk and whey powder (MW) or containing 220 g (TL), 440 g (TM) or 660 g (TH) yeast protein (Toprina)/kg total protein*

(Regression equations relating abomasal outflow (y) to the proportion of yeast protein in the milk substitute diets (x): $y = a + bx$)

	Diets					Linear coefficients†		
	MW	TL	TM	TH	SEM (6 df)	*a*	*b*	SE *b*
Intake (l)	4·53	4·48	4·58	4·43	—	—	—	—
Outflow (l):								
0–1 h	1·11	1·32	1·29	1·16	—	—	—	—
1–2 h	0·71	0·77	0·63	0·94	—	—	—	—
2–4 h	1·33	1·68	1·33	1·37	—	—	—	—
4–6 h	1·21	1·00	1·16	1·19	—	—	—	—
6–8 h	1·02	0·72	0·88	0·67	—	—	—	—
Total	5·38	5·48	5·30	5·33	—	—	—	—
Apparent secretion (l)	1·36	1·00	0·71	0·89	—	—	—	—
Outflow (g):								
PEG	3·96	3·78	4·00	4·16	0·158	—	—	—
TN	19·7	20·8	22·0	22·9	2·47	—	—	—
PN	6·9	8·3	8·7	10·8	0·73	6·85	5·48*	1·488
Fat	84·4	90·1	92·5	90·9	12·13	—	—	—
Enzyme activity (μmol tyrosine/min):								
Pepsin (*EC* 3.4.23.1)	1388	897	1818	1120	226·1	—	—	—
Chymosin (*EC* 3.4.23.4)	2498	2645	2485	2352	454·9	—	—	—
Outflow (mmol):								
K^+	145	167	170	184	8·7	148·6	54·8*	17·68
Na^+	243	262	276	279	17·3	—	—	—
Cl^-	513	540	493	470	45·4	—	—	—

Proportion of intake recovered in 8 h:								
K^+	0·62	0·62	0·58	0·61	—	—	—	—
Na^+	1·26	1·46	1·40	1·54	—	—	—	—
Cl^-	2·31	2·55	2·37	2·33	—	—	—	—
Apparent secretion (mmol):								
Na^+	50	83	78	98	—	—	—	—
Cl^-	292	328	285	268	—	—	—	—
Outflow of acid:								
pH	—	—	—	—	0·36	3·5	0·8**	0·23
0–2 h	4·5	4·9	5·3	5·4	—	—	—	—
2–4 h	3·5	3·6	4·1	4·5	—	—	—	—
4–6 h	2·9	3·9	3·5	3·8	—	—	—	—
6–8 h	3·2	2·5	2·5	2·7	—	—	—	—
Titratable acidity (mmol)	373	351	334	383	72·3	—	—	—
(Cl^-–Na^+) outflow (mmol)	270	277	217	191	33·4	—	—	—

PEG, polyethylene glycol; TN, total nitrogen; PN, protein N.
* $P < 0·05$, **$P < 0·01$, ***$P < 0·001$.
† Regression coefficients and their standard errors are actual values $\times 10^3$.

The effect of dietary treatments was only significant for PN, K^+ and pH, which showed linear effects with increasing proportion of Toprina in the diet. There was a tendency for the total amount of PEG in the digesta during the 8 h after a meal to increase with greater amounts of Toprina in the diet. As in Expt 1, the relative outflows of TN, PN and fat for the four diets were broadly similar, with outflow increasing as the level of Toprina in the diet was increased. Expressing PN and fat outflows as recoveries emphasized the increased outflow with greater amounts of Toprina in the diet. Nearly 0·4 more of the PN intake and 0·14 more of the fat intake were recovered after diet TH was given than after the control diet was given.

No clear pattern of change in pepsin and chymosin activities between diets occurred, although chymosin activity tended to decrease with greater amounts of Toprina in the diet. As in Expt 1, there was approximately twice as much chymosin activity as pepsin activity, the ratio being 1·91 for Expt 2 and 1·94 for the same three calves in Expt 1.

The total outflow and concentration of K^+ and Na^+ in the digesta increased and the total outflow of Cl^- slightly decreased with greater amounts of Toprina in the diet. The increase in outflow of K^+ with increasing amounts of Toprina was the reverse of that which occurred in Expt 1 with Pruteen. When expressed as the proportion of K^+ recovered there was no increase with greater amounts of Toprina in the diet. Expressing the Na^+ and Cl^- results as apparent secretions rather than as total outflows made no difference to the observed effects of increasing Na^+ and decreasing Cl^- outflow with greater amounts of Toprina in the diet.

The total amount of (Cl^-–Na^+), as a measure of acidity, decreased with increasing amounts of Toprina in the diet. Expression of the results as apparent secretion of (Cl^-–Na^+) did not alter this effect, since the intakes of these ions, unlike those in Expt 1, were similar for all four diets. When there were greater amounts of Toprina in the diet, the pH of the digesta was higher immediately after feeding and lower after about 5 h. The TA of the digesta decreased when diets TL and TM were given but increased after diet TH was given. The same pattern was observed when differences in TA intake were taken into account.

Interaction between pattern of outflow and concentration of Toprina in the diet. There were significant diet × time after feeding interactions for PN and K^+ outflow and for chymosin activity.

DISCUSSION

The results for the two experiments were broadly similar with an increase in the outflow of TN, undigested PN, fat and Na^+ and a decrease in proteolytic activity and Cl^- with increasing amounts of SCP in the diet. The differences between treatments in the Toprina experiment were not as marked as those in the Pruteen experiment with only three as opposed to ten of the twelve variables measured showing a treatment effect. Since Toprina has been shown to be less digestible than Pruteen in growth trials (Stobo & Roy, 1977), and the only differences between the two experiments were the source of the SCP and the age of the calves, it seems likely that the greater differences between treatments found in Expt 1 were an age effect, presumably because the calf can adapt its digestive function as it gets older (Roy & Stobo, 1975).

The pattern of outflow of digesta with time after feeding was similar to that observed by Mylrea (1966*a*), Mathieu (1968), Tagari & Roy (1969) and Leibholz (1975), and did not differ between diets. However, there was a reduction in the amount of apparent secretion as the amount of SCP in the diet was increased. This effect was also observed after feeding 'severely' rather than 'mildly' heat-treated milk (Tagari & Roy, 1969; Ternouth *et al.* 1974) and after feeding diets based on soya-bean or fish protein (Ternouth *et al.* 1975), although in the experiments of Ternouth *et al.* (1974, 1975) the cannula was distal to the bile duct.

The increase in outflow of TN, undigested PN and fat that occurred with increasing amounts of SCP included in the diet is similar to observations reported after feeding 'severely' rather than 'mildly' heat-treated milk (Tagari & Roy, 1969; Johnson & Leibholz, 1976) and after feeding diets containing other non-milk proteins, such as soya bean, fish, field-bean (*Vicia faba*) protein and whey protein concentrate (Colvin *et al.* 1969; Toullec *et al.* 1971; Guilloteau *et al.* 1975, 1979). The changes observed in the outflows of TN, PN and fat are consistent with the reduced coagulation of the diets containing SCP. Certainly, when milks are given that either do not clot or produce flocculent curds, there is reduced protein digestion (Tagari & Roy, 1969; Toullec *et al.* 1974), although Toullec *et al.* (1974) found that the digestibility was only markedly reduced before 1 month of age. As the calf gets older, the increase in abomasal and pancreatic proteases (Huber *et al.* 1961; Gorrill *et al.* 1967, Ternouth *et al.* 1976) is sufficient to cope with the faster protein flow.

Garnot *et al.* (1974, 1977) observed a reduction in chymosin secretion after feeding non-milk proteins but pepsin activity was not affected. Williams *et al.* (1976), however, obtained a reduction in both chymosin and pepsin activity when non-milk proteins were given. This apparent discrepancy is probably associated with the different methods used to determine the activities. Garnot *et al.* (1974, 1977) first separated the chymosin and pepsin from abomasal washings on a DEAE-cellulose column and determined activity from the clotting times of a solution of κ-casein. The method of Williams *et al.* (1976) used in the present experiments involved the measurement of the release, by juice from a gastric pouch, of tyrosine from a haemoglobin solution at pH 2·1 and pH 3·5 to give pepsin and chymosin activities respectively. A significant reduction in both chymosin and pepsin activities was found after giving diets containing increasing amounts of Pruteen and there was a tendency towards reduced chymosin activity when diets containing Toprina were given. The activity of chymosin was twice as great as the activity of pepsin; Garnot *et al.* (1974, 1977) also found chymosin activity higher than that of pepsin whereas Williams *et al.* (1976) found the reverse. Hill *et al.* (1970) and Henschel (1973) observed that the relative amounts of pepsin and chymosin secreted varied considerably among calves. The values for mean pepsin and chymosin activities in Expt 2 were 1·13 and 1·14 respectively of those in Expt 1 for the same three calves. Williams *et al.* (1976) found that chymosin production decreased with age whereas pepsin production was unaffected. In contrast, Garnot *et al.* (1977) observed a slow increase in pepsin but no change in chymosin production with age. However, the results of those two experiments and the present experiment are in agreement in that there was a decrease in total proteolytic activity with addition of non-milk proteins to the diet.

The decrease in K^+ and increase in Cl^- concentrations with time after feeding were similar to the findings of Mylrea (1966*b*) and Ternouth (1971). Both these authors also noted an increase in Na^+ concentration with time after feeding, but in the present experiment a decrease in Na^+ concentration occurred. However, the concentration of Na^+ in the diets used by Mylrea (1966*b*) and Ternouth (1971) was considerably lower than that in the present experiment. It is possible that these low levels may have caused stimulation of Na^+ secretion, whereas the high levels used in the present experiment could have caused inhibition of Na^+ secretion. From Table 3, it is clear that Pruteen relative to Toprina has a much higher concentration of Na^+ and lower concentration of K^+.

The recoveries of K^+, Na^+ and Cl^- during the 8 h collection period for the milk-control diet, MW, fell within the range of values obtained by Mylrea (1966*b*). For Cl^- there was little difference in 8-h recoveries between the Toprina treatments, which had similar dietary Cl^- concentrations. However, for the Pruteen treatments Cl^- recovery was 2·4 of Cl^- intake for diet MW and 12·6 of Cl^- intake for diet IVH, the latter having a very low Cl^- concentration.

The addition of either Toprina or Pruteen to the diet had a similar effect on the total

concentration of Na^+ and Cl^- as well as on the other variables measured. This suggests that the reason for the difference between the Toprina and Pruteen diets in the recovery of Na^+ and Cl^- is due to the abomasum compensating for the dietary imbalance of the Pruteen diets. There is presumably a stimulation of Cl^- secretion and an inhibition of Na^+ secretion. This would explain the very large increase in the recovery of Cl^- and decrease in recovery of Na^+ after giving Pruteen diets. It could also account for the large apparent secretion of (Cl^-–Na^+) despite the overall decrease in acidity of the digesta when Pruteen diets were given.

The inclusion of non-milk proteins in milk-substitute diets has been shown to reduce the acid concentration in the digesta (Tagari & Roy, 1969; Ternouth *et al.* 1975; Williams *et al.* 1976). In the present experiment, the pH was higher for the first 7 h after giving diets containing SCP and lower from 7 to 8 h when compared with the control diet. This probably arose because more dietary constituents of the SCP diets than of the control diets had left the abomasum by 7 h after feeding, thus leaving a greater proportion of acidic endogenous secretions in the abomasum. The concentrations of TA and (Cl^-–Na^+) also showed the increased acidity of abomasal outflow with time after feeding, agreeing with the results of Mylrea (1966*a*) and Tagari & Roy (1969).

From the results of the present experiments it is clear that inclusion of SCP in diets for preruminant calves results in a reduction in enzymic secretion, acid secretion and abomasal proteolysis. The reduced proteolysis is associated with a faster outflow of proteins from the abomasum giving less time for proteolysis to occur, a reduction in the secretion of the enzymes necessary to digest the proteins and a change in acidity that may result in less than optimum conditions for proteolysis.

These results may partially explain observations made on intact calves which show that levels of inclusion higher than 100 g SCP/kg (about 200 g protein/kg total protein) cause reduction in performance, digestibility and health, particularly in calves under 4 weeks of age and if the diets are offered in large quantities (Stobo & Roy, 1977; Hinks, 1977, 1978). These findings could partly be explained by reduced protein digestibility in the abomasum.

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