The nutritive value of non-milk proteins for the preruminant calf. The effect of replacement of milk protein by soya-bean flour or fish-protein concentrate

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1. Milk-substitute diets, in which 330–360 (L) or 610–700 (H) g/kg milk protein was replaced by protein from a thermo-alkali-treated soya-bean flour (SF) or from fish-protein concentrate (FPC), were compared in an experiment involving fifty Friesian calves, of which half were supplemented with a growth promoter, Grofas, known to have bacteriostatic properties. The liquid diets were fed *ad lib.* from 48 h of age until 136 kg live weight.

2. Seven calves, given non-milk protein, died or were removed from the experiment. There was little difference between treatments in the incidence of diarrhoea or in other observations on health of surviving calves, but those given non-milk protein maintained a lower mean rectal temperature.

3. Weight gain was reduced, especially during the first 3 weeks of life, by inclusion of non-milk protein. The reduction was greater for SF than for FPC, and greater at the H level. Supplementation with Grofas improved weight gain for calves given non-milk protein, but tended to reduce that for calves given milk protein.

4. Apparent digestibility of dry matter and protein was reduced when non-milk protein was used. The reduction was greatest at a young age, greater for SF than for FPC and greater at the H level. Apparent digestibility of fat was most markedly reduced with FPC, especially at 1 week of age, and was increased by Grofas supplementation. Digestibility of the carbohydrate in SF was low at 1 week but increased with age. Grofas supplementation caused a marked reduction in the disappearance of SF-carbohydrate in the alimentary tract. Apparent absorption of ash and calcium were reduced by non-milk protein, especially by FPC. The reduction in absorption of ash from SF was moderated by Grofas supplementation. Although Ca retention for calves given SF at the H level was very low, no bone defects were observed.

5. Nitrogen retention was slightly lower for calves given non-milk protein, but the efficiency of retention of apparently digested N was greater for calves given non-milk protein because of the much reduced urinary N excretion associated with a lower apparently digested N intake.

6. Dressed-carcass weight and 'killing out' percentage were lower for calves given non-milk protein, especially SF at the H level. Adrenal weights were markedly increased by feeding SF especially at the H level. Pancreas weight was greater for calves given SF than for those given FPC.

7. Weight of abomasal contents at slaughter was much lower for calves given non-milk protein. Weight of intestinal tissue and of total tissue in the alimentary tract were markedly increased by non-milk protein, especially by SF at the H level, and reduced by Grofas supplementation. The increased weight was associated with increased thickening of the walls of both the small and large intestine, which was possibly associated with hypertrophy of muscle cells.

8. It is concluded that up to 360 g/kg milk protein could be replaced by protein from this thermo-alkali-treated SF, and up to 610 g/kg milk protein from this FPC without markedly affecting performance, especially if an effective growth promoter is included in the diet. The beneficial effect of Grofas appeared to result from the reduction in the fermentation of the oligosaccharides of SF, which was reflected in higher digestibility particularly of fat and absorption of Ca and in reduced thickness of the intestines.

The replacement of milk protein by alternative sources of protein in the diet of the preruminant calf may be desirable on economic grounds. However, such replacement may be disadvantageous for two reasons. First, the digestion of dietary protein and absorption of amino acids may be markedly reduced and secondly, the absorbed amino acids may not meet the requirements of the calf. In particular, the feeding of soya-bean flour (SF) may have detrimental effects due to the presence of trypsin inhibitor, haemagglutinins, a large content of oligosaccharides (which probably cannot be utilized by the calf), and phytin. A number

of treatments of SF, including toasting accompanied by acid or alkali treatment (Colvin & Ramsey, 1968, 1969; Colvin, Lowe & Ramsey, 1969) or extraction with alcohol (Gorrill & Thomas, 1967; Nitsan, Volcani, Hasdai & Gordin, 1972; Smith & Sissons, 1975), has been claimed to overcome the detrimental effects of SF. Thermo-alkali treatment has been shown to increase the rate of inactivation of trypsin inhibitor (Wallace, Bannatyne & Khaleque, 1971) and to be more effective at a lower temperature than thermo-acid treatment (Coblentz, 1975).

Replacement of casein by SF or fish-protein concentrate (FPC) impairs curd formation in the abomasum, and in comparison with milk protein, both cause a reduction in rennin and pepsin secretion. Moreover diets containing SF, but not FPC, reduce gastric acid secretion (Williams, Roy & Gillies, 1976). In addition, although the rate of outflow of fluid from the abomasum is unaffected, replacement of 400 g/kg milk protein by that from SF or FPC results in a much shorter retention time of protein in the abomasum with a resultant decrease in proteolysis (Ternouth, Roy, Thompson, Toothill, Gillies & Edwards-Webb, 1975). There appears to be no compensation by the pancreas, since the volume of secretion and protease activity are reduced (Gorrill & Thomas, 1967; Gorrill, Thomas, Stewart & Morrill, 1967; Ternouth *et al.* 1975). In general, the calf is more able to tolerate these changes as it becomes older.

In older calves, the presence of haemagglutinins in the blood has been detected (van Adrichem & Frens, 1965; van Leeuwen, Weide & Braas, 1969; Smith & Wynn, 1971; Smith & Sissons, 1975), especially when milk protein has been completely replaced by protein from SF, and when successive feeds of SF interspersed with feeding of normal milk diets have been the routine. It has been suggested that under such conditions, gastric stasis occurs during the first 1–2 h after feeding, followed by rapid passage through the small intestine, and that this phenomenon is associated with the presence of high levels of antibody in the blood (Smith & Sissons, 1975).

FPC, either partially hydrolysed, or produced by solvent extraction of the fat, has proved more satisfactory, although the latter has been associated with muscular dystrophy (Makdani, Huber & Michel, 1971; Michel, Makdani, Huber & Sculthorpe, 1972) arising from the residual amount of oil in the FPC, which consists largely of polyunsaturated fats.

The majority of experiments on the use of SF (Ramsey & Willard, 1975) and FPC (Huber, 1975) in calf diets has been made with very restricted levels of intake, but Paruelle, Toullec, Frantzen & Mathieu (1972) studied the performance of three calves and their digestibility of a thermochemically treated SF supplying 700–750 g/kg protein of a milk substitute, containing chlortetracycline and given at *ad lib.* levels from 15 d of age to 150 kg live weight. The same group of workers (Toullec, Paruelle & Patureau-Mirand, 1973) also gave a large number of calves a diet containing partially-hydrolysed FPC over the same period. Neither of these products gave as good results as milk protein. In addition, Smits, Vreman, Boeve, Bon & Nieboer (1974) studied the digestibility of the protein in various FPC, and obtained quite high values, but the age at which the trials were made is not disclosed.

In view of the changes in digestive function brought about by SF and FPC, an experiment was done to study the performance of calves given milk-substitute diets at *ad lib.* levels in which 330–360 or 610–700 g/kg protein was supplied either by a thermo-alkali-treated SF or by FPC. Since it was expected that digestibility of protein would be reduced by the non-milk protein sources, the diets were formulated to contain more protein than was necessary to sustain the maximum genetic potential for growth of calves given milk protein. Moreover, as the replacement with non-milk protein might lead to a proliferation of an adverse microbial flora in the intestines, especially in the young calf, half the number of calves on each treatment was supplemented with a growth promoter that had bacteriostatic properties.

METHODS

Plan of experiment

The experiment, of randomized block design, was made with fifty Friesian male calves between March 1972 and March 1974 and consisted of ten treatments in each of five replications.

The diets, all containing 200 g fat/kg dry matter, were as follows: reconstituted, spray-dried skim-milk powder, with (MPG) and without (MP) the growth promoter Grofas; 560 g/kg protein in dried skim-milk replaced by SF (360 g) and spray-dried whey powder (200 g), with (SLG) and without (SL) Grofas; 890 g/kg protein in dried skim-milk replaced by SF (700) and spray-dried whey powder (190), with (SHG) and without (SH) Grofas; 560 g/kg protein in dried skim-milk replaced by FPC (330) and spray-dried whey powder (230), with (FLG) and without (FL) Grofas; 880 g/kg protein in dried skim-milk replaced by FPC (610) and spray-dried whey powder (270), with (FHG) and without (FH) Grofas.

Diets

Colostrum. The calves were removed from their dams before sucking and were placed on experiment within 8 h of birth. Before 48 h of age, each calf was given 7 kg whole colostrum from the first two milkings after parturition of Friesian cows.

Milk substitutes. The composition of the diets is given in Table 1. For all diets, an ultra-high-fat milk powder (640 g fat/kg dry matter (DM)) (Roy, Stobo, Gaston, Shotton & Ganderton, 1973) was used to supply supplementary fat, consisting of a mixture of beef tallow, palm oil and soya-bean lecithin (421, 182 and 37 g/kg DM, respectively), minerals and vitamins. For the calves given the growth promoter, quinoxaline-1,4-di-*N*-oxide (Grofas, which has now been withdrawn by the manufacturer; ICI Pharmaceutical Division, Alderley Park, Cheshire) was incorporated into the ultra-high-fat milk powder at the rate of 3·28 g Grofas/kg, and the resulting mixture was mixed with unsupplemented ultra-high-fat milk powder (1:9, w/w) to give 328 mg Grofas/kg. With an inclusion rate of 300 g ultra-high-fat milk powder/kg diet, the final concentration of Grofas was 98 mg/kg milk-substitute powder.

The spray-dried skim-milk powder (Volac Ltd, Royston, Herts.) used in diets MP, MPG, SL, SLG, FL and FLG was subjected to a mild pre-heating treatment before spray-drying and contained 170 g non-casein-nitrogen/kg total N. The SF was solvent-extracted and had been subjected to a thermo-alkali treatment to remove 'hidden' trypsin inhibitor (Sorbasoy Special; British Arkady Co. Ltd, Manchester) and was the same product as used by Williams *et al.* (1976) in studies of gastric secretion. The FPC (Protanimal; Astra-Ewos AB, Mölndal, Sweden) was the same product as that used by Ternouth *et al.* (1975) and Williams *et al.* (1976), except that the batch used for experimental blocks nos. 2–5 inclusive of the present experiment contained a suspending agent. Spray-dried whey powder (Krafco; Kraft Food Ltd, London W1) was included in the diets containing non-milk protein so that similar concentrations of protein and carbohydrate were present in all diets. During the course of the experiment, four to eight batches of each ingredient were used.

DL-methionine was added to diets containing SF; 1·5 g/kg milk-substitute powder for diets SL and SLG and 2·7 g/kg milk-substitute powder for diets SH and SHG to bring the total methionine content to that of diet MP and MPG, namely 7 g/kg milk-substitute powder.

All diets were reconstituted at the rate of 1 kg milk-substitute powder:6 kg water to give a DM content of 137 g/kg.

Table 1. *Composition of milk-substitute diets fed to calves*

Diet ...	MP	SL	SH	FL	FH
Ingredient (g/kg DM)					
Ultra-high-fat milk powder*	300	290	282	310	310
Spray-dried, skim-milk powder	700	311	—	299	—
Soya-bean flour†	—	197	375	—	—
Fish-protein concentrate‡	—	—	—	123	212
Spray-dried whey powder§	—	200	340	268	478
DL-methionine	—	1·6	2·8	—	—
DM content (g/kg) of:					
Diet as fed	138	137	137	138	138
Powdered diet	969	962	956	966	963
Composition of DM (g/kg)					
Fat	199	201	206	205	206
Protein (nitrogen × 6·38 for milk nitrogen × 6·25 for non-milk)	312	291	282	288	270
Lactose (anhydrous)	396	330	264	370	354
Carbohydrate other than lactose	—	86	160	28	51
Ash	69	68	67	82	92
Ca	11	8	6	14	16
Non-casein milk N in total dietary N (g/kg)	173	166	148	190	208
Proportion of dietary protein as (g/kg):					
Non-milk protein	—	358	702	327	605
Non-casein protein	200	560	891	563	876

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively.

* Containing 640 g fat/kg DM, mineral supplement (/kg), (magnesium 1·0 g, iron 351 mg, manganese 140 mg, copper 35 mg, cobalt 0·36 mg, zinc 70 mg, iodine 0·42 mg), vitamin supplement (/kg), (retinol 14700 μg, cholecalciferol 306 μg, D-α-tocopherol 70 mg, cyanocobalamin 106 μg) and antioxidant (butylated hydroxyanisole 112 mg/kg).

† Sorbasoy special; hexane-extracted, and thermo-alkali-treated to destroy 'hidden' trypsin inhibitor, containing (g/kg DM) 58 fat, 353 carbohydrate, 528 protein, 60 ash (British Arkady Co. Ltd, Manchester).

‡ Protanimal; solvent-extracted herring meal, ground, 140 US mesh, containing (g/kg DM) 12 fat, 771 protein, 160 ash (Astra-Ewos A.B., Mölndal, Sweden).

§ Krafco (Kraft Foods Ltd, London W.1).

Calves

Collection, housing and management of the calves were as described by Roy, Stobo, Gaston & Greatorex (1970), the individually-penned calves being offered the diets at 38° by bucket *ad lib.* in two equal feeds daily at 08.15 and 16.30 hours. Calves had access to the milk substitute for 20 min at each feed, the quantity offered being increased by 0·5 kg milk substitute/feed every alternate day, provided all the milk substitute given had been consumed for four consecutive feeds. The environmental temperature and relative humidity were maintained at 17° and 65% respectively.

The calves were weighed weekly and routine records of body temperature and consistency of faeces were made daily to 14 d of age and at 1 week intervals thereafter. In addition, daily observations of each calf were made, and if necessary further information was recorded.

Digestibility and N and calcium balance trials were made on four replications of calves beginning at 1, 4 and 10 weeks of age, using the methods described by Roy, Gaston, Shillam, Thompson, Stobo & Greatorex (1964). The calves were slaughtered on reaching 136 kg live weight, the measurements made after slaughter being similar to those described by Roy, Stobo & Gaston (1970). In addition, on four replications, the digestive tract was ligated to isolate the contents of the reticulo-rumen, omasum, abomasum and intestines.

After separation, the component parts were weighed, cut open and the contents removed; after being washed, the tissues were allowed to drain for 6 h before they were weighed. For the final two replications, and also for some replacement calves (see below), the intestine was also ligated at the ileo-caecal valve, and the weights and contents of the small and large intestine recorded separately. In these calves, the length of the small intestine, large intestine, and caecum blind sac from the ileo-caecal valve were measured by placing the tissues vertically next to a metre rule.

Analytical methods

The chemical methods used for the diets, urine and faeces were those described by Roy *et al.* (1964).

Statistical analysis

The transformation of certain values before analysis was as described by Roy *et al.* (1964). All statements in the text of the form 'were different from' or 'there was clear evidence that' indicate a statistical difference of at least $P < 0{\cdot}05$.

RESULTS

Health and mortality

Five calves died during the course of the experiment and two further calves were slaughtered in extremis. All had received diets containing non-milk protein. Six of these calves were replaced; one had been given diet FL (*Salmonella typhi-murium* septicaemia, 24 d of age), one had been given diet FHG (*S. dublin* septicaemia and congestion of the lung, 17 d of age), two had been given diet SH (*Aspergillus fumigatus* infection of the lung, 72 d of age; *S. typhi-murium* localized intestinal infection, 95 d of age) and two had been given diet SHG (dehydration, enlarged thyroid and a fair growth of non-haemolytic *Escherichia coli* in the intestines, 91 d of age; almost pure growth of lactobacilli in intestines and enlarged mesenteric lymph nodes, 14 d of age). The seventh calf, given diet FL, died at 101 d of age, death being associated with calf diphtheria (*Fusiformis necrophorus*), which involved ulceration of the tongue and abomasum, a congested ileum and a thickened and oedematous colon. As the calf appeared normal up to 91 d, the results obtained up to this stage of the experiment were used in the analysis. One of the calves given diet SHG (slaughtered at 91 d) and one calf given diet SH (slaughtered at 72 d) that were examined immediately after slaughter in extremis showed evidence of atrophy of the intestinal villi. In addition, one otherwise normal calf given diet FLG showed a grossly enlarged left salivary gland, the inflammation being confined to the interstitial tissue.

Throughout the course of the experiment a particular phage type of *S. typhi-murium* (Phage type U163; definitive type 95; Salmonella Reference Laboratory, Public Health Laboratory, Colindale) was present in the calf pens. If it was suspected from the appearance of blood in the faeces or from abnormally high rectal temperature that a calf might be excreting salmonella, samples of the faeces were checked for the presence of the organisms.

In spite of the tendency for a higher mortality rate of calves given non-milk protein, there was no evidence that inclusion of non-milk protein in the diet had any effect on the number of calves that were excreting salmonella, although there was a slight indication that supplementation with Grofas may have resulted in some reduction in the incidence of excretion by calves given milk protein or SF.

The incidence of diarrhoea, even during the first 14 d of life (mean 1 d), and of nasal and eye discharge was low and did not differ between treatments, although there was a tendency ($P < 0{\cdot}1$) for Grofas to reduce the over-all incidence of diarrhoea in calves given SF. The incidence of a high rectal temperature ($> 39{\cdot}33°$) was greater for calves given SF than for

Table 2. *Effect of source of protein and Grofas† (G) on performance of calves given milk-substitute diets*

(Mean values for five calves/group)

Diet‡ ...	Treatment	MP	SL	SH	FL	FH	Pooled SE of mean	Statistical significance of effect: G within milk	MP v. non-milk protein	SF v. FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G
Birth wt (kg)	Without G	41·4	38·0	40·5	41·1	38·3	1·81			*			*			
	With G	39·3	40·1	35·3	41·7	43·9										
Total dry matter intake (kg)	Without G	147·4	156·8	198·1	153·9	171·1	5·90		**	***	***			**		
	With G	149·6	154·2	178·0	142·6	158·7										
Final live wt (kg)	Without G	144·6	139·6	132·3	136·6	136·8	2·73									
	With G	138·2	140·6	137·7	138·4	139·8										
Age on day before slaughter (d)	Without G	82	97	119	89	102	4·4		*	***	**	**		*	**	
	With G	90	92	97	80	87										
Live-weight gain (kg/d)																
Birth – 3 weeks	Without G	1·11	0·85	0·67	0·88	0·66	0·089		**					*		
	With G	0·98	0·90	0·88	1·05	0·80										
Birth – slaughter	Without G	1·27	1·06	0·80	1·08	0·97	0·048	*	***	*	**	**	*	*	**	
	With G	1·12	1·10	1·05	1·22	1·11										
Relative wt gain ($k \times 10^2$)§	Without G	1·54	1·36	1·02	1·36	1·26	0·056		**	*	*	***	**	*	*	
	With G	1·42	1·38	1·41	1·53	1·35										
Feed conversion ratio (kg dry matter/kg gain)	Without G	1·43	1·54	2·19	1·62	1·74	0·073		***	*	***	**	**	*		
	With G	1·51	1·54	1·74	1·48	1·65										

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ $k = \dfrac{\log_e \text{final wt} - \log_e \text{birth wt}}{\text{age (d)}}$.

those given FPC, and supplementation with Grofas reduced the incidence in calves given milk protein. Calves given non-milk protein had significantly lower mean rectal temperatures (38·84°) than those given milk protein (38·96°, pooled SE 0·055°). A high proportion of calves showed lung lesions at slaughter, but there was no difference between treatments in their incidence or severity.

Growth rate

The results are given in Table 2. Since final live weight did not differ between treatments and daily live-weight gain was reduced by the inclusion of non-milk protein in the diet, age at slaughter was significantly greater for calves given non-milk protein and significantly greater for calves given SF than for those given diet FPC. Grofas supplementation reduced the age at slaughter for calves given non-milk protein, especially at higher levels of replacement, but reduced the growth rate of calves given milk protein.

Digestibility and N and Ca balance

Faecal DM *concentration.* With the level of passive immunity afforded by the feeding of 7 kg colostrum, there was no evidence that non-milk protein had any effect on the DM concentration of the faeces, even at 1 week of age (Table 3). Moreover, the DM concentration decreased for all treatments from 4 to 10 weeks of age. The faeces of calves given FPC were of significantly higher DM content than those of calves given SF, and were even drier when the FPC diets were supplemented with Grofas.

DM *digestibility.* Daily DM intake, after adjustment for differences between treatments in live weight, was increased by high inclusion rates of non-milk protein (Table 3). Apparent digestibility of DM was markedly reduced with non-milk protein in the diet, the reduction being greater for SF than FPC and greater for higher levels of inclusion. However, as the calves became older, digestibility of DM of the non-milk protein sources improved, the highest value (0·92) being that for diet FLG at 10 weeks. Nevertheless, digestibility of non-milk protein was not as high as for milk protein, even at 10 weeks of age.

Fat digestibility. Although the fat provided in the ultra-high-fat milk powder was the same for all diets, digestibility of fat was reduced in the diets containing non-milk protein, especially FPC; at 1 week of age, digestibility of fat for diet FH was only 0·44 compared to 0·81 for diet MP (Table 3).

Protein digestibility. Digestibility of protein reflected that of DM except that Grofas supplementation enhanced the digestibility of SF (Table 3).

Carbohydrate digestibility. Digestibility of lactose was unaffected by the inclusion of FPC, but the presence of 353 g carbohydrates other than lactose/kg DM in the SF reduced digestibility, especially at 1 week of age (Table 3). Even so, by 4 weeks of age, it appeared that the digestibility or disappearance due to fermentation of the carbohydrate in SF was quite high. Grofas supplementation caused a marked reduction in the apparent digestibility of these carbohydrates but not of lactose, since the greater the quantity of SF carbohydrates in the diet, the greater was the reduction in digestibility.

Ash absorption. Apparent absorption of ash was reduced when diets contained non-milk protein; this occurred with FPC even at the low level (Table 3). However with SF, low levels of inclusion caused a smaller reduction than high levels. Grofas supplementation increased ash absorption for diets containing SF, especially at high levels of inclusion, but had no significant effect on diets containing FPC.

Ca absorption. Apparent absorption of Ca was lower for non-milk protein diets and decreased with age, but unlike ash absorption, it did not differ between SF and FPC (Table 3). Higher levels of non-milk protein reduced absorption, but with SF, this was moderated by supplementation with Grofas.

Table 3. *Effect of source of protein and Grofas† (G) on digestibility of milk-substitute diets fed to calves*

(Mean values, with their standard errors where indicated, for four calves/group)

Diet‡ ...	Treatment	MP	SL	SH	FL	FH	Pooled SE of treatment mean within weeks	Statistical significance of effect: G within milk	MP v. non-milk protein	SF v. FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G	Weeks	Weeks × treatments
Live wt (kg)																		
1 week	Without G	50·5	46·1	46·0	48·5	43.5												
	With G	48·8	48·7	45·1	52·5	48·3												
4 weeks	Without G	76·4	67·1	61·3	70·8	59·4												
	With G	70·4	71·8	64·4	75·4	68·7	3·77										***	***
10 weeks	Without G	133·0	110·3	91·0	116·2	99·4												
	With G	115·5	120·4	110·2	127·4	116·8												
Dry matter (DM) intake (kg/d)																		
1 week	Without G	1·13	1·05	1·06	1·11	1·07												
	With G	1·12	1·21	1·27	1·23	1·16												
4 weeks	Without G	1·70	1·47	1·41	1·60	1·46												
	With G	1·47	1·54	1·68	1·65	1·68	0·109					*					***	
10 weeks	Without G	2·17	1·81	1·72	1·73	1·93												
	With G	1·86	1·78	2·19	2·15	2·02												
Over-all§	Without G	1·48±0·069	1·48±0·060	1·60±0·070	1·45±0·060	1·66±0·068					**			*			—	—
	With G	1·46±0·060	1·44±0·061	1·78±0·061	1·52±0·066	1·60±0·060												
Faecal DM (g/kg)																		
1 week	Without G	189	179	195	179	202												
	With G	168	169	168	206	204												
4 weeks	Without G	188	198	173	200	193												
	With G	167	174	186	233	216	15·5			***					*		***	
10 weeks	Without G	143	121	136	133	194												
	With G	147	130	150	185	192												
Apparent digestibility																		
DM																		
1 week	Without G	0·91	0·78	0·74	0·81	0·76												
	With G	0·89	0·85	0·77	0·83	0·76												
4 weeks	Without G	0·95	0·89	0·80	0·87	0·83												
	With G	0·95	0·88	0·77	0·89	0·85	0·017		***	**	***			**			***	*
10 weeks	Without G	0·92	0·88	0·81	0·89	0·89												
	With G	0·95	0·89	0·84	0·92	0·89												
Over-all‖	Without G	0·92±0·011	0·86±0·011	0·79±0·011	0·86±0·011	0·83±0·011												
	With G	0·94±0·011	0·88±0·011	0·78±0·011	0·87±0·011	0·83±0·011			***	**	***			**			—	—
Fat																		
1 week	Without G	0·81	0·62	0·66	0·56	0·44												
	With G	0·76	0·79	0·81	0·61	0·50												
4 weeks	Without G	0·89	0·87	0·73	0·71	0·60												
	With G	0·90	0·87	0·86	0·77	0·68	0·042		***	***		***		**	*		***	*
10 weeks	Without G	0·82	0·78	0·73	0·75	0·79												
	With G	0·91	0·85	0·91	0·83	0·79												
Over-all¶	Without G	0·83±0·022	0·77±0·023	0·72±0·023	0·68±0·022	0·61±0·022												
	With G	0·87±0·023	0·84±0·022	0·84±0·024	0·72±0·023	0·65±0·023			***	***		***		**			—	—

Table 3 (*cont.*)

(Mean values, with their standard errors where indicated, for four calves/group)

Diet‡ ... Treatment		MP	SL	SH	FL	FH	Pooled SE of treatment mean within weeks	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L × G	Within FPC L	Within FPC G	Within FPC L × G	Weeks	Weeks × treatments
Apparent digestibility																		
Protein																		
1 week	Without G	0·86	0·71	0·60	0·74	0·71												
	With G	0·90	0·84	0·71	0·79	0·66												
4 weeks	Without G	0·94	0·84	0·66	0·83	0·75												
	With G	0·94	0·84	0·62	0·84	0·78	0·030		***	***	***	*		**			***	*
10 weeks	Without G	0·92	0·84	0·66	0·86	0·87												
	With G	0·95	0·87	0·76	0·92	0·86												
Carbohydrate																		
1 week	Without G	1·00	0·89	0·86	0·99	0·96												
	With G	0·95	0·88	0·79	0·97	0·96												
4 weeks	Without G	1·00	0·95	0·92	0·99	1·00												
	With G	1·00	0·92	0·81	1·00	1·01	0·013		***	***	***	***	***				***	**
10 weeks	Without G	1·00	0·97	0·96	1·00	0·99												
	With G	0·99	0·94	0·87	1·00	0·99												
Apparent absorption																		
Ash																		
1 week	Without G	0·88	0·74	0·71	0·71	0·71												
	With G	0·84	0·82	0·77	0·74	0·71												
4 weeks	Without G	0·90	0·84	0·72	0·75	0·72												
	With G	0·90	0·84	0·75	0·77	0·71	0·027		***	**	***	•					*	
10 weeks	Without G	0·77	0·79	0·68	0·74	0·73												
	With G	0·85	0·76	0·77	0·76	0·71												
Calcium																		
1 week	Without G	0·87	0·53	0·43	0·56	0·58												
	With G	0·77	0·68	0·55	0·59	0·56												
4 weeks	Without G	0·85	0·72	0·42	0·60	0·56												
	With G	0·83	0·71	0·54	0·64	0·53	0·048		***		***	**	**	*			**	
10 weeks	Without G	0·58	0·68	0·31	0·60	0·50												
	With G	0·74	0·56	0·57	0·61	0·47												
Ca retention (g/d)																		
1 week	Without G	11·0	4·5	2·5	8·9	9·9												
	With G	9·7	6·9	3·9	10·3	10·4												
4 weeks	Without G	15·9	8·7	3·4	13·5	13·2												
	With G	13·4	8·9	5·1	14·7	14·1	1·35		***	***	***						***	
10 weeks	Without G	14·4	10·2	3·0	14·6	15·7												
	With G	15·1	8·4	7·0	18·2	15·1												
Over-all††	Without G	12·6±0·56	11·9±0·78	10·2±1·12	9·5±0·67	7·5±0·91												
	With G	13·3±0·55	11·8±0·74	11·2±0·96	9·3±0·87	6·1±1·10			***	***	*			***			—	—

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ Adjusted for differences between treatments in live wt.

‖ Adjusted for differences between treatments in DM intake.

¶ Adjusted for differences between treatments in fat intake.

†† Adjusted for differences between treatments in Ca intake.

Table 4. *Effect of source of protein and Grofas† (G) on the nitrogen metabolism of calves given milk-substitute diets*

(Mean values, with their standard errors where indicated, for four calves/group)

Diet‡ ... Treatment	MP	SL	SH	FL	FH	Pooled SE of treatment mean within weeks	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G	Weeks	Weeks × treatments
Metabolic body size ($W^{0.75}$) (kg)																	
1 week Without G	18·9	17·7	17·6	18·4	16·9												
With G	18·4	18·4	17·4	19·5	18·3												
4 weeks Without G	25·8	23·4	21·0	24·4	21·4	0·92		*		*			*	*		***	***
With G	24·3	24·7	22·7	25·6	23·9												
10 weeks Without G	39·1	34·0	29·4	35·4	31·5												
With G	35·2	36·3	34·0	37·9	35·5												
Live-wt gain (kg/d)																	
1 week Without G	0·93	0·69	0·61	0·72	0·62												
With G	0·89	1·06	0·95	0·95	0·65												
4 weeks Without G	1·19	1·00	0·83	0·99	0·98	0·164										*	
With G	0·91	1·03	0·93	1·09	0·92												
10 weeks Without G	1·11	1·19	0·82	0·50	1·00												
With G	0·78	0·73	1·17	1·20	0·92												
Dry matter intake (g/$kg^{0.75}$ d)																	
1 week Without G	58·4	59·2	60·1	60·5	63·1												
With G	60·7	65·3	73·3	63·7	63·3												
4 weeks Without G	65·8	62·4	64·5	65·2	68·2	3·34				**	*						
With G	60·6	62·3	73·8	64·7	70·3												
10 weeks Without G	54·8	53·3	58·6	49·0	61·4												
With G	53·3	49·3	64·3	56·9	56·8												
N intake (g/d)																	
1 week Without G	55·0	48·2	47·6	50·6	45·9												
With G	54·8	55·3	57·1	56·0	49·6												
4 weeks Without G	83·0	67·4	63·5	72·6	62·7	5·09		*			*					***	
With G	71·7	70·4	75·3	74·8	71·9												
10 weeks Without G	106·4	83·3	77·3	78·7	83·0												
With G	90·8	81·7	98·1	97·7	86·5												
Faecal N (g/d)																	
1 week Without G	5·6	14·1	19·2	12·8	13·1												
With G	5·1	8·9	16·9	11·4	16·7												
4 weeks Without G	5·3	11·1	21·6	12·1	15·2	1·88		***	***	***			*				*
With G	4·4	11·5	29·0	11·6	15·9												
10 weeks Without G	7·2	12·6	25·4	10·7	11·0												
With G	4·5	10·3	23·4	8·3	11·7												
Apparently digested N (g/d)																	
1 week Without G	49·5	34·1	28·4	37·8	32·7												
With G	49·7	46·4	40·3	44·5	32·9												
4 weeks Without G	77·7	56·3	41·9	60·5	47·5	5·33		***		*	*					***	
With G	67·3	58·9	46·3	63·2	56·0												
10 weeks Without G	99·1	70·8	51·9	68·0	72·0												
With G	86·3	71·4	74·8	89·4	74·8												
Over-all§ Without G	65·0±1·39	57·8±1·26	48·2±1·31	58·6±1·25	57·2±1·29			***	***	***			*				
With G	66·0±1·24	60·2±1·24	47·7±1·29	60·3±1·28	55·7±1·24												

Table 4 (*cont.*)

(Mean values, with their standard errors where indicated, for four calves/group)

Diet‡ ... Treatment		MP	SL	SH	FL	FH	Pooled SE of treatment mean within weeks	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G	Weeks	Weeks × treatments
Urinary N (g/d)																		
1 week	Without G	16·4	12·3	9·6	12·3	9·7												
	With G	18·6	12·8	10·4	14·8	10·3												
4 weeks	Without G	31·3	21·2	16·3	22·3	19·4												
	With G	32·2	22·1	16·2	26·9	22·1	2·96		***	*				*	*		***	*
10 weeks	Without G	57·3	32·7	27·5	38·9	31·0												
	With G	50·2	39·5	36·0	48·0	40·3												
Over-all§	Without G	31·3±1·77	23·5±1·61	20·4±1·68	25·6±1·59	22·3±1·65			***	**	**			*				
	With G	33·0±1·58	25·3±1·58	18·7±1·64	28·0±1·63	24·7±1·58												
Urine wt (g/kg$^{0·75}$)																		
1 week	Without G	247	250	248	260	278												
	With G	246	262	290	268	289												
4 weeks	Without G	278	275	262	279	308												
	With G	280	263	314	310	312	16·8			*	*			*			***	
10 weeks	Without G	235	210	224	200	279												
	With G	240	201	268	255	262												
N balance (g/d)																		
1 week	Without G	33·1	21·8	18·9	25·5	23·1												
	With G	31·1	33·6	29·8	29·7	22·5												
4 weeks	Without G	46·4	35·1	25·6	38·2	28·0												
	With G	35·2	36·7	30·1	36·3	33·8	4·65		*								***	
10 weeks	Without G	41·8	38·1	24·3	29·1	40·9												
	With G	36·0	31·9	38·7	41·5	34·4												
Over-all‖	Without G	28·5±2·09	34·3±1·63	34·3±2·05	32·4±1·62	35·4±1·69			***									
	With G	27·3±1·78	33·2±1·61	35·5±1·63	30·4±1·72	32·4±1·62												
Biological value¶																		
1 week	Without G	0·75	0·75	0·79	0·77	0·81												
	With G	0·70	0·80	0·83	0·75	0·79												
4 weeks	Without G	0·67	0·71	0·72	0·72	0·69												
	With G	0·60	0·71	0·75	0·66	0·70	0·015††		***					*			***	—
10 weeks	Without G	0·51	0·64	0·59	0·54	0·66												
	With G	0·51	0·56	0·62	0·56	0·56												

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ Adjusted for differences between treatments in N intake.

‖ Adjusted for differences between treatments in apparently digested N.

¶ For values assumed for metabolic faecal N and endogenous urinary N, see p. 178.

†† SE based on variation due to treatments × weeks.

Ca retention. Since no attempt was made to equalize the Ca intakes on the various diets, absolute retention of Ca or retention expressed relative to metabolic body-weight ($W^{0.75}$, kg) for diets containing SF was much lower, especially at high levels of inclusion, than for diets containing milk protein or FPC (Table 3). Although retention of Ca by calves given diet SH was low, no overt signs of rickets or other bone disorders were observed. Daily Ca retention values increased with age, but Grofas supplementation was without effect. After adjustment for differences between treatments in Ca intake, retention of Ca was higher for calves given diets containing SF than for those given diets containing FPC, indicating a greater efficiency of absorption of Ca at the lower levels of Ca intake that occurred with SF.

N retention. Although live-weight gain was lower throughout the experiment for calves given non-milk protein, live-weight gain during the balance periods did not differ significantly between treatments because of the high variability (Table 4).

Although N intake was somewhat lower and faecal N excretion was higher for non-milk-protein diets, urinary N losses were considerably reduced, especially at high levels of substitution. As a result N balance, although slightly lower for calves given non-milk protein, did not differ between diets containing FPC or SF and was unaffected by level of inclusion of non-milk protein.

When adjusted for differences between treatments in apparently digested N, N balance of calves given milk protein was lower than for all the diets containing non-milk protein. The apparently digested N of the non-milk protein was thus being retained more efficiently than that of the milk protein, because the level of digestible N intake from the milk protein was, as planned, excessive in relation to the energy supply. This was confirmed by the biological values, which were calculated using a metabolic faecal N value of 1·9 g N/kg DM intake and an endogenous urinary N value of 190 mg N/kg $W^{0.73}$ equivalent to 175 mg N/kg $W^{0.75}$ (Roy *et al.* 1964; Roy, Stobo, Gaston & Greatorex, 1970), and which at all ages were higher for the non-milk protein diets.

Measurements made at slaughter

Carcass. Although there was no difference between treatments in live weight at slaughter, calves given non-milk protein, especially at high levels of SF inclusion, had lower dressed-carcass weights (CW) and 'killing out' percentage (Table 5). When values were expressed relative to $CW^{0.75}$, no difference between treatments was found in perirenal fat deposition, except for an interaction between level of inclusion of SF and Grofas supplementation, in mesenteric fat deposition (over-all mean $\pm$ SE, $68 \pm 2{\cdot}1$ g/kg $CW^{0.75}$) or in the weights of kidneys ($24 \pm 0{\cdot}35$ g/kg $CW^{0.75}$) or skin ($257 \pm 3{\cdot}4$ g/kg $CW^{0.75}$). However, adrenal weight on a per kg $CW^{0.75}$ basis was markedly increased by feeding SF, especially at high levels of inclusion. This increase in adrenal weight was not associated with differences between treatments in the incidence of diarrhoea or of a high rectal temperature or in the severity of lung lesions. Although not significant, Grofas tended to increase the adrenal weight of calves given milk protein. Pancreas weight, either absolute or in terms of $CW^{0.75}$ was significantly greater for calves given SF than for those given FPC, even when adjusted for treatment differences in the incidence of diarrhoea throughout the experimental period, with which pancreas weight was positively correlated.

Alimentary tract contents. The weights of contents in the various parts of the alimentary tract were analysed in absolute terms and also as proportions of the total weight of contents. Only the absolute values are presented in Table 6. The salient feature was the much reduced weight of abomasal contents at slaughter of the calves given non-milk protein. After adjustment for differences between treatments in age at slaughter, non-milk protein, with the exception of diet FLG, also caused a reduction in the weight of contents of the intestines. However, total alimentary tract contents did not differ between treatments because of the

Table 5. *Effect of source of protein and Grofas† (G) in milk-substitute diets fed to calves on measurements made at slaughter*

(Mean values, with their standard errors where indicated, for five calves/group, except for diet FL without G for which the missing value for one calf was calculated by the method of Yates (1933))

Diet‡ ...	Treatment	MP	SL	SH	FL	FH	Pooled SE of mean	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G
Age at slaughter (d)	Without G	83	98	120	90±5·0	103	4·4									
	With G	91	93	98	81	88										
Live-wt at slaughter (kg) (*A*)	Without G	140·2	134·8	127·4	134·5±3·11	132·1	2·75									
	With G	134·8	136·3	132·9	132·9	134·4										
Dressed carcass wt (kg) (*B*)	Without G	85·1	81·1	72·4	81·2±2·34	79·6	2·07		*		**					
	With G	82·5	81·7	78·3	79·8	82·3										
Dressed carcass wt (kg)§	Without G	80·7±0·79	80·5±0·74	77·0±0·80	80·9±0·84	80·9±0·74				***	**					
	With G	81·9±0·74	80·1±0·75	79·1±0·74	80·6±0·74	82·1±0·74										
Killing out percentage (100 *B*/*A*)	Without G	60·5	60·1	56·6	60·3±0·71	60·2	0·63		•	**	***					
	With G	61·2	59·9	58·9	60·0	61·3										
Killing out percentage§	Without G	59·8±0·58	60·0±0·54	57·4±0·58	60·2±0·61	60·5±0·54				***	**					
	With G	61·1±0·54	59·7±0·55	59·0±0·54	60·2±0·54	61·2±0·54										
Perirenal fat (g/kg carcass $wt^{0·75}$)	Without G	49	52	35	51±6·2	51	5·5						*			
	With G	40	44	50	46	48										
Pancreas wt (g/kg carcass $wt^{0·75}$)‖	Without G	4·38	4·83	5·46	4·06±0·521	4·15	0·445			**						
	With G	4·73	4·98	4·74	3·53	3·83										
Pancreas wt (g/kg carcass $wt^{0·75}$)¶	Without G	4·33±0·401	4·52±0·416	5·30±0·405	3·97±0·470	4·25±0·402				**						
	With G	4·74±0·400	5·09±0·402	4·98±0·410	3·59±0·401	3·93±0·402										
Adrenal wt (g/kg carcass $wt^{0·75}$)	Without G	0·289	0·337±0·0211		0·392	0·310±0·0211	0·309	0·0187		***	**					
	With G	0·335	0·320		0·373	0·317	0·287									

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ Adjusted for differences between treatments in live wt at slaughter.

‖ Four calves/treatment.

¶ Adjusted for differences between treatments in incidence of diarrhoea (birth-slaughter).

Table 6. *Effect of source of protein and Grofas† (G) in milk-substitute diets fed to calves on the weight of contents of the alimentary tract at slaughter*

(Mean values, with their standard errors where indicated, for four calves/group, except for diet FL without G for which the missing value for one calf was calculated by the method of Yates (1933))

Diet‡ ...	Treatment	MP	SL	SH	FL	FH	Pooled SE of mean	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G
Wt of contents:																
Reticulo-rumen (kg)	Without G	1·66	1·04	1·00	1·13±0·277	1·09	0·236	•								
	With G	0·75	1·00	1·00	1·06	1·83										
Omasum (g)	Without G	38	0	13	48±20·7	38	17·7									
	With G	0	13	0	13	0										
Abomasum (g)	Without G	700	413	138	393±145·1	188	123·9		***							
	With G	763	375	153	175	338										
Intestines (kg)	Without G	1·58	1·61	2·31	1·35±0·256	1·49	0·219			*	*					
	With G	1·88	1·41	1·69	1·75	1·14										
Intestines (kg)§	Without G	1·90±0·226	1·57±0·195	1·49±0·348	1·49±0·233	1·21±0·218			*					**		
	With G	1·94±0·196	1·55±0·200	1·68±0·194	2·10±0·230	1·27±0·199										
Total (kg)	Without G	3·98	3·06	3·46	2·92±0·500	2·80	0·427									
	With G	3·39	2·80	2·84	3·00	3·30										

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ Adjusted for differences between treatments in age at slaughter.

Table 7. *Effect of source of protein and Grofas*† *(G) in milk-substitute diets fed to calves on the fresh weight of the alimentary tract of calves at slaughter*

(Mean values, with their standard errors where indicated, for four calves/group, except for diet FL without G for which the missing value for one calf was calculated by the method of Yates (1933))

Diet‡ ...	Treatment	MP	SL	SH	FL	FH	Pooled SE of mean	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G
Wt at slaughter ($kg^{0.75}$)	Without G	40·8	39·7	37·3	39·5±0·89	39·0	0·76				*					
	With G	39·6	39·9	39·0	39·2	39·6										
Wt of tissue (g/kg slaughter $wt^{0.75}$)																
Reticulo-rumen	Without G	20	22	23	21±1·2	23	1·0								*	
	With G	20	21	21	19	20										
Omasum	Without G	4·9	4·7	5·4	4·6±0·45	5·4	0·38						**	*		
	With G	5·4	5·6	3·8	4·5	5·4										
Abomasum	Without G	17	15	15	17±1·5	16	1·3									
	With G	16	14	14	15	16										
Intestines	Without G	100	123	174	111±7·7	124	6·6		***	***	***	***	**			
	With G	91	113	121	102	112										
Intestines§	Without G	109±6·9	122±6·0	151±10·6	115±7·1	116±7·6			***	*	*	•				
	With G	93±6·0	117±6·1	121±5·9	112±7·9	116±7·1										
Total	Without G	142	165	217	152±9·2	169	7·8		***	**	***	***	**			
	With G	132	153	160	140	153										
Total§	Without G	154±8·2	163±7·1	189±12·6	157±8·4	160±7·9			**							
	With G	134±7·1	158±7·2	160±7·0	152±8·3	157±7·2										

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ Adjusted for differences between treatments in age at slaughter.

Table 8. *Effect of source of protein and Grofas† (G) in milk-substitute diets fed to calves on the weight of tissue and length of the small and large intestines*

(Mean values, with their standard errors where indicated, for two calves/group, except for diet SF without G for which the missing value for one calf was calculated by the method of Yates (1933))

Diet‡ ...	Treatment	MP	SL	SH	FL	FH	Pooled SE of mean	Statistical significance of effect: G within milk	MP *v.* non-milk protein	SF *v.* FPC	Within SF L	Within SF G	Within SF L×G	Within FPC L	Within FPC G	Within FPC L×G
Age at slaughter (d)	Without G	80	90	139	92±15·2	115	10·4				*					
	With G	100	92	98	80	89										
Small intestine (/kg slaughter wt$^{0·75}$)																
Wt (g)	Without G	63	78	136	70±9·1	84	6·3		**	**	**	***	**			
	With G	59	75	71	65	71										
Wt (g)§	Without G	84±9·6	86±5·9	87±19·9	77±7·6	63±9·5		*								
	With G	55±5·0	82±5·5	70±4·9	86±9·4	81±6·2										
Length (m)	Without G	0·72	0·78	1·05	0·74±0·077	0·85	0·053				*	*				
	With G	0·71	0·73	0·77	0·71	0·82										
Length (m)§	Without G	0·90±0·078	0·86±0·048	0·61±0·161	0·80±0·061	0·67±0·077		*								
	With G	0·68±0·041	0·78±0·044	0·76±0·039	0·89±0·076	0·91±0·050										
Wt:length (g/m)	Without G	2·1	2·6	3·7	2·4±0·26	2·5	0·18		**	**	*	**	**			
	With G	2·1	2·6	2·4	2·4	2·2										
Large intestine (/kg slaughter wt$^{0·75}$)																
Wt (g)	Without G	37	41	61	35±7·3	40	5·0			**	*					
	With G	34	44	53	37	36										
Length (m)	Without G	0·12	0·13	0·16	0·13±0·011	0·15	0·007		*		*					
	With G	0·13	0·13	0·15	0·13	0·14										
Wt:length (g/m)	Without G	7·2	8·2	10·8	7·0±1·15	6·9	0·79			**						
	With G	6·8	8·5	9·2	7·0	6·9										
Caecum (/kg slaughter wt$^{0·75}$)																
Length (mm)	Without G	8·1	8·5	11·8	7·9±1·13	9·9	0·78				•					
	With G	8·6	8·5	9·7	8·4	9 7										
Length (mm)§	Without G	10·6±1·23	9·6±0·76	6·0±2·54	8·7±0·96	7·4±1·21										
	With G	8·2±0·64	9·3±0·70	9·6±0·62	10·8±1·20	10·9±0·79										

MP, milk protein; SL, SH, soya-bean flour at low and high levels of inclusion respectively; FL, FH, fish-protein concentrate at low and high levels of inclusion respectively; SF, soya-bean flour; FPC, fish-protein concentrate.

*$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

† Growth promoter, quinoxaline-1,4-di-*N*-oxide; ICI Pharmaceutical Division, Alderley Park, Cheshire.

‡ For details, see Table 1.

§ Adjusted for differences between treatment in age at slaughter.

high variability. Grofas supplementation reduced the amount of fluid in the rumen of calves given milk protein.

Alimentary tract tissue. Alimentary tract tissue weights were analysed as absolute values, as proportions of the total alimentary tract tissue weight and as values expressed on a $W^{0.75}$ basis. Only the last-mentioned values are presented in Table 7.

Non-milk protein had no effect on abomasal tissue weight. However, for rumen and omasal tissue weight, several differences were apparent. Within FPC, Grofas increased rumen tissue weight and high levels of inclusion increased omasal tissue weight, whereas with SF, high levels of inclusion tended to decrease omasal weight.

The weight of intestinal tissue and therefore of total tissue in the alimentary tract was markedly increased by non-milk protein, especially by higher levels of inclusion of SF and was reduced by Grofas supplementation. This difference was still apparent when the values were adjusted for differences between treatments in age at slaughter.

In the final two replications of the experiment, additional measurements were made on the small intestine and large intestine separately and the results are given in Table 8. Small intestine weight expressed on a per kg slaughter weight$^{0.75}$ basis and length of small intestine also expressed on this basis, when adjusted for differences between treatments in age at slaughter, were unaffected by treatments other than a reduction in the value of both variables when milk protein was supplemented with Grofas. However, the value for weight: length, a measure of the thickness of the small intestine, was markedly increased by non-milk protein, especially by SF at high levels of inclusion and was reduced when SF was supplemented with Grofas. Similar findings of increased weight, length and weight:length for the large intestine were obtained with increasing levels of inclusion of SF.

The adjustments made for age at slaughter were calculated using the combined regression coefficients, since with values available from only two calves/treatment, no comparison of the regression between treatments was feasible. However, as it seemed possible that the regression might differ in slope and position between treatments, a further analysis was made in which all available values were grouped according to diet as follows: milk protein, four calves; non-milk protein, eighteen calves; SF, eleven calves; SF without Grofas, six calves; and SF with Grofas, five calves. The results differed from those given in Table 8, in that weight of small intestine expressed per kg $W^{0.75}$, after adjustment for difference in age at slaughter, was greater for calves given non-milk protein or SF. Moreover even with this method of adjustment, weight:length for the small intestine was increased when SF was fed.

DISCUSSION

The salient feature of the experiment was that replacement of milk protein in a milk-substitute diet by SF supplying 360 g/kg protein or by FPC supplying 330 g/kg protein was possible, but with some detriment to the health and performance of the calf. In general, higher levels of replacement, i.e. 700 g protein from SF or 610 g protein from FPC/kg total protein, were more detrimental than the lower levels, but FPC was more satisfactory than SF, especially at the higher rate of inclusion. Moreover, the reduction in digestibility and of growth rate was greatest in the neonatal calf and all deaths that occurred were of calves given non-milk protein.

At the level of Ig given to the calves in colostrum and with the high volume of air space for each calf and high air extraction rate in the experimental calf house (Roy, Stobo, Gaston, Ganderton, Shotton & Ostler, 1971), there was no indication of an increased incidence of diarrhoea or of low faecal DM concentration during the first 3 weeks of life of calves given non-milk protein. However, there was a slight tendency for calves given SF unsupplemented with Grofas to show a greater incidence of diarrhoea throughout the whole

experimental period. Similarly calves given SF had a greater incidence of a high rectal temperature than those given FPC.

The lower mean rectal temperature of calves given non-milk protein was associated with their lower digestible energy intake. The poor digestibility of non-milk protein in the neonatal calf is probably associated with a short retention time in the abomasum, due to lack of coagulation and to the reduction in gastric and pancreatic protease secretion that also occurs (Ternouth *et al.* 1975; Williams *et al.* 1976). Digestibility of non-milk protein improves with age; thus the calf appears to adapt itself to lack of coagulation. Gastric acid secretion and pancreatic protease secretion are also known to increase with age (Ternouth & Roy, 1973).

To maintain a constant concentration of protein in the different diets, replacement of the skim-milk solids with non-milk protein necessitated the inclusion of increasing quantities of spray-dried whey powder with increasing quantities of non-milk protein. At the high levels of inclusion of SF and FPC, the only casein in the diet was that contained in the ultra-high-fat milk powder. However, there was no evidence that high levels of whey had an adverse effect on the calves; in fact diet SH, which resulted in the poorest performance, contained a much lower proportion of whey protein than did diet FH. Possibly, the low intake of soluble milk protein could have been a contributory cause to the poor performance of calves given diet SH. Whey has been shown to be a poorer stimulator of oesophageal groove function than milk protein, SF or FPC (Guilhermet, Mathieu & Toullec, 1973) and there has been some suggestion that certain FPC may pass into the rumen and cause bloat (Makdani *et al.* 1971). There was little evidence that rumen contents at slaughter were greater for calves given FPC, but there was evidence of increasing development of omasal tissue with increasing level of FPC possibly arising from the trapping of insoluble FPC particles within the omasal laminas on their passage to the abomasum.

There was no evidence in this experiment or in that of Ternouth *et al.* (1975) that, at the levels of SF used, gastric stasis had occurred after feeding as has been shown with complete replacement of milk protein by SF, when such a diet is given intermittently to calves being reared on whole milk (Smith & Sissons, 1975).

Although protein digestibility of diets containing SF was reduced even when supplemented with Grofas, fat digestibility was much less affected even at high inclusion rates of SF; indeed at 10 weeks, diet SHG had the same fat digestibility as diet MPG. This suggested that the presence in the diet of a small amount of soya-bean oil, rich in polyunsaturated fatty acids, tocopherols and phosphatides, was having some beneficial effect on fat digestibility. In contrast, the inclusion of FPC had a marked effect in reducing fat digestibility and it can only be concluded that this was associated with the high ash content of the FPC, causing increased faecal excretion of Ca and Mg soaps (Raven & Robinson, 1958). If this was so, it is not indicated in the Ca absorption values for diets containing FPC, which were no lower than those containing SF. However, the low absorption of Ca from SF compared with that from milk may have been due to the presence of phytin.

No supplementary Ca was included in the diets containing SF, since it seemed possible that there was sufficient for the calves' needs. Animals tend to maintain constancy in Ca retention by adjusting the efficiency of absorption in relation to Ca intake. This appeared to occur with FPC diets, which contained large amounts of Ca, but it certainly did not occur with diets containing SF, where absorption of Ca was much lower than that for the Ca in milk. This may have been due to the phytin content of soya bean, which accounts for 70% of the total phosphorus, and is not only unavailable for simple-stomached animals but interferes with the absorption of Ca, iron and zinc (Taylor, 1965; Anon, 1967; O'Dell, 1969). Thus, Ca retention for calves given diets SH and SHG was very low (0·25–0·33 of that obtained with milk protein); however, calves given diet SHG gained 1·05 kg/d and there

was no sign of bone malformation, possibly because of the rather high level of vitamin D included in the diets.

The efficiency of retention of apparently digested N was, surprisingly, higher for the diets containing SF supplemented with methionine or FPC, than for those containing milk protein. This finding is substantiated by the calculated biological values (Table 4) which were higher for the non-milk protein than for milk protein. However, as both the protein content of the diets containing non-milk protein and the apparent absorption of N from those diets were lower than for the diets based totally on milk protein, the non-milk protein is more likely to have been limiting and under such conditions urinary N excretion was reduced.

Extrapolating the values for apparent digestibility of protein found in this experiment to a value corresponding to either 100% inclusion of a mixture of non-milk protein and whey protein at a ratio of 4·08:1 for SF and 2·57:1 for FPC, or to 100% inclusion of non-milk protein, gives coefficients for apparent protein digestibility of 0·61 for the SF plus whey and 0·76 for the FPC plus whey mixtures, and of 0·52 and 0·70 for SF and FPC respectively. From these values, it is estimated that the apparent digestibility coefficient for whey protein is 0·94.

The increased weight of adrenals of calves given SF especially at high levels of replacement, could have been a response to 'stress' or could be due to disturbance in electrolyte balance. Hawkins, Roy, Shillam, Greatorex & Ingram, (1959) observed a tendency for adrenal weight at 3 weeks of age to be positively correlated with the previous incidence of diarrhoea, and to increase in calves dying from a localized intestinal infection with *E. coli* after profuse diarrhoea (Roy, Shillam, Hawkins, Lang & Ingram, 1959). Such deaths are associated with a hyperkalaemia. However, in the present experiment no relationship was obtained between adrenal weight and the preceding incidence of diarrhoea, and it is possible that the increased adrenal weights could have resulted from the necessity to maintain homoeostasis in a situation where the SF contained a very high concentration of potassium, 20 g/kg DM, compared to 12 g K/kg DM in milk.

Supplementation with Grofas in general appeared to have a slightly detrimental effect on calves given milk protein, but was very beneficial for calves given non-milk protein. Although the spectrum of bacteriostatic activity of Grofas is not known, it seems probable that it was controlling an adverse flora in the intestinal tract of calves given non-milk protein, but possibly having a slightly adverse effect on the balance of the intestinal flora when used with milk protein.

Thus, with milk protein, Grofas had the effect of reducing live-weight gain over the whole experimental period. It also tended to reduce digestibility of lactose and N retention, reduced the length and weight of the small intestine, and reduced the fluid content, probably of salivary origin, in the rumen at slaughter. Grofas, however, did reduce the incidence of a high rectal temperature.

In contrast, the effects of Grofas with non-milk protein were to increase live-weight gain, to reduce age at slaughter and to reduce feed conversion ratio for diets containing SF, especially at the high rate of inclusion. In addition during the balance trials, Grofas supplementation resulted in an increase in faecal DM concentration for calves given FPC; DM intake, apparent digestibility of protein and absorption of ash and Ca for calves given SF; and digestibility of fat for calves given SF or FPC.

However, the digestibility of the carbohydrate in SF was markedly reduced by Grofas, which suggests that hydrolysis or fermentation of the oligosaccharides by the microflora may have been prevented. As a result of the high digestibility of N in calves given FPC supplemented with Grofas, urinary N excretion was increased.

There was no evidence that Grofas improved N retention or the biological values of the

protein at the levels of dietary protein:energy used, nor did it have any effect on the weight of contents of the alimentary tract at slaughter. It did, however, have a slight effect in reducing the weight of rumen tissue in calves given FPC and a very marked effect in reducing the weight and proportion of intestinal tissue in the alimentary tract of calves given SF. In particular, the increased thickness of the intestinal wall, as measured by the value for weight:length, for calves given SF, especially at high levels, was reduced by Grofas. Histological studies on seven calves (A. Turvey, personal communication) indicated that this increase in thickness was largely due to muscle tissue and, on the basis of a decrease in number of muscle nuclei/unit area, appeared to be associated with an increase in cell size rather than in cell numbers. Grofas supplementation tended to reduce the proportion of muscular tissue, particularly in the duodenum and ileum.

It seems probable that the increase in muscle cell size was associated with the extra work necessary in moving a greater bulk of digesta through the intestine, when a diet of low digestibility was given. Indeed the number of muscle nuclei/unit area for duodenal sections for the seven calves examined was positively related to DM digestibility at 10 weeks of age (r 0·77, $P < 0·05$). However, the relationships of digestibility with jejunal and ileal sections were not quite significant (r 0·61 and 0·67 respectively). It is not possible to say how far poor digestibility per se increased thickening or was the result of thickening or how far poor digestibility was associated with the presence of an adverse microflora, which is suggested by the beneficial action of Grofas. Although it is well known that germ-free animals have thinner-walled intestines than conventional animals (Coates, 1973), in that situation, it is due partly to a smaller proportion of lymphoid tissue, but mainly to a reduction in connective tissue, particularly the lamina propria, within the villi. In the present experiment, the proportion of lymphoid tissue tended to be slightly increased by Grofas supplementation and there was little evidence that the proportion of villi tissue was reduced, although some villous atrophy appeared to have occurred in two calves slaughtered in extremis. Moreover, the area of villi, lamina propria and lymphatic tissue in the duodenum and jejunum tended to be lower for calves given SF.

The beneficial effect of Grofas on both Ca and fat absorption for calves given SF, which contained some residual soya-bean oil, would suggest that Grofas may have reduced the hydrogenation of unsaturated fatty acids by the gut microflora and thus prevented the faecal excretion of Ca soaps of C16:0 and C18:0 fatty acids.

In conclusion, it would appear that the risks associated with replacement of milk protein are such that up to 360 g/kg protein could be replaced by the thermo-alkali-treated SF and up to 610 g/kg protein by the FPC without markedly affecting performance, especially if an effective growth promoter is included in the diet.

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