Br. J. Nutr. (1966), **20**, 373

Comparative studies on the digestive physiology of sheep fed on semi-purified or roughage–concentrate diets

1. Food and water intake, rumen volume and rates of parotid secretion

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(*Received* 24 *November* 1965—*Accepted* 30 *December* 1965)

1. The food and water intake and diurnal variations in the rumen contents were measured in sheep with rumen fistulas receiving semi-purified or pelleted roughage–concentrate diets. The rates of secretion and composition of parotid saliva of the sheep on both diets and of sheep fed on hay were determined. The sheep had continuous access to the diet. 2. The mean daily intakes of the semi-purified and roughage–concentrate diets were 920 and 2234 g respectively. The corresponding water intakes were 2·50 and 6·05 l. 3. None of the sheep ruminated on either diet. The weight of total contents and of dry matter in the rumen was highest in animals on the roughage–concentrate diet, and marked diurnal fluctuations, which reflect the eating habits of these animals, were observed. The semi-purified diet appeared to have a very marked water-retaining capacity in the rumen. 4. The estimated daily total secretion of both parotid glands was 2·7, 5·4 and 11·5 l. in sheep fed on the semi-purified diet, roughage–concentrate and hay respectively. No appreciable differences were observed in the composition of parotid saliva between the three groups of sheep. In sheep receiving hay, saliva secretion was lower during eating than between periods of eating, during part of which time the sheep presumably ruminated. 5. It is concluded that the observed differences in parotid secretion reflected differences in the physical form of the diet.

Semi-purified diets are being used to an increasing extent in studies on the nutrition and physiology of ruminants. Owing to the complex nature of microbial fermentation and synthesis in the rumen, the formulation of semi-purified diets for ruminants has been extremely difficult. Studies with these diets are justified only if they support a microbial population and function comparable with those found in the rumen of animals receiving more usual diets.

Lawlor, Smith & Beeson (1963) evaluated existing semi-purified diets for growing–fattening sheep in terms of growth rate, feed efficiency and general appearance. The studies to be reported were designed to compare the microbial population and function, and certain other aspects of the digestive physiology of sheep receiving semi-purified and pelleted roughage–concentrate diets. Diets lacking unground roughages have been observed to result in a marked reduction, and in some instances a total absence of rumination. The absence of rumination presumably causes a serious depression of salivation and consequently the loss of saliva as the most important buffer in the rumen. This communication reports the food and water intake, the amount of contents in the rumen and the rate of secretion and composition of parotid saliva.

EXPERIMENTAL

Animals and diets. Merino cross-bred wethers aged about 2 years old fitted with permanent rumen cannulas of 1 in internal diameter were used throughout the experi-

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ments. Samples of the rumen contents were removed through a polyvinyl chloride tube. The tube was connected to a suction flask operated with a hand pump. The sheep were housed in individual wooden pens with slatted wooden floors. No bedding was used since animals receiving the semi-purified diet tended to eat bedding. After an initial period of 4–6 weeks when they were given hay, three sheep were fed on the semi-purified diet over a period of 11 months. The remaining sheep were fed on either the pelleted roughage–concentrate diet or medium-quality hay.

Table 1. *Composition (%) of the semi-purified diet*

Isolated soya-bean protein	14·8
Cellulose powder (Mikro-Technik GmbH, Miltenberg, Main, Germany)	40·0
Starch	17·3
Glucose (Gervais, Rosenheim, Germany)	17·3
Soya-bean oil	3·0
Choline chloride	0·1
Vitamin mixture*	0·5
Major-mineral mixture†	6·4
Trace-mineral mixture‡	0·6

* Providing (per kg diet): vitamin A 4000 i.u., vitamin D 880 i.u., vitamin E 33 mg, inositol 110 mg.

† Providing (per 100 g diet): $CaHPO_4.2H_2O$ 3·152 g, $K_2CO_3.1\frac{1}{2}H_2O$ 2·050 g, $MgSO_4.3H_2O$ 0·698 g, NaCl 0·500 g.

‡ Providing (per kg diet): $CuSO_4$ (anhydrous) 26·0 mg, $FeSO_4.2H_2O$ 596·6 mg, $Na_2B_4O_7.10H_2O$ 124·6 mg, $MnSO_4.H_2O$ 64·6 mg, $ZnSO_4.7H_2O$ 137·7 mg, KI 19·6 mg, $2CoCO_3.3Co(OH)_2$ 0·18 mg, MoO_3 0·51 mg.

Table 2. *Composition (%) of the pelleted roughage–concentrate diet*

Medium-quality ground hay (32 % crude fibre)	50
Ground maize	20
Ground barley	10
Ground oats	13
Soya-bean meal	5
Mineral mixture*	2
Vitamin A	100000 i.u./100 g
Vitamin D	13000 i.u./100 g

* Containing (per 100 g): bone meal 38·0 g, $CaCO_3$ 32·0 g, NaCl 24·5 g, $MgSO_4.7H_2O$ 5·0 g, trace-mineral mixture (Fe, Cu, Mn, Co) 0·5 g.

The compositions of the semi-purified and the roughage–concentrate diets are given in Tables 1 and 2. Owing to the rather high glucose and starch content, it was not possible to pellet the semi-purified diet; the diet was given in powder form to the animals. Addition of B vitamins to the semi-purified diet was shown significantly to improve the growth rate of lambs (Lawlor *et al.* 1963). In the present experiments mature sheep maintained over a prolonged period on a diet free from B vitamins were examined for any signs of a B vitamin deficiency. Both diets were given *ad lib.* and the sheep had access to food throughout the 24 h, since the intake of semi-purified diets by sheep is slow and irregular. Measurements were made of the daily food and water intake and live weight. Throughout certain experiments food intake was recorded every 2 h from 07.00 to 23.00 h.

Determination of volume of reticulo-rumen contents. The total water content of the reticulo-rumen, which for brevity will be referred to as the rumen, was determined at 07.00, 10.00, 14.00, and 18.00 h by infusing into the rumen 100 ml of a 5 % (w/v) solution of polyethylene glycol (PEG) with a molecular weight of 4000. Samples of rumen fluid were taken immediately before and 1, 2 and 3 h after the PEG infusion. The concentration of PEG in the samples was determined turbidimetrically by the method of Corbett, Greenhalgh, Gwynn & Walker (1958) and the theoretical concentration at zero time was deducted by extrapolation. The volume of rumen water was calculated by the method of Hydén (1961). Simultaneously the dry-matter percentage of the rumen contents was determined on rumen samples obtained through the cannulas. The weights of total content and of dry matter were calculated.

Measurement of parotid secretion. The operation technique of Ash & Kay (1959) was adopted using narrow polyethylene tubing inserted into the parotid duct. The tubing was connected to an automatic measuring apparatus, a detailed description of which was recently given by Brüggemann, Walser-Kärst & Giesecke (1965). This apparatus provided continuous automatic records of the salivary flow in 10 ml fractions. On leaving the recording system, the saliva was continuously pumped into the rumen through the rumen cannula. The individual experiments usually continued for 72 h.

Analysis of saliva. Samples of parotid saliva were obtained from each sheep at intervals during 12 h collection periods. All samples were deproteinized before analysis. Sodium was precipitated as the zinc uranyl acetate complex by the method of Snell & Snell (1959). The colour of the dissolved complex was developed with ammonium thiocyanate as described by those authors and read in a photometer using a 420 nm filter. Potassium was precipitated as the silver cobaltinitrite complex, and the colour of the dissolved complex was developed with ammonium thiocyanate by the method given by Hinsberg & Lang (1957). A 590 nm filter was used for photometric reading. Bicarbonate was determined as CO_2 by the titration method of Neish (1952). Phosphate was determined by the photometric method of Fiske & Subbarow (1925) and calculated as HPO_4^{2-}. Chloride was determined by titration using the method of Hinsberg & Lang (1957). The concentrations of these constituents were expressed as m-equiv./l. parotid saliva.

RESULTS

The mean daily food and water consumptions of sheep receiving the semi-purified and the roughage–concentrate diets are given in Table 3. The food and water consumption of the sheep given the pelleted roughage–concentrate was very high compared with that of the sheep on the semi-purified diet. Water consumption seemed to be directly proportional to dry-matter intake, with sheep E a remarkable exception. The daily gains of the sheep fed on the semi-purified diet fluctuated greatly between sheep and ranged from 50 to 160 g throughout the experimental periods. The three animals fed on the roughage–concentrate diet maintained a steady rate of growth during the period and averaged 270 g daily gain. Results of the determinations of rumen contents in the sheep are given in Table 4. The weight and percentage of dry matter in the rumen tended to be higher in sheep fed on the roughage–concentrate diet. No consistent variations were observed between the sheep on either diet throughout the day. The rumen contents of the sheep on both diets were very homogenous, as they usually are when finely ground or pelleted diets are given.

Table 3. *Mean values with their standard errors for the daily food and water intake of sheep fed on the semi-purified diet and pelleted roughage–concentrate diets*

Diet	Sheep	Food intake (g)	Water intake (l.)
Semi-purified	B	689·3 ± 0·043	1·34 ± 0·09
	D	1039·2 ± 0·304	3·46 ± 0·17
	G	1031·4 ± 0·050	2·70 ± 0·18
	Mean	920·0	2·50
Roughage–concentrate	C	2535·7 ± 0·174	5·05 ± 0·22
	E	1689·3 ± 0·128	7·12 ± 0·22
	H	2478·6 ± 0·177	5·98 ± 0·39
	Mean	2234·5	6·05

The variation in consistency of the rumen contents of the sheep fed on the semi-purified diet was particularly noteworthy. In some instances the rumen contents were extremely frothy, whereas in others they were variably viscous. In animals fed on the pelleted roughage–concentrate diet the rumen contents were extremely uniform, with no variation from animal to animal. The consistency of the faeces was normal with both diets.

None of the sheep were observed to ruminate on either diet, but the animals given the semi-purified diet chewed wood from their pens; these sheep often spent as much time chewing the wood as consuming their food. After 11 months on the semi-purified diet sheep B, D and G showed no signs of B-vitamin deficiency.

Rate of secretion and composition of parotid saliva. The mean rates of secretion of parotid saliva together with the daily food intake on days of experiment are recorded in Table 5. There were very marked differences between the diets. When long hay was given to sheep C and chopped hay to sheep I no obvious difference in the rates of paraotid secretion between these animals was noted. It would appear from Table 5 that no correlation existed between the daily food consumption and the quantity of parotid saliva secreted. The mean rates of salivary secretion were 4·6, 2·9 and 8·1 l. parotid saliva/kg food in sheep receiving the semi-purified diet, roughage–concentrate diet or hay respectively; mean values for daily water intake were 1·63, 4·26 and 2·18 l. or 2·6, 2·3 and 1·7 l. water/kg food respectively.

The mean concentrations of the various electrolytes in the parotid saliva are given in Table 6. No differences in composition were observed between samples of saliva collected during the day or at night. Some of the more important observations made during the continuous automatic recording of the salivary flow are presented in Figs. 1–3.

The diurnal pattern of salivary secretion throughout the day for one sheep of each group is shown in Fig. 1. Irrespective of the diet, there were five to six peaks of maximum parotid secretion distributed over the day at intervals of about 2–4 h.

In order to compare the secretion patterns of both parotid glands in the same animal, sheep G was first cannulated on the left side, and 2 weeks later on the right side. The flow patterns obtained with both parotids over a 24 h period are plotted in Fig. 2. Agreement between the secretion patterns was as close as could be expected.

Table 4. *Diurnal variations in total water, total dry-matter content and percentage dry matter in the rumen of sheep fed on the semi-purified and pelleted roughage–concentrate diets*

			Rumen contents*			
Time	Diet	Sheep	Water (kg)	Dry matter (kg)	Total (kg)	Dry matter (%)
07.00 h	Semi-purified	B	5·4	1·01	6·4	15·8
		D	3·6	0·72	4·3	16·8
		G	7·3	0·96	9·1	10·5
		Mean	5·4	0·90	6·6	14·4
	Roughage–concentrate	C	5·6	1·06	6·7	15·8
		E	4·8	0·89	5·7	15·5
		H	7·9	1·49	9·4	15·8
		Mean	6·1	1·15	7·3	15·7
10.00 h	Semi-purified	B	5·6	0·94	6·5	14·4
		D	7·3	1·22	8·5	14·4
		G	5·0	0·77	5·8	13·2
		Mean	6·0	0·97	6·9	14·0
	Roughage–concentrate	C	6·9	1·51	8·4	18·0
		E	4·5	0·93	5·4	17·3
		H	8·7	1·84	10·5	17·5
		Mean	6·7	1·45	8·1	17·9
14.00 h	Semi-purified	B	4·7	0·64	5·3	12·0
		D	4·2	0·90	5·1	17·6
		G	4·3	0·66	4·9	13·5
		Mean	4·4	0·73	5·1	14·4
	Roughage–concentrate	C	4·9	0·93	5·8	16·0
		E	4·6	0·85	5·4	15·7
		H	7·7	1·47	9·2	16·0
		Mean	5·7	1·08	6·8	15·9
18.00 h	Semi-purified	B	4·2	0·92	5·1	18·0
		D	6·5	0·95	7·4	12·8
		G	4·4	0·76	5·2	14·6
		Mean	5·0	0·89	5·9	15·1
	Roughage–concentrate	C	6·0	1·27	7·2	17·6
		E	5·1	1·04	6·2	16·8
		H	8·3	1·71	10·0	17·1
		Mean	6·5	1·34	7·8	17·2

* Based on samples of rumen content taken at intervals after the intraruminal infusion of a polyethylene glycol marker (see p. 375).

To ascertain the influence of food intake on the pattern of parotid secretion, the food consumption of sheep G was measured over 2 h periods. The results are summarized in Fig. 3. It will be noted from the graph that the food intake (g/h) and the rate of secretion of parotid saliva (ml/h) followed an inverse pattern. Somewhat similar patterns were obtained with other sheep fed on the different diets, despite the irregular feeding habits of the individual animals.

Table 5. *Mean rates of secretion of parotid saliva and daily food intake in seven sheep receiving semi-purified and roughage–concentrate diets, or hay*

Diet	Sheep (parotid)*	Parotid secretion: Determined (ml/h)	Parotid secretion: Estimated total† (l./24 h)	Food intake on days of experiment (kg/24 h)
Semi-purified	G (l)	53·8	2·6	0·9
		48·3	2·3	0·6
		50·0	2·4	0·3
	G (r)	62·1	3·0	0·8
		49·6	2·4	0·3
	K (l)	58·3	2·8	0·4
	L (l)	67·9	3·3	1·1
	Mean	61·4	2·7	0·6
Roughage–concentrate	J (l)	106·0	5·1	1·0
		100·0	4·8	1·8
		122·5	5·9	1·9
	N (l)	103·3	5·0	2·3
		123·8	5·9	2·1
		112·9	5·4	2·0
	Mean	111·4	5·4	1·9
Hay‡	C (l)	219·6	10·5	Not recorded
		251·5	12·1	
		235·3	11·3	—
	I (r)	208·3	10·0	1·4
		239·2	11·5	1·1
		285·0	13·7	1·4
	Mean	239·8	11·5	1·3

* Letters in parentheses refer to left and right parotids.
† Calculated by doubling the values obtained for the single glands.
‡ Sheep C received long hay and sheep I chopped hay.

Table 6. *Mean composition of parotid saliva (m-equiv./l.) during periods of 12 h in three sheep receiving three diets*

Diet	Na^+	K^+	HCO_3^-	HPO_4^{2-}	Cl^-
Semi-purified:					
Mean	152	14	125	54	15
Range	138–158	8–19	107–144	47–73	11–20
Roughage–concentrate:					
Mean	153	14	135	62	7
Range	148–158	8–16	123–144	52–73	7–9
Hay:					
Mean	153	6	128	36	7
Range	151–157	5–7	111–148	33–38	5–8

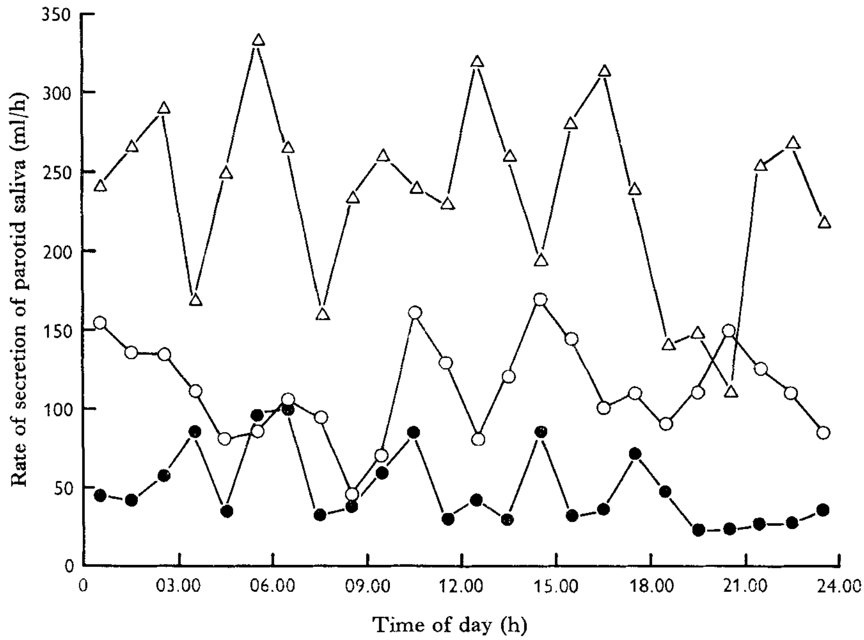

Fig. 1. Diurnal variation in the secretion of parotid saliva of sheep fed on different diets. ●—●, sheep G, semi-purified diet; ○—○, sheep J, roughage-concentrate diet; △—△, sheep I, hay. The experimental points are means of the values obtained on 3 successive days.

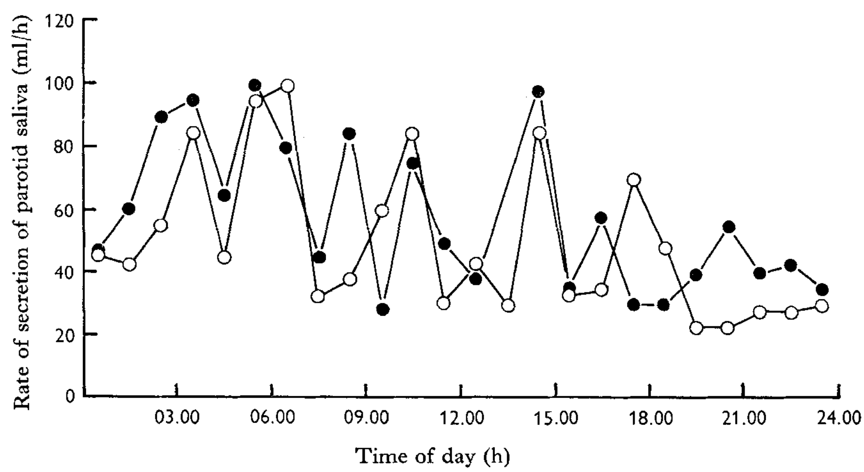

Fig. 2. Diurnal variation in the secretion of parotid saliva from both parotid glands of sheep fed on the semi-purified diet. ●—●, right gland; ○—○, left gland.

DISCUSSION

The mean daily consumption of the semi-purified diet by the sheep was considerably lower than that reported earlier (Lawlor *et al.* 1963). This difference was largely because the sheep used here were fully matured wethers 2 years old at the beginning of the experiment, whereas young growing, fattening lambs were used earlier.

It may be suggested that the omission of B vitamins from the diet adversely influenced the food intake. Even though there is no direct evidence to the contrary, we think that this suggestion is unlikely, especially since the consumption of the B vitamin-free diet in the studies with fattening lambs (Lawlor *et al.* 1963) was much higher than the intake of the similar diet in the present experiment.

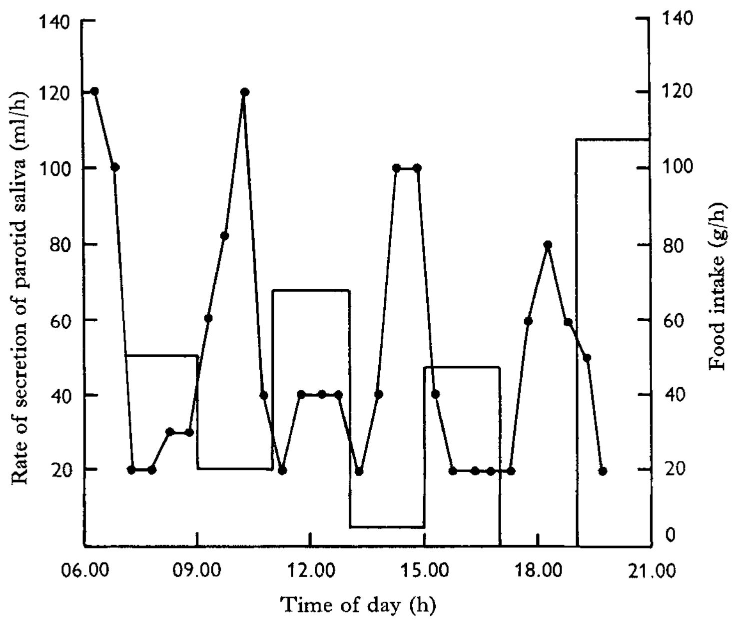

Fig. 3. Diurnal variation in the secretion of parotid saliva of sheep G and in its consumption of the semi-purified diet. ●—●, secretion from right gland. The corresponding food intake measured over 2 h periods is shown as a histogram.

The water intake per kg food with both diets was almost identical, being 2·71 l. for sheep on the semi-purified diet and 2·69 l. for those on the pelleted roughage–concentrate. A variation in water-retaining capacity of the semi-purified diet was probably the cause of the variation in viscosity and consistency of the rumen contents. The variations with time in the total dry-matter content in the rumen of each animal seemed to reflect the eating habits of the sheep, especially those fed on the pelleted roughage–concentrate. They invariably consumed more food between 07.00 and 09.00 h and between 15.00 and 17.00 h.

The pelleted roughage–concentrate diet contained approximately 20 % crude fibre. It is well known that crude fibre as such does not stimulate rumination. In earlier studies by Agrawala, Duncan & Huffman (1953) and later by Bell & Lawn (1957) a complete lack of rumination was observed in ruminants fed on semi-purified diets. Oltjen, Sirny & Tillman (1962) found that sheep fed on semi-purified diets ruminated for 46 ± 5 min/day. This value is very low, and the authors emphasize that the rumination periods were very irregular and short, averaging 2–5 min in length. In our studies the sheep on the semi-purified diet spent a considerable time chewing wood.

The mean daily rates of parotid secretion were clearly influenced by the nature of the food given to the animals, but no correlation was evident between the amounts of saliva secreted daily and the amounts of food. This result is in agreement with the observations of Kay (1960). The rates of parotid secretion which we found for sheep fed on hay greatly exceeded those which Kay (1960) and Wilson & Tribe (1963) reported. The differences may have been due to differences in body-weights of the animals, since it has been shown by Kay (1960) that there appears to be a positive correlation between parotid secretion and body-weight.

The absence of rumination and the physical form of the diets were probably the main factors responsible for the reduction in salivation in the sheep fed on either the semi-purified or roughage–concentrate diets. Furthermore, the differences in the rates of parotid secretion between these groups of sheep can probably be attributed to the physical form of the diets. Wilson & Tribe (1963) concluded that particles of a length greater than 0·5 mm were necessary to promote normal parotid secretion. In our studies the particle size of the cellulose used in the semi-purified diet was less than 200 μm. It is therefore interesting that the daily rate of parotid secretion in the sheep fed on this diet was about four times that reported by Wilson & Tribe for sheep fed on very finely ground hay. It is unlikely that the roughage–concentrate pellets *per se* stimulated increased salivary flow since we observed that the pellets immediately disintegrated on entering the rumen.

The composition of the parotid saliva agrees reasonably well with that of the 24 h collection of saliva reported by Kay (1960). Small differences in the mean content of K^+ and Cl^- as well as HPO_4^{2-} may well be attributed to the varying levels and availability of these components in the different diets.

The patterns of parotid secretion revealed marked changes in flow rates during the 24 h of collection. In sheep receiving hay the periods of maximum secretion did not coincide with periods of eating, but contained the periods of rumination. It has been convincingly demonstrated with cattle (Bailey & Balch, 1961) and sheep (Wilson, 1963) that the periods of eating and rumination coincide with those of increased saliva secretion; the present results suggest that saliva was appreciably greater during rumination than during eating. Similarly occurring peaks, but of lesser amplitude, were observed in sheep fed on the semi-purified and roughage–concentrate diets. This observation was of particular interest with the semi-purified diet. Comparison of the patterns of food intake and parotid secretion (Fig. 3) suggested a very definite tendency for increased salivary secretion to coincide with periods of reduced food intake and vice versa. It is also possible that with either the semi-purified diet or the roughage–concentrate pellets the animals may have regurgitated small amounts of rumen contents and thereby stimulated parotid secretion.

One of us (M.J.L.) is greatly indebted to the Alexander von Humboldt-Stiftung for the fellowship award to carry out this work. Thanks are due to Professor Dr Dr Johs. Brüggemann for providing the facilities in his Institute and for his advice and encouragement. We thank Miss Jutta Demme for her valuable technical assistance.

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Printed in Great Britain