PARTICLE SEPARATION IN THE RETICULO-RUMEN

T.M. Sutherland*

SUMMARY

Examination of the partitioning of particles between the dorsal and ventral regions of the rumen and the buoyancy properties of the particles themselves gives quantitative support for a major role of mechanical entrapment in the dorsal raft in restricting the availability of particles for outflow from the reticulo-rumen. The effect is greatest for large particles and diminishes with time after feeding.

Separation of particles on their buoyancy properties during the quiescent period of reticular contractions, although it plays a part in preventing the passage of large buoyant particles, is insufficient to account for observed reticular-omasal differences in particle size distribution.

Possible filtration mechanisms at the level of the reticulum and omasum are discussed.

INTRODUCTION

Within the reticulo-rumen particles may be several centimetres in length although, even immediately after feeding most of the ingesta are much smaller. Few large particles escape from the forestomach to the small intestine and most of this restriction to large particle outflow occurs before the omasum (Pearce 1967; Reid et al. 1977); Poppi et al. 1980; Ulyatt 1983; Reid 1984; Moir 1984; Ulyatt et al. 1984). The comminution of the ingesta to produce particles which are appropriate for outflow is being intensively studied and the relative importance of mastication during eating, rumination and microbial attack have recently been defined in some instances (Ulyatt et al. 1984; Waghorn et al. 1986).

Rather less clear at present are the nature and relative importance of the mechanisms which lead to the preferential retention of large particles within the forestomach. Mechanical retention in the raft of dorsal rumen contents (Uden and van Soest 1982; van Soest 1982); entrapment and channelling by the walls of the 'honeycomb cells' of the reticulum (Reid 1984; Moir 1984); retention at the reticulo-omasal orifice itself with the unguiform papillae acting as filtering and discriminating devices (Ehrlein 1980) are all potential mechanisms but their relative contributions are yet to be defined.

The importance of specific gravity as a factor determining the rate of passage of materials through the gut has been repeatedly demonstrated (King and Moore 1957; Campling and Freer 1962; desBordes and Welch 1984). Recently I introduced a number of experimental approaches to the study of the sedimentat ion/f lotat ion behaviour of rumen and reticular particles and discussed the relevance of these to the problem of differential particle

*Address: Department of Biochemistry, Microbiology and Nutrition, The University of New England, Armidale N.S.W. 2351, Australia

outflow (Sutherland 1987). In the present paper I will use the particle size distribution analyses available to localise more precisely where differential particle retention is occurring and try to define therefrom the relative importance of the specific mechanisms which have been proposed.

Particle distribution within the rumen

The broad features of the distribution of particles and their sizes in the rumen have been known for a long time. Excellent quantitative data are contained in the study of Evans et al. (1973) on cows fed a diet of hay. Dorsal and ventral rumen samples from anterior and posterior sites and ventral reticular samples were analysed by wet sieving techniques. I have pooled their data and calculated from it 'distribution coefficients' i.e. ratios of dorsal to ventral concentrations (see Sutherland 1987).

TABLE 1: Distribution coefficients (ratios of dorsal to ventral rumen concentrations) of rumen particles of three group sizes from three hay fed cows (calculated from data of Evans et al. 1973).

Sampling times measured from mid-feeding (h)	Coarse particles 2.4mm	Medium particles 0.6 - 2.4mm	Small particles 0.075 - 0.6mm
2.0	14.3 ± 1.6	7.1 ± 1.4	3.7 ± 0.4
4.5	15.2 ± 5.5	6.8 ± 2.4	4.2 ± 0.4
7.5	12.6 ± 5.2	7.7 ± 1.2	3.3 ± 0.9
11.5	7.5 ± 0.3	4.2 ± 0.8	2.7 ± 0.4
19.5	4.8 ± 2.2	2.9 ± 0.5	1.5 ± 0.2
23.5	3.7 ± 0.3	2.8 ± 0.2	1.2 ± 0.1

Two relationships emerge from the data in Table 1. First the distribution coefficient declines for all particle sizes with time after feeding. The small rise seen between 2 h and 4.5 h is presumably due to the fact that feeding continues during this interval. For the values from 4.5 h onwards the regression lines between $\log_{10}D$ where D is distribution coefficient and t is time in hours are:

for large particles $\log_{10}D = 1.2663 - 0.0308t$ $r^2 = 0.77$

for medium particles $\log_{10}D = 0.9554 - 0.0234t$ $r^2 = 0.76$

for small particles $\log_{10}D = 0.7431 - 0.0287t$ $r^2 = 0.92$

The second relationship is that distribution coefficient is positively related to particle size.

I have done similar experiments with sheep fed a diet of dry lucerne chaff. The results of these are given in Table 2.

The general trends seen in Table 2 are similar to those in the cattle experiments except that in the sheep experiments there are no significant differences in the concentrations between dorsal and ventral rumen for all

TABLE 2: Distribution coefficients of rumen particles of different sizes from sheep fed 1.2 kg lucerne chaff once daily.

Time from start of feeding (h)	4.0mm	2.0mm	1.0mm	0.5mm	0.25mm	Fines
3.0	15.7±6.1	9.8±2.3	8.2±1.8	5.6±0.8	4.6±0.7	2.7±0.4
6.0	8.7±3.2	6.2±1.6	5.6±1.2	3.9±0.8	3.3±0.5	2.0±0.2
12.0	8.0±1.8	5.1±1.0	5.6±0.9	3.2±0.4	2.9±0.3	1.8±0.1
24.0	4.2±1.8	3.2±0.6	1.1±0.2	0.9±0.1	1.0±0.1	1.1±0.1

but the largest particles at 24 h after feeding. The proportions of the larger particles are by this stage very small - about 2% of all particles in the rumen. There are not enough sampling periods to test the mathematical nature of the time relationships but the sieve size relationships are as follows:

at 3 h $\log_{10}D = 0.9184 + 0.4472 \log_{10}S$, $r^2 = 0.80$

at 6 h $\log_{10}D = 0.7454 + 0.3757 \log_{10}S$, $r^2 = 0.53$

at 12 h $\log_{10}D = 0.6785 + 0.3424 \log_{10}S$, $r^2 = 0.55$

in which D is distribution coefficient and S is sieve size in mm.

These experiments show in a quantitative way that the large particles are preferentially concentrated in the dorsal contents and relatively less available in the ventral contents than smaller particles. It seems clear that the material presented to the reticulo-omasal orifice originates in the ventral reticular contents (Reid 1984; Moir 1984; Ehrlein 1980) and that the ventral reticular contents interchange with the contents of the cranial sac and hence the ventral rumen (Wyburn 1980; Waghorn and Reid 1977). The distribution between dorsal and ventral contents is thus a factor in the differential passage of the various particle sizes. It can be readily shown that the effect of the dorsal raft on the probability of a particle escaping with the fluid outflow is given by:

$$\frac{R \cdot D_w + (1-R)}{R \cdot D_p + (1-R)}$$

where D_w and D_p are the distribution coefficients for water and the particle respectively and R is the fraction of the total rumino-reticular content constituted by raft (Sutherland 1987). By putting known values of D_w and D_p and likely values of R into this expression it is readily shown that inclusion within the dorsal raft can be an important mechanism of differential particle retention. .

It is perhaps worthwhile examining a little more closely the causes of the differential distribution of particles in the raft. If retention in the

raft were simply due to the flotation/sedimentation characteristics of the particles themselves dorsal enrichment would exist' solely for those particles with an intrinsic tendency to float. Thus increased concentration within the raft of particles of density greater than that of rumen fluid is clear evidence of mechanical entrapment. As will be shown in the next section nearly all of the small particles in the rumen have functional densities greater than that of rumen liquid yet are clearly concentrated in the dorsal contents as long as the raft persists.

Flotation/sedimentation properties of rumen particles

A variety of techniques have been applied to the measurement of the density of feed materials and the ruminal particles resulting from their ingestion. Evans et al. (1973) used continuous density gradients of ethanol and carbon tetrachloride at 37°C with a 30 minute settling period. Welch and his co-workers (Welch 1982; Welch and Smith 1978) have adapted pyknometer techniques to the problem.

The effective density of rumen particles has contributions from solid, liquid and gaseous components with the ratio of the first to the last of these being a major determinant. Calculation shows that the gas phase of particles undergoing ruminal digestion turns over extremely rapidly. Removing the particles from their rumen environment will tend to result in changes to their rates of gas production and gas loss and so gas content. Thus unless the behaviour of the particles is examined quickly under conditions simulating those in vitro density determinations may be inaccurate and possibly misleading. These constraints make the determination of density very difficult and neither the method of Evans et al. (1973) nor that of Welch (1982) meet the requirements of measuring the densities of the particles in their metabolically active state, although both have some value in the comparison of particles of different sizes. Both methods lead to the conclusion that particles increase in density as particle size decreases. Evans et al. (1973) were able to show that particles with lower densities were preferentially concentrated in the dorsal regions.

In examining the relationship between size and buoyancy properties a simple separation into buoyant and non-buoyant fractions followed by wet sieve analysis can be useful. A clean separation of floating from sedimenting particles for all but the most fine can be achieved by a 2 minute incubation after dilution in artificial saliva at 40°C (Sutherland 1987). When sheep were fed lucerne chaff once daily the percentage proportions (mean ± SE) of buoyant particles in the size groups 4.0mm; 2.0mm; 1.0mm; 0.5mm and 0.25 mm were for the period 3-6 h after feeding 89.5±3.7; 85.5±1.6; 52.9t6.3; 20.1±1.1 and 7.4±1.7. Examination of ventral rumen contents gave similar proportions for the same size groups. Comparisons of these values with the distribution coefficients shown in Table 2 demonstrate that particles below 1mm sieve size are found in the dorsal regions in concentrations at least an order of magnitude higher than would be expected from their buoyancy properties. This shows very clearly the importance of mechanical entrapment in the raft as a retention factor. A similar conclusion can be drawn from observations made on the rumen ingesta of sheep fed meadow hay (Sutherland, unpublished) and are inherent in the data of Evans et al. (1973).

The role of the reticulum

In 1984 Moir and Reid simultaneously published their ideas for a role of the reticulum in differential particle flow. The main features of the Moir-Reid hypothesis are that as the reticulum remains stationary during most of a cycle of rumino-reticular contract ions flotation of lighter larger particles dorsally and settling of smaller denser particles towards the reticular pole may occur; the small amount of digesta transferred to the reticulo-omasal orifice per cycle flows there at the end of the second contraction of the reticulum and so originates in material sedimented to the reticular pole during the quiescent period; in the contracted state the reticules become raised and form channels that empty towards the reticulo-omasal orifice with flow over the reticules constituting the sieving process.

In samples drawn from the ventral reticulum of sheep fed once daily the particulate DM% was 3.1±0.2; 3.5±0.3; and 3.6±0.4 for samples drawn at 3, 6, and 12 h after feeding, although by 24 h particulate DM% had risen to 6.3±0.4 (Sutherland 1987). For at least the first half of such a feeding cycle the ventral contents are quite fluid. Experiments in vitro show that migration rates are sufficient at these concentrations to leave at least the immediate vicinity of the reticular pole depleted of buoyant particles. The percentage distribution among sieve sizes of the sedimenting particles from the ventral reticulum is compared with that of whole ventral reticular contents, ventral rumen contents and dorsal rumen contents in Table 3.

The results show the relative loss of larger particles in going from dorsal to ventral rumen contents but that there is little difference in distribution of sizes between ventral rumen and ventral reticulum. Removal of floating particles from the ventricular samples further diminishes the proport ion of large part ic les, with less than 4% at any time being larger than 2.0mm sieve size. When this last distribution series is compared with those found for omasal and abomasal samples by Waghorn et al. (1986) for similar sheep fed the same quantities of lucerne chaff, they nevertheless have greater quantities of particles of lmm sieve size and larger. It is evident that an additional 'sieving' mechanism must operate that is not dependent purely on specific gravity. The preferential retention of larger particles on the reticules of the reticulum would provide such a mechanism.

The Moir-Reid hypothesis is attractive in not only providing a mechanism for differential particle retention but also plausible explanations for several distinctive features of the physiology of the reticulum and for the nature of the detailed anatomy of its inner surface. Radiological observations of the movements of sand in the reticulum are consistent with the hypothesis but there is a paucity of direct evidence. The reticular-omasal differences in distribution of particle sizes could also be explained by filtration at the reticulo-omasal orifice itself with the unguiform papillae constituting a self-clearing grid, although McBride et al. (1984) from their endoscopic observations suggest that when patent the opening is too wide for such a function.

A role for the omasum in preventing the passage of large particlesto the hind gut has often been suggested from the fact that large particles are often found trapped between the omasal leaves. Weston and Cantle (1984) have shown a more rapid rate of passage for small as compared with large particles.

TABLE 3: Distribution of particle sizes as percentages of total particulate DM in the sample for dorsal ruminal, ventral ruminal, ventral reticular and sedimenting ventral reticular contents from sheep fed 1.2 kg lucerne chaff once daily.

Site	Sieve size	Percentage in size class Time after feeding (h) 3	6	12	24	SEM
Dorsal rumen	4.0	6.8	6.7	5.1	0.6	± 0.5
	2.0	18.8	14.8	11.9	1.5	± 0.9
	1.0	13.8	13.7	15.0	4.2	± 0.6
	0.5	11.4	13.3	14.7	19.0	± 0.9
	0.25	24.0	23.2	22.4	42.2	± 1.0
	Fines	25.3	28.4	30.9	32.6	± 1.9
Ventral rumen	4.0	3.5	3.9	3.3	1.3	± 0.9
	2.0	6.6	5.9	4.6	1.6	± 0.7
	1.0	8.8	8.4	7.7	5.0	± 1.0
	0.5	10.1	12.7	13.6	22.4	± 1.7
	0.25	24.5	25.4	26.8	38.6	± 1.8
	Fines	46.6	43.8	44.0	31.1	± 3.1
Ventral reticulum	4.0	3.5	2.3	2.2	1.0	± 0.9
	2.0	5.6	5.1	3.4	1.1	± 0.9
	1.0	9.1	10.6	9.2	8.0	± 1.2
	0.5	9.8	13.5	10.4	17.3	± 1.8
	0.25	27.3	27.4	29.2	40.5	± 2.6
	Fines	44.9	41.1	45.7	32.1	± 3.1
Sediment ventral reticulum	4.0	1.2	0.8	0.7	0.3	± 0.3
	2.0	2.3	2.1	1.4	0.4	± 0.4
	1.0	8.1	9.6	8.0	6.6	± 1.1
	0.5	11.0	14.9	11.4	18.0	± 2.0
	0.25	32.6	32.3	34.0	44.9	± 3.0
	Fines	44.8	40.50	44.5	29.7	± 3.3

If back-flow from omasum to reticulum were non-selective an omasal sieving function would operate. A sieving mechanism implies enrichment with respect to large particles in the digesta at the location of the mechanism compared to the immediate source of the material. The relative compositions for omasal (Waghorn et al. 1986) and sedimentable reticular contents (Table 3) indicate that if such as omasal mechanism exists it can only play a very minor role in comparison to the sieving mechanism within the reticulum or at its exit. The observations of McBride et al. (1984) leave the Moir-Reid hypothesis as providing the most probable explanation of the post-ruminal sieving action.

CONCLUSIONS

The ability of the ruminant forestomach to retain large particles is exhibited through a whole range of conditions from those of the free ranging

grazing or browsing animal to that of the stalled animal fed once daily. It seems clear that a variety of mechanisms are simultaneously involved. In sheep fed lucerne chaff once daily dorsal raft formation and mechanical entrapment, intrareticular sedimentation/flotation and retention on the reticular reticules as proposed by Moir and Reid all appear to be significant mechanisms. Under other circumstances some of these mechanisms may diminish in importance while the remainder may increase and other mechanisms such as that of omasal entrapment and back-flow may come into action. The answer to the question "how does the forestomach prevent the exit of large particles?" is one that has several answers and involves the quantitation of the contributions of the various mechanisms under a whole range of conditions.

REFERENCES

CAMPLING, R.C. and FREER, M. (1962). Br. J. Nutr. 16: 507.

desBORDES, C.K. and WELCH, J.G. (1984). J. Anim. Sci. 59: 470.

EHRLEIN, H.-J. (1980). In "Forestomach motility in ruminants", p. 1, Publikationen zu Wissenschaftlichen Filmen, Sektion Medizin, Serie 5, Nummer 9, Film C 1328. Gottingen: Institut fur den Wissenschaftlichen Film.

PEARCE, G.R. (1967). Aust. J. Agric. Res. 18: 119.

POPPI, D.P., NORTON, B.W., MINSON, D.J. and HENDERSON, R.D. (1980). J. Agric. Sci., Camb. 94: 275.

REID, C.S.W. (1984). In "Ruminant Physiology: Concepts and Consequences", p. 79, ed. S.K. Baker (University of Western Australia Press: Perth).

REID, C.S.W., ULYATT, M.J. and MUNRO, J.A. (1977). Proc. N.Z. Soc. Anim. Prod. 37: 173.

SUTHERLAND, T.M. (1987). In "Aspects of Digestive Physiology of Ruminants", p. 81, ed. A Dobson (Cornell University Press: Ithaca N.Y.).

UDEN, P. and van SOEST, P.J. (1982). Anim. Feed Sci. Tech. 7: 35.

ULYATT, M.J. (1983). In "Fibre in Human and Animal Nutrition", p. 103, eds. G. Wallace and L. Bell (The Royal Society of New Zealand: Wellington).

ULYATT, M.J., WAGHORN, G.C., JOHN, A., REID, C.S.W. and MUNRO, J. (1984). J. Agric. Sci., Camb. 102: 645.

van SOEST, P.J. (1982). "The Nutritional Ecology of the Ruminant*' (O & B Books, Corvallis, Oregon).

WAGHORN, G.C. and REID, C.S.W. (1983). N.Z. J. Agric. Res. 26: 289.

WAGHORN, G.C., REID, C.S.W., ULYATT, M.J. and JOHN, A. (1986). J. Agric. Sci., Camb. 106: 287.

WELCH, J.G. (1982). J. Agric. Sci. 54: 885.

WELCH, J.G. and SMITH, A.M. (1978). J. Anim. Sci. 46: 309.

WESTON, R.H. and CANTLE, J.A. (1984). Canad. J. Anim. Sci. 64: (Supplement) 322.

WYBURN, R.S. (1980). In "Digestive Physiology and Metabolism in Ruminants", p. 35, eds. Y. Ruckebush and P. Thivend (MTP Press: Lancaster, England).