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Abomasal emptying in sheep as related to the amount of protein entering the abomasum

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1. In sheep, provided with re-entrant cannulas in the caudal duodenum and with an infusion tube into the abomasal fundus, emptying of the liquid phase of the abomasal contents was studied with [^{57}Co]EDTA. Radioactivity was measured continuously with Geiger–Müller counters placed along the re-entrant cannulas.

2. It appeared that first-order kinetics could be applied to the elimination of [^{57}Co]EDTA from the abomasum. Mean retention time of this label in the abomasum was 40–50 min.

3. Infusion of a suspension of soya-bean protein in saline (9 g sodium chloride/l) caused an initially decreased rate of abomasal emptying, but after 48 h an adaptation had occurred to the original rate of emptying producing an increased volume of abomasal contents and increased flow-rate of digesta into the duodenum.

For simple-stomached animals and man, but also for immature ruminants in their pre-ruminating state, emptying of gastric contents is fairly well understood. It is generally accepted that liquid emptying of both the abomasum and simple stomach occurs exponentially (Bell, 1980). Development of the forestomachs in ruminants causes a distinct change in the function of the abomasum. Both the different composition of the ration and the fermentation of the feed constituents in the forestomachs, together with the forestomachs acting as a volume buffer, result in a flow of digesta into the abomasum which differs in composition and is also more continuous in comparison with that in the pre-ruminating state. Due to this continuous inflow of digesta from the forestomachs, the adult abomasum is never empty and the continuous presence of digesta in the abomasum causes a steady secretion of abomasal juice (Hill, 1955; McLeay & Titchen, 1974). In sheep it has been found that digesta are acidified in the abomasum to a pH of about 3 (Van Bruchem, 1977). In comparison with the reticulo-rumen, digesta are retained in the abomasum for a considerably shorter period.

From the abomasum the digesta pass into the duodenum by a fairly continuous process. This emptying process may be governed by factors originating from the abomasum itself and from the small intestine. Opinions differ as to whether receptors involved in the feedback regulation of abomasal emptying are localized in the proximal part of the small intestine only. Possibly interspecies differences exist, but in the pre-ruminant calf receptors affecting abomasal emptying were found to be widely distributed in the duodenum and jejunum (Bell & Holbrooke, 1979).

Proteins have been found to affect abomasal antral motility (Van Bruchem, 1977). This changed motility pattern possibly leads to a different emptying pattern of the abomasum, thus affecting not only the digestion of proteins in the abomasum but also the delivery of digesta in the small intestine for digestion there. In the present experiments the retention of the digesta in the abomasum was estimated in sheep which received an additional supply of soya-bean protein introduced directly into the abomasum.

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METHODS

Animals

Emptying of the digesta from the abomasum was studied with two Texel wethers, aged 2–4 years and with a body-weight of about 60 kg. They were provided with a silastic infusion tube into the abomasal fundus (Van Bruchem, 1977) and with re-entrant cannulas into the caudal duodenum beyond the entry of the common bile and pancreatic duct. The cannulas used were prepared of hard PVC (12 mm i.d.). During the experiments the sheep were kept individually in metabolism cages with water available in excess and care was taken that the experiments were started after the sheep had recovered from surgery and had been well accustomed to the whole experimental routine.

Experimental conditions

The effect of intra-abomasal infusions of suspensions of soya-bean protein (Promine-D; Central Soya Chemurgy, Chicago) in saline (9 g sodium chloride/l) on the emptying of the digesta from the abomasum was determined in two sheep. A suspension, containing either 25 (S1) or 100 (S2) g soya-bean protein/l, was infused into the abomasum through the silastic infusion tube at a rate of about 60 g/h. Thus about 1·5 or 6 g soya-bean protein/h were added to the abomasal digesta or about 25 or 100% of the rate of passage of protein through the abomasum under control conditions (without intra-abomasal infusion of soya-bean protein). A set of three experiments per week was conducted with each sheep, starting on Monday with the control experiment (C) without intra-abomasal infusion. Subsequently, on Wednesday, treatment T1 was done 4 h after the onset of the intra-abomasal soya-bean protein infusion. This infusion was continued until Friday, when treatment T2 was carried out after 52 h infusion of soya-bean protein. For each sheep, treatments S1 and S2 were applied alternately for a period of 1 week each, starting for one sheep with the S1 treatment and for the other with the S2 treatment. Since both treatments S1 and S2 were preceded by a control experiment, there were twice as many control experiments as infusion experiments. Two control experiments had to be excluded. For calculation of the volume of the abomasal contents in these weeks the infusion experiments were compared with the control experiments nearest in time. With one sheep, seven S1 and seven S2 treatments were applied and with the other, five S1 and five S2 treatments, two less because of technical problems with the re-entrant cannulas of this sheep. With both sheep a set of three experiments was carried out with infusion of saline alone.

During the experiments the sheep received a daily ration of 600 g chopped hay and 300 g mixed concentrates, together containing 128 g crude protein (nitrogen × 6·25). With this ration together with the protein introduced additionally into the abomasum during the experiments, body-weight of the sheep remained stable.

Experimental procedure

The retention time of the digesta in the abomasum was studied by injection of an emitter of soft gamma rays into the abomasum. For this purpose, ^{57}Co with a main gamma ray of 122 keV and a half-life of 271 d was chosen as a tracer. For each experiment, 0·5 mg Co^{2+} (0·05 mCi; 1·85 MBq), chelated to EDTA was injected through the silastic infusion tube into the abomasum. Since the sheep were fed twice daily at 08·00 and 17.00 hours the experiments were always started at the same time of the day, at 09.30 hours.

The ^{57}Co activity in the duodenal digesta was measured using two Geiger–Müller (GM) counters, placed along the tube connecting the duodenal cannulas. The GM counters were shielded from the body of the sheep with 4 mm lead to minimize the effect of ^{57}Co inside

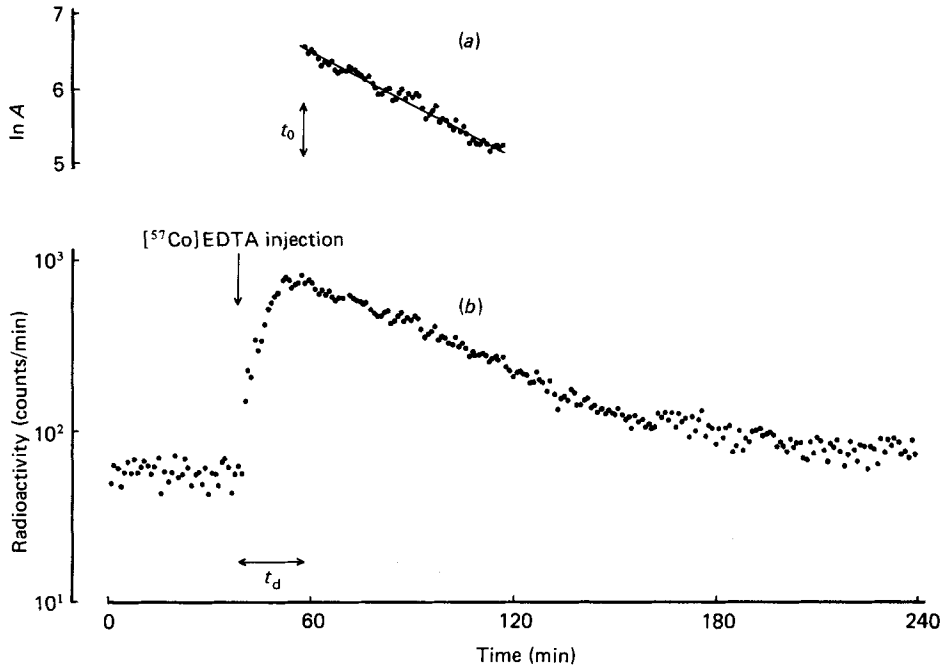

Fig. 1. Diagrammatic representation of the ^{57}Co-radioactivity curve in duodenal digesta after a single injection of [^{57}Co]EDTA into the abomasum (*b*). In plot *a* the fit is expressed between the logarithm of the background-corrected ^{57}Co-radioactivity (ln *A*) in duodenal digesta and the regression line obtained by solving the following equation:

$$\ln A = \ln A_0 - kt = 7{\cdot}427 - 0{\cdot}0206\, t,$$

where ln A_0 is the intercept on the *Y* axis, *k* is the rate constant of fractional emptying and *t* is time (min). t_0, Time interval with the highest counting rate; t_d, retention time of digesta in the cranial duodenum.

the sheep. Measurement of the ^{57}Co activity was started in advance of the injection of the radioactive label and was continued for a time interval of about 6 h (Fig. 1).

Calculations

The GM counters were connected in such a way that the numbers of discharges of each tube were combined and recorded on a per minute basis (counting rate in counts/min).

Counting was started before intra-abomasal injection of [^{57}Co]EDTA and the pre-injection background was recorded as shown in Fig. 1. Subsequently, after injection of the ^{57}Co, radioactivity in the duodenum increased gradually to a maximum level of activity. Radioactivity then decreased steadily until a new steady-state level was reached, the post-injection background. This post-injection background appeared to be higher than the pre-injection background activity, caused by incomplete shielding of the ^{57}Co activity present inside the sheep by the lead placed between the GM counters and the sheep. Subsequent experiments were carried out at least 2 d after the previous experiment because more than 90% of the injected dose of ^{57}Co was recovered in the faeces in 2 d. The post-injection background was regarded as the background during the experiment. This background was determined over a time interval of 30 min and the mean value thus obtained was subtracted from the counting rates of sixty intervals of 1 min, starting with the time interval with the highest counting rate (t_0). Then this background-corrected duodenal

Table 1. *The mean retention time of the digesta in the abomasum* (t_a), *the relative volumes of abomasal contents* (V), *the relative flow-rates of digesta into* (F) *and the retention times of the digesta in the cranial duodenum* (t_d) *under control conditions* (*C*) *and as affected by intra-abomasal addition of soya-bean protein at two rates of infusion* (*S1*, *S2*) *4 h* (*T1*) *and 52 h* (*T2*) *after the start of infusion*

(Mean values with their standard errors)

		t_a (min)		*V*		*F*		t_d (min)	
Treatment	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C	22	41·9[a]*	2·6	1·00[a]	—	1·00[a]	0·058	19·8[a]	1·6
S1.T1	12	45·3[a]	3·5	1·18[a]	0·10	1·09[ac]	0·079	24·8[ac]	2·2
S1.T2	12	41·9[a]	3·5	1·13[a]	0·10	1·08[ac]	0·079	20·7[ac]	2·2
S2.T1	12	63·2[b]	3·5	1·58[b]	0·10	0·97[a]	0·079	25·5[bc]	2·2
S2.T2	12	46·1[a]	3·5	1·42[b]	0·10	1·24[bc]	0·079	19·4[ac]	2·2

* Values within columns sharing common superscript letters were not significantly different.

^{57}Co-radioactivity (*A*) was plotted on a semilogarithmic scale *v.* time (*t*), as shown in plot *a* of Fig. 1. A straight line appeared to fit best. Correlation coefficients were in general higher than 0·95. The regression line thus obtained gave the index *k* and the intercept on the *Y* axis (ln A_0). As the relation between ln *A* and *t* was obviously linear, the emptying pattern of the abomasum was considered as a single exponential, in accordance with the following equation:

$$A_t = A_o \times e^{-kt}.$$

In this equation, *A* represents the background-corrected counting rate (counts/min) and *k* the rate constant of fractional emptying of the abomasum (/min). With A_o an indication was obtained of the volume of the abomasal contents (*V*; ml) and then the flow-rate of digesta from the abomasum into the duodenum was calculated as $V \times k$ (ml/min). Also, as an index of abomasal emptying, the interval between the time of injection of [^{57}Co]EDTA into the abomasum and the time of the highest duodenal ^{57}Co-activity (t_0) was determined (t_d; min). For statistical analysis of the results a two-sampled analysis of variance was applied, considering differences between sheep (Nie *et al.* 1975).

RESULTS

With the present experimental set-up the volume of the fluid abomasal contents could not be estimated precisely, since the counting efficiency of the GM counters could not be determined. Therefore the volume of the fluid abomasal contents was approximated as follows. Within the set of weekly experiments the A_0 values for treatments T1 and T2 were compared with that of the control experiment, and the T1 and T2 abomasal volumes were expressed as proportions of the control abomasal volume. For treatments T1 and T2 duodenal flow-rates were expressed in a similar way.

In Table 1 the results have been summarized. It appears that shortly after the start of the infusion of the 100 g soya-bean protein/l suspension into the abomasum (treatment $S_2 . T_1$) the mean retention time of the fluid digesta in the abomasum (t_a) calculated as $1/k$, was significantly increased. The flow-rate of digesta from the abomasum (*F*) was not yet increased and as a consequence the relative volume of the abomasal contents (*V*) was increased. The retention time of the digesta in the cranial duodenum (t_d) was slightly but

Table 2. *The mean retention time* (t_a) *and relative volumes* (V) *of the digesta in the abomasum, the relative flow-rates of digesta into* (F) *and the retention times of digesta in the cranial duodenum* (t_d) *under control conditions* (*C*) *and after 4 h* (*T1*) *and 52 h* (*T2*) *of intra-abomasal infusion of saline* (*9 g sodium chloride/l*)

Treatment	*n*	t_a (min)	*V*	*F*	t_d (min)
C	2	43·1	1·00	1·00	24·0
T1	2	40·4	1·00	1·07	18·0
T2	2	33·0	0·96	1·22	29·0
SEM (2 df)		3·0	0·013	0·08	2·5

There was no significant difference between values for C, T1 and T2.

significantly increased. After a more prolonged period of infusion (treatment T2), the mean retention time of the digesta in the abomasum returned to a level not significantly different from the control (C) level. The relative volume of abomasal contents remained significantly increased and the flow-rate had evolved to a level slightly higher than that under control conditions. The retention time of the digesta in the cranial duodenum (treatment S2.T2) had returned to the control level.

With the lower level of soya-bean protein infusion, the factors studied showed a comparable pattern of dependence on the protein infusion but the effects were less pronounced and did not show significant differences from control observations.

To decide whether the effects obtained were caused by the soya-bean protein or by the saline in which the soya-bean protein had been suspended, in two sets of experiments only saline was infused, at a rate comparable to that applied with the soya-bean protein suspensions. In contrast to soya-bean protein there was a tendency for the infusion of saline to reduce the mean retention time and the volume of the digesta in the abomasum (Table 2).

DISCUSSION

Although the propulsive activity of the abomasum in the adult ruminant is probably similar to that in the pre-ruminating state because the mechanisms controlling the alimentary tract are probably established early in ontogeny, the filling of the abomasum and composition of its contents change remarkably during the development of the forestomachs. The digesta reaching the abomasum in mature ruminants are composed of predigested feed residues. Dry-matter content and thus viscosity of the digesta leaving the omasum are quite low and the protein consists for the greater part of microbial protein, synthesized in the forestomachs. Considerable amounts of volatile fatty acids are present in abomasal digesta although smaller amounts reach the duodenum (Van Bruchem, 1977).

Control of the propulsion of the digesta from the abomasum into the duodenum depends on the amount and nature of the abomasal contents and also on neurally and hormonally mediated feedback mechanisms from the small intestine (Bell & Mostaghni, 1975). In sheep, proteins were found to affect abomasal secretory activity and the flow-rate of digesta to the duodenum and, shortly after the start of the protein infusion, abomasal, antral and pyloric motility were also affected (Van Bruchem, 1977).

In the present study, fractional rates of emptying of the fluid phase of the abomasal contents were found to correspond rather well with those determined by Grovum & Williams (1973) using [^{51}Cr]EDTA. However, Faichney & Griffiths (1978) determined

fractional rates of abomasal emptying with [^{51}Cr]EDTA which were considerably lower than those derived from our experiments.

Shortly after the start of the 100 g soya-bean protein/l infusion (treatment S2.T1), when the amount of protein entering the abomasum was about doubled, the retention time of the digesta in the abomasum was highly significantly increased. This was probably a result of the feedback information gained from small intestinal receptors in response to changes in the acid and protein content of the digesta, since the flow-rate of digesta into the duodenum had not yet increased.

Also t_d was slightly increased and thus the filling of the proximal duodenum would also be increased. Ignoring any changes in duodenal, pancreatic and bile secretions, it can be calculated that 213 ml and 264 ml of digesta would be distending the duodenum cranial to the re-entrant cannulas in treatments C and S2.T1 respectively. This indicated a possible increase of 11% in the distension of the duodenum when soya-bean protein was infused intra-abomasally and would probably cause increased excitation of mechanical receptors.

From previous results (Van Bruchem, 1977; Van Bruchem & Van 't Klooster, 1980) it can be deduced that the increase in duodenal flow of digesta caused by intra-abomasal infusion of a protein suspension generally exceeded the infusion and was caused by the secretion of additional abomasal juice. Thus after 4 h of infusion (treatment T1) considerably more than the 240 g infusate should have been retained in the abomasum additionally, since F had not yet increased. As can be seen from Table 1, this was not the case, and as a consequence it may be suggested that with treatment S2.T1 not only the emptying rate of the abomasum was inhibited but that of the forestomachs as well, although the latter not to such an extent that feed intake was affected.

After the more prolonged period of infusion (treatment S2.T2) a filling of the duodenum of about 257 ml can be calculated, hardly deviating from that of treatment S2.T1 (264 ml). Most surprisingly an increased duodenal flow of digesta was observed (treatment S2.T2). Presumably an increased steady-state flow-rate of digesta from the abomasum was induced by adaptation of small intestinal receptors together with an enhanced stimulation of abomasal emptying caused by the increased filling of both the abomasum and the forestomachs. There was still a highly significantly increased volume of abomasal contents but with t_a no longer deviating from that under control conditions. This is in accordance with the previous finding that abomasal, antral and pyloric motility were not affected after a more prolonged period of soya-bean protein infusion (Van Bruchem, 1977).

At a lower rate of soya-bean protein infusion the effects obtained were much less pronounced; in comparison with the control conditions no significant contrast was obtained. Thus the magnitude of the effects depends on the amount of soya-bean protein supplied intra-abomasally, and with a lower rate of infusion the new steady-state level of gastrointestinal activity is presumably attained earlier.

With the infusion experiments a part of the effect might possibly be caused by the saline in which the soya-bean protein had been suspended, since in the control experiments no intra-abomasal infusate was applied. However, in other mammals an increased rate of gastric emptying has been found with non-hypertonic saline solutions (Hunt & Pathak, 1960; Bell & Razig, 1973). Due to the limited number of experiments it was not proven unequivocally that infusion of saline results in both a decreased mean retention time and decreased volume of digesta in the abomasum, but it seems evident that saline in non-hypertonic concentrations is certainly not inhibiting abomasal emptying. Thus inhibition of abomasal emptying as induced by infusion of suspensions of soya-bean protein in saline is caused by the soya-bean protein, the inhibiting effect probably being even stronger than can be deduced from Table 1, since these effects may be regarded as the resultant of both the soya-bean protein and the saline effect.

So with considerably higher amounts of protein entering the abomasum the conclusion seems justified that the retention of the digesta in the abomasum will not be affected drastically. Thus a considerably increased steady-state input of protein will not coincide with a decreased peptic digestion in terms of the time interval available.

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