THE FLOWPATHS TAKEN BY GROUND SUPPLEMENTS IN THE STOMACHS* OF SHEEP**

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OPSOMMING: DIE VLOEIPAAIE VAN GEMAALDE SUPPLEMENTE IN DIE VOORMAE VAN SKAPE

Die doel van hierdie proef was om te bepaal of gemaalde supplemente na die abomasa van skape gelei kan word deur middel van die retikulêre groewe. As rumenfermentasie hierdeur vermy kan word, sal geskikte supplemente meer doeltreffend gebruik word. Die studie is in twee dele uitgevoer. Nadat ongeveer die helfte van die rumininhoud deur 'n groot rumen-kannula verwyder is, kon die retikulêre groef duidelik waargeneem word. Wanneer 'n mengsel van 98 g mieliemeel en 2 g NaCl ingeneem is, het die groef selde gefunksioneer. In die tweede gedeelte is die vloeipaaie gevolg, in skape met rumen en abomasale kannulas, met behulp van gemerkte supplemente. Die oplosbare fraksie van 'n supplement is gemerk met die oplosbare merker ⁵¹Cr-Ł (Cr gekompleks met etieleendiamientetraasynsuur). Die growwe partikels is gemerk met die partikulêre merker ¹⁰³Ru-P (tris (1,10-fenantrolien) Ru (III) chloried). Die skape is *ad lib.* gekerfde koringhooi aangebied en gemerkte supplemente van dieselfde tipe as die bogenoemde een. Daar is gebruik gemaak van twee vloeipaaie. Normaalweg het 'n supplement deur die cardia uitgestort. Cr-E het dan met die waterfraksie van die retikulô-rumen inhoud gemeng. Die gemerkte mieliepartikels (met 'n S.G. van 1,45) het gesink na die onderste laag van hierdie digesta. Tweedens het die retikulêre groef 'n paar keer gesluit. 'n Supplement is dan direk na die abomasum gelei. Met ons huidige kennis kan ons nie 'n droë supplement saamstel wat die groef altyd sal sluit nie.

SUMMARY:

The object of this experiment was to find out whether ground supplements could be conveyed to the abomasa of sheep via the reticular grooves. If ruminal fermentation could be avoided in this way, suitable supplements would be more efficiently utilized. The flowpaths of the supplements in the stomachs were followed in two ways. Firstly about half of the contents of a rumen was removed through the large rumen fistula. In this way the reticular groove could be observed. When supplements of 98 g of maize meal plus 2 g NaCl were ingested the grooves were seldom functional). The second method involved following the flowpaths of marked supplements in the stomachs of sheep fitted with rumen and abomasal cannulae. The soluble fraction of a supplement was marked with the soluble marker 51 Cr-E (Cr complexed with ethylenediaminetetra – acetic acid). The contrasting coarse particles were marked with the particulate marker 103 Ru-P (tris (1, 10 – phenanthroline) Ru (III) chloride). Sheep were offered chopped wheaten hay *ad lib.* and marked supplements similar to the one above. Two flowpaths were followed. Normally a supplement was ejected through the cardia. Cr-E became associated with the water fraction of the reticulo-rumen digesta. The marked maize particles by contrast (that had an S.G. of 1,45) sank to the bottom of this digesta. On a few occasions the reticular groove was activated. A supplement was then conveyed directly to the abomasum. Our present knowledge does not permit us to compile a dry supplement which will consistantly activate this groove.

Ruminants can be at a nutrient advantage if supplements containing protein (Reis & Schinckel, 1964; Reis, 1969), amino acids (Reis, Tunks & Downes, 1973) or starch (Morgan, 1975) are not fermented in their rumens. The results of Morgan (1969) suggest that ground maize-based supplements could be conveyed directly to the abomasa of mature sheep. He concluded that sheep had a similarly keen appetite for the supplements as for milk which activated their reticular groove reflexes. This study was therefore undertaken to investigate how and to what extent such supplements are conveyed directly to the abomasa of sheep.

Procedure

Receipt of MS. 31.3.1977

1. Plan of experiment

The experiment was divided into two sections. First the reticulo-rumens of the sheep were observed while consumed 98 g of maize meal + 2 g NaCl. Secondly suitably marked supplements which included maize meal and NaCl were offered to sheep and the flows of the markers from the rumen or abomasum were quantified after ingestion.

2. Sheep and their management

For mature Merino wethers weighing about (ca.) 45 kg were used, except where it is mentioned that four 1-year old Border Leicester x Merino wethers weighing ca. 40 kg were used. They were each fitted with an abomasal cannula and a rumen cannula that was 10 cm

^{*} Stomach refers to rumen, reticulum, omasum and abomasum

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in diameter. The sheep were offered a basal diet of chopped wheaten hay *ad lib*. and received a 100 g supplement once per day which contained maize that was hammermilled through a 6,4 mm screen.

About half the contents of the rumen of each mature sheep was removed through the cannula, to permit the reticular groove to be observed during the time that each sheep consumed 98 g of maize meal plus 2 g NaCl (salt). This was repeated daily for 30 days and during the last 14 days the observations were recorded. Over the next 20 days these sheep were each offered one of the following supplements per day in the following sequence: 98 g of maize meal (MM) + 2 g salt, 97 g MM + 3 g salt, 93 g MM + 2 g salt + 5 g dry molasses, 98 g MM + 2 g salt and 25 g MM + 0.5 g salt. These are referred to as maize supplements. Markers were added to these supplements when their flows in the stomach were required to be quantified after consumption. For 14 days, the 1 year old sheep were similarly offered in sequence 98 g MM + 2 g salt, 93 g MM + 2 g salt + 5 g molasses and 25 g MM + 0,5 g salt. The flow of a maize supplement in the stomach was similarly measured with the aid of markers.

3. Definitions of even-flow, shunting and by-pass

Even-flow of a marker from an organ occurs only after the marker has been completely mixed with digesta in that organ. The rate at which a marker evenflows from the reticulo-rumen to the abomasum is therefore dependent on the mean retention time of the marker in the reticulo-rumen. This rate is ca. 100 times slower than the rate at which a marker flows to the abomasum by shunting or by-pass.

Shunting is the flow of a marker from or past the reticulum. Shunting happens before even-flow and can be due to one or both of the following: First shunting can occur because of the high concentration of a marker near the reticulo-omasal orifice. Secondly shunting happens when the reticular groove conveys the marker directly to the abomasum.

By-pass of the "rumen" only occurs when the reticular groove conveys ingesta directly to the abomasum.

4. Measurement of the mean retention times of markers and the volume of digesta in the abomasum

The mean retention times of the markers in the abomasum and the volume of digesta in this organ are required in section 5.2 to calculate the quantities of markers included in a supplement that were shunted to the abomasum. For this the markers were prepared in a similar way to what they were when included in a supplement. Doses of 50 μ Ci of 51 Cr-E (Cr complexed with ethylenediaminetetra – acetic acid) were added to 1 g of 52 Cr-E and dried at 40°C for 24 hours. The coarse particles in a sample of maize meal similar to that used in the supplements was separated from the finer fraction with the aid of a 1 mm² sieve. Soluble and sus-

pendable matter was removed from the coarse particles by washing under a tap for 18 hrs. These particles were dried in a forced draught oven at 40°C for 6 hours. (There were 550 maize particles/g and their density was 1,45). Twenty grams of particles were soaked for 24 hours in 200 ml of a Ru-P (Ru-labelled tris (1,10-phenanthroline) ruthenium (III) chloride) solution containing 0,4 μ Ci of 103Ru and 1,1 mg of Ru-P/ml. The particles were then washed under tap water for 15 sec. and dried in a forced draught oven at 40°C for 24 hours.

To measure the mean retention times of the markers and the volume of digesta in the abomasum, 1 g of Cr-E containing 50 μ Ci of ⁵¹Cr and 0,2 g of Ru-P marked maize particles were isolated at the bottom of a polypropylene tube. After the rumen of a welltrained sheep was partially emptied, the tip of the tube was fed through the reticulo-omasal orifice via the rumen fistula, until it was felt to be ca. 5 cm into the abomasum. The tube was gently washed out with 20 ml of filtered abomasal digesta and the rumen contents replaced in less than 4 minutes. Immediately afterwards each sheep was offered 98 g of maize meal + 2 g salt which was consumed in ca. 7 minutes. About 20 ml of abomasal digesta was collected at 15, 30, 60, 90, 120 and 180 minutes after the markers were infused into the abomasum. The volume of digesta in the abomasum and the mean retention time of a marker were calculated according to the method of Weston & Hogan (1967). The samples were radio-assayed as described by Tan, Weston & Hogan (1971).

- 5. Measurement of the percentage of the dose of a marker that even-flowed or was shunted in the stomach
- 5.1 The technique

The dose of each marker added to a supplement was prepared as follows: One gram of maize particles marked with 10^3 Ru-P plus 1 g of Cr-E containing 50 μ Ci of 51Cr (both prepared as in section 4) were mixed into a maize supplement. Such preparations allowed the flow of the particulate and contrasting soluble fractions in a supplement to be respectively measured in the stomach (Morgan, 1975). A marked supplement was offered to a sheep at 0 hours and ca. 20 ml of abomasal digesta was taken at + 0,5 + 1 and + 2 hours. At + 3 hours ca. 30 ml of rumen liquor was withdrawn and immediately afterwards an equivalent dose of 51Cr-E to that used above was infused *per rumen.* At + 5, + 6 and + 7 hours ca. 30 ml of rumen liquor was withdrawn.

5.2 Calculation of the percentage dose of a marker shunted to the abomasum

In all of the calculations used in this paper steady state conditions in the stomach are taken to occur,

as feed was available at *ad lib*. intake (Morgan, 1975). A control treatment was one where the consumed markers even-flowed from the reticulo-rumen. This was taken to occur when less than 5% of the dose of a marker added to a supplement was in abomasal digesta at + 0,5 hours. 103Ru counts in abomasal digesta were multiplied by a correction factor of 1,5 to obtain actual 103Ru counts in the abomasum (see Morgan, 1975).

To extrapolate the percentage dose of a marker in a supplement that was shunted to the abomasum in the shunt measurement of Table 1 let:

v	=	volume of digesta in abomasum (from sec-						
		tion 4). •						
с	=	counts of the marker/ml of abomasal di-						
		gesta.						
d	=	total counts in a dose of the marker.						
%	=	percentage dose of the marker in the abo-						
		masum.						
С	=	% in the control.						
Т	=	total 🚿 when shunting occurred.						
В	=	% due to shunting alone.						
m	=	counts derived from a definite marker.						
t	=	a definite collection time.						
	đ	c x v x 100						

Now $\% = \frac{c \times v \times 100}{d}$

From the slope obtained from the mean retention time of the marker in abomasal digesta (see section 4.) and from $T_{m+0.5 h}$, $T_{m0 h}$ can be calculated.

But
$$B_{mt} = T_{mt} - C_{mt} x (100 - \frac{T_{m0}h}{100})$$

From the logs of $B_m(t=0.5, +1 \text{ and } +2 \text{ h})$. $B_m 0 \text{ hr}$ can be extrapolated. This is the percentage dose of the marker shunted to the abomasum at 0 h. From the slope of this graph the mean retention time of the shunted marker in the abomasum can be determined.

The percentage dose of the marker in the abomasum at 0 hr of the control measurement i.e. C_{m0h} for Table 1 was extrapolated from the logs of $C_{m(+0.5, +1 \text{ and } +2 \text{ h})}$. Most of the $C_{m+0.5h}$ was probably due to even-flow. For an upper limit, which is an overestimate of C_{m0hr} , the slope obtained from the mean retention time of a marker in the abomasum (see section 4) and $C_{m+0.5h}$ can be used to calculate C_{m0hr}

5.3 Calculations of the percentage of the dose of a soluble marker in the rumen after 3 hours

Equal volumes of rumen liquor were counted.

- Let A = Counts in rumen liquor at + 3 hours, prior to the infusion of the second dose of marker.
 - B = Extrapolated counts in rumen liquor at + 3 hours, due to A plus the second dose of marker (obtained by back extrapolating the log of the counts in rumen liquor, withdrawn at + 5, + 6 and + 7 hours).

C = Counts in rumen liquor at + 3 hours, due to the second dose of marker.

Then B - A = C.

The percentage of the dose of the marker added to the supplement and in the rumen after 3 hours = $\frac{A}{C} \times 100$

Results

1. Observations in the reticulo-rumen and the paths taken by ingested supplements

The form of the reticular groove (groove) was observed to be S-shaped and less than 50 mm in length from the cardia (the terminal opening of the oesophagus) to the reticulo-omasal orifice, which was situated halfway up the median wall of the reticulum.

The groove was able to form a tube of ca. 10 mm in diameter. The pillars or lips of the groove were more muscular posteriorly and were normally relaxed, but closed. When sheep swallowed the lips were felt to close more tightly and they tightened with every reticular contraction.

When a bolus of the supplement was ingested it took one of two paths. In the great majority of cases the cardia rosetted and 20-30 ml of the broth-like masticate, with a density of ca. 1,25 was expelled into the area above and proximal to the reticulo-ruminal fold. Following this a cycle of contractions ensued, which was initiated by the reticulum contracting in two stages and expelling an estimated 200 ml of its 300 ml volume of digesta (that had a density of ca. 1,1) into the rumen. During the second stage of a reticular contraction, the reticular-omasal orifice was felt to open. Almost immediately after the two-stage reticular contraction, the rumen underwent a primary contraction (see Sellers and Stevens, 1966). The cycle was repeated ca. 3 times per minute. No building up of coarse maize particles was felt to occur in the reticulum. They were felt in the bottom layer of reticulo-rumen digesta. In a minority of the cases when the bolus was ingested, the cardia did not rosette but was partially forced open by the masticate and some seconds afterwards when the pillars of the groove relaxed, masticate in the groove seeped out along the length of the lips and flowed into the reticulum. This was repeated over several consecutive swallows. In what seemed to be an extention of this, a sheep consumed the supplement for more than 3 minutes and although it swallowed no masticate appeared in the reticulorumen.

Sheep required less than 10 minutes to consume a supplement.

2. Parameters associated with the flow of a marked supplement in the stomach

When introduced *per abomasum* via the rumen fistula of sheep number 4, the mean retention times of

51Cr-E and of the 103Ru-P marked maize particles were 0,53 hours (r = 0,99) and 1,04 hours (r = 0,97) respectively, while the volume of digesta in the abomasum was 0,33 litres in each case, provided that 103Ru counts were multiplied by a correction factor of 1,5 (see Morgan, 1975).

In the majority of cases when the sheep were offered a marked maize supplement, 35% (± 4) of the dose of ⁵¹Cr-E had flowed out of the rumen within 3 hours. The mean retention time of Cr-E in the rumen was 7,2 hr. The markers present in the abomasum at + 0,5 hours probably even-flowed there, but if it is assumed that they were there as a result of shunting then an upper limit of no more than 5% (± 0.3) of the dose of ⁵¹Cr-E and 2% (\pm 0,02) of the dose of ¹⁰³Ru-P could have been shunted to the abomasum (see section 5.2). A typical example of these control measurements is shown in Table 1, when sheep number 4 consumed a marked supplement of 98 g maize meal + 2 g salt. However, considerable shunting of the markers to the abomasum occurred when a similar supplement was subsequently offered to the same sheep. In the latter case 66% of the dose of 51Cr-E and 68% of the dose of ¹⁰³Ru-P were extrapolated to be shunted to the abomasum at 0 hours. From the disappearance of these shunted markers in the abomasum, their respective mean retention times were 0,5 hours (r = 0,98) and 1,0 hour (r = 0.96) and the volume of digesta in the abomasum was 0,34 litres in both cases, which is in agreement with the data above when the markers were introduced into the abomasum.

Table 1

Markers in the rumen or abomasum after ingestion of marked supplements

Measure- ment	Marker	% dose of a marker	% dose of a marker in aboma- sum at (h)			
		at $+ 3^*$	0	+0,5	+1	+2
Control	Cr-E	60**	0**	2	6	11
	Ru-P		0**	0,7	1,5	1,9
Shunt	Cr-E	21**	66**	26	12	5,2
	Ru-P		68**	41	26	10

Time in hours after ingestion of a marked supplement.
Data extrapolated as described in sections 5.2 and 5.3.

A second partial shunting of the markers to the abomasum occurred when a 1 year old sheep was offered a marked supplement of 98 g maize meal + 2 g salt. In this case 64% of the dose of 51 Cr-E had flowed out of the rumen within 3 hours and the percentage of the doses of the markers extrapolated to be shunted to the abomasum at 0 hours were intermediate between those of the control and when shunting occurred in Table 1.

There were two paths available to a marked supplement of 98 g maize meal + 2 g NaCl in the sheep's stomach. The first and mostly used path was that of ejection of the boli through the cardia. Three pieces of information indicate what happened to the contrasting soluble or particulate fractions of the supplements after that. First an average of 35% of a dose of Cr-E include in a supplement had flowed out of the rumen in 3 hours. As the mean retention time (tm) of Cr-E in the rumen was 7,2 hours this is very nearly the quantity of marker expected to have even-flowed out of the rumen. Second from the percentage doses of the markers in the abomasa of the sheep at +0.5 hours and their tm's there, an upper limit of 5% or 2% of the respective doses of Cr-E or Ru-P could have been shunted to the abomasa when the supplements were consumed. Finally the coarse maize particles (with a density of 1,45) in boli did not accumulate in the reticulum but could be felt at the bottom of the reticulo-rumen digesta. Thus it is concluded that after ejection the boli were rapidly removed from the reticulum by the turbulance generated by the reticulo-rumen contractions and mixed with rumen digesta. After that the markers even-flowed to the abomasum.

A second path resulted in a large loss of Cr-E from the rumen after 3 hours and considerable shunting of the markers to the abomasum in two sheep. What needs to be hypothesized from these results when the shunting occurred, is whether deposition of the markers near the orifice in an immotile reticulo-rumen and their subsequent high concentrations in digesta flowing to the omasum was responsible for the shunting, or whether activation of the reticular groove resulted in the markers by-passing to the abomasum. Since the mean retention times and regression co-efficients of the markers infused per abomasum or calculated to be shinted to the abomasum were almost identical, no shunting of the markers took place later than 0,5 hours after supplementation. From Table 1, at + 0,5 hours 2% and 26% of the doses of Cr-E in the supplement were in the abomasum in the control or when shunting occurred respectively. Thus 26% - 2% = 24% was shunted. If this 24% had been shunted to the abomasum at + 0,5 hours, then 76% of this marker would have remained in the rumen. From the control 40% of 76% = 30% of the marker would have even-flowed out of the runnen in 3 hours. Thus in 3 hours the sum of the dose of Cr-E that even-flowed out of the rumen plus the quantity that was shunted to the abomasum at + 0,5 hours would have been 30% + 24% = 54%. However 79% of the dose of Cr-E had flowed out of the rumen in 3 hours when shunting occurred. Thus shunting occurred prior to + 0.5 hours. As time approached 0 hours i.e. when the supplement was offered the extrapolated shunting of the dose of Cr-E approached 66%. If 66% of the Cr-E was shunted to the abomasum at 0 hours, then 34%remained to even-flow out of the rumen and by the same reasoning as above, 40% of 34% = 13% would have

even flowed out of the rumen in 3 hours. Thus in 3 hours the sum of the dose of Cr-E that would have evenflowed out of the rumen plus the quantity that was shunted to the abomasum at 0 hours would have been 13% + 66% = 79%. This is the same quantity of the marker not found in the rumen after 3 hours. Therefore a percentage of the dose of Cr-E approaching 66% was shunted to the abomasum at a time approaching that when the supplement was offered.

How did this shunting happen? When the reticular groove becomes operational motility in the reticulorumen ceases (Schalk & Amadon, 1928). From this it can be hypothesized that the groove may have been activated sufficiently, when shunting occurred, to stop motility but insufficiently to close the lips of the groove so that boli are not conveyed through the orifice. The latter is consistent with the observation in the present experiment that boli sometimes oozed out of the cardia and through the lips of the groove. Under these circumstances it may further be hypothesized that the brothlike boli could partially fill up the reticulum and the uppermost layer would flow out of the office. This could only occur if the orifice were dilated. Because of the position of the orifice, which is situated half way up the wall of the reticulum, it is difficult to see how 66%of a dose of Cr-E have been shunted out in this way. In addition Ru-P marked particles would sink to the bottom of the reticulum because of their relative density and would thus not flow out of the orifice. If this hypothesis desecribed the shunting then abomasal digesta

would contain shunted Cr-E and little Ru-P, but as this was not the case the hypothysis was rejected.

It is concluded that shunting of the markers in the ground supplement past the foregut (from Table 1) was due to the activation of the reticular groove for the following 4 reasons: First the groove was observed to convey boli from a supplement down its entire length. This indicates that such a supplement was able to stimulate the groove. Secondly the explanation for the shunted Ru-P marked particles found in abomasal digesta after offering the marked supplement seems to be that of direct conveyance there via the groove.

Thirdly the shunted markers reached the abomasum prior to + 0,5 hours after supplementation and more specifically for Cr-E at a time approaching 0 hours. Finally approximately equal percentages of the markers were extrapolated to have reached the abomasum at 0 hours. This is to be expected if part of the supplement was conveyed to the abomasum via the groove.

None of the maize supplements were able to consistently activate the groove which may be due to the fact that they were not as appetizing to sheep as milk. It is consequently concluded that the prevention of fermentation in the rumen by the reticular groove conveying a ground supplement directly to the abomasum is not a reliable method of increasing the efficiency of supplementation at present.

The considerable economic benefits promised by ground supplements that are conveyed directly to the abomasum suggests that further attention should be given to research in this field.

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