Effect of starch fermentation in the rumen on voluntary intake of roughage and kinetics of digestion*

J.P. Pienaar,** C.Z. Roux and G.P. Kühn

Animal and Dairy Science Research Institute, Private Bag X2, Irene 1675, Republic of South Africa

Received 19 May 1989; revised 8 November 1989; accepted 15 May 1990

The effect of starch fermentation in the rumen on the kinetics of roughage digestion, was studied using 12 sheep fed three roughages, viz. lucerne hay, maize cob leaves and wheat straw. The amount of starch infused per day was increased from 0 to 600 g/d in steps of 20 g/d over 30 days. The amount of starch infused was delivered at two rates; either as the daily amount infused over 12 h, or over 24 h. The well known negative effect of starch fermentation on roughage intake and digestion was observed when the lucerne and maize cob leaf diets were fed, but not when the wheat straw diet was used. The rate at which starch was infused affected the intake of maize cob leaves in a variable manner. The slow infusion rate led to a very small negative effect, whereas the fast rate of starch infusion resulted in a large negative effect. Regardless of diet, the negative effects of starch on intake could not be ascribed to reduced rumen fill, nor to a reduced concentration of rumen ammonia. Furthermore, pH of the rumen contents was not lowered. Rate of passage of non-fermentable OM was decreased by starch infusion on one diet (maize cob leaves) only, even though the mean retention time (MRT) of water was significantly influenced by starch infusion on all the diets. Although starch fermentation negatively affected many aspects of roughage digestion, the paramount factor appears to be a reduced rate of roughage digestion.

Die invloed van fermentasie van stysel in die rumen op die verteringskinetika van ruvoer is met 12 skape ondersoek. Drie verskillende ruvoere, nl. lusernhooi, mieliekopblare en koringstrooi is gebruik. Stysel is in die rumen geïnfuseer teen hoeveelhede wat oor 30 dae vermeerder is vanaf 0 tot 600 g/d met stappe van 20 g/d. Die stysel is teen twee tempo's toegedien, sodat die daaglikse hoeveelheid oor 12 h of oor 24 h geïnfuseer is. Die welbekende negatiewe effek van styselfermentasie op ruvoerinname en verteringstempo is waargeneem met die lusern- en mieliekopblaardiëte maar nie met die koringstrooidieet nie. Die tempo waarteen die stysel geïnfuseer is het 'n veranderende effek op die inname van mieliekopblare gehad. Feitlik geen effek is waargeneem met die stadige infusietempo nie terwyl 'n groot negatiewe effek waargeneem is met die vinnige infusietempo. Hierdie negatiewe effekte is nie veroorsaak deur 'n verlaging in rumenvulling, of deur 'n té lae rumen-ammoniakkonsentrasie nie. Die pH van die rumeninhoud is ook nie verlaag nie. Die tempo van deurvloei van nie-fermenteerbare organiese materiaal is slegs op een dieet (mieliekopblare) verlaag, alhoewel daar betekenisvolle effekte op die retensietyd van water by al drie diëte waargeneem is. Alhoewel daar baie faktore betrokke was by die negatiewe effek van kragvoerfermentasie op ruvoervertering, blyk dit asof 'n verlaagde tempo van ruvoervertering die belangrikste faktor was.

Keywords: Concentrate, interaction, kinetics, roughage, rumen.

* Part of a Ph.D. thesis submitted to the Department of Animal Science, University of Natal.

** To whom correspondence should be addressed.

The negative effect of a concentrate supplement on the voluntary intake of roughage and on digestibility is not new (Hamilton, 1942). The explanation most generally proposed is the well documented negative effect of a low pH on the activity of cellulolytic microbes (Ørskov & Fraser 1975; Mertens & Loften, 1980; Mould, Ørskov & Gauld, 1983& 84).

There are, however, also other possible causes. The rumen protozoal population may be stimulated by starch supplementation (Eadie & Mann, 1970). These protozoa significantly depress rumen bacterial populations and thus indirectly decrease the fermentation rate of roughage diets. The fact that fibre digestion is sometimes depressed by starch fermentation without a concomitant decrease in pH (Henning, van der Linden, Mattheyse, Nauhaus, Schwartz & Gilchrist, 1980; Mould, Ørskov & Mann, 1983&84), suggests that carbohydrates may specifically affect roughage digestion.

Evidence from the literature indicates that the processing of grains may modify their effect on the voluntary intake of roughage (Ørskov & Fraser, 1975). There is also clear evidence that the processing of grains influences their fermentation rates (Hungate, 1966; Ørskov & Fraser 1975; Liebenberg, Meissner & Pienaar, 1979). Therefore, provided that no other nutrients are limiting, the amount and rate of starch digested and the type of roughage used (Dixon, 1986) must be the key to understanding the interaction between roughage and concentrate digestion in the rumen.

Very little is known about the effect of concentrate supplementation on the *in vivo* digestion and the outflow kinetics of fibre. The few studies which have addressed this problem (Mould & Ørskov, 1983&84) did not allow the authors to separate the physical effects of the grain from the effect of carbohydrate fermentation on the passage of roughage and the kinetics of fermentation. Thus, another approach should be followed to separate these two factors. Application of their *in sacco* results to the *in vivo* situation could also be confounding. Since concentrate supplements are normally more readily consumed than roughage, roughage intakes may be suppressed after concentrates have been consumed. This may be due to mere animal behaviour.

In this study, the physical effect of grain as well as the palatability effect of starch concentrates was eliminated by infusing very finely ground starch, which is completely available to microbial attack and has a very short retention time, into the rumen. By this method, the physical effect of the grain particles on roughage fermentation in, and passage from, the rumen was completely eliminated and the effect of carbohydrate fermentation in the rumen could be examined

in isolation. Care was also taken to ensure that other nutrients such as minerals, protein, or rumen ammonia were maintained at adequate levels during the experiment.

The aim of this experiment was, therefore, to study the effects of different amounts of starch, fermented at two rates, on roughage intake and on the kinetics of roughage passage and fermentation.

Experimental Procedure

Animals, diets and treatments

Twelve nearly mature SA Mutton Merino wethers, fitted with large rumen cannulae (83 mm ID), were kept indoors in metabolism cages for the duration of each experiment, and were fed regularly every 4 h by automatic self-feeders. These sheep were randomly allocated to six experimental treatments within each period as described under 'Experimental design and data analysis'. The animals were adapted to diets, metabolism cages and water infusion for more than 30 days during experimental period 1, and for 24 days during experimental period 2, before starch infusion was commenced.

Three experimental diets, which consisted mainly of either lucerne hay, dry maize cob leaves or wheat straw, each hammer-milled through a 6-mm sieve, were used. They were supplemented with non-protein nitrogen, protein and micro and macro minerals according to the NRC (1975) specifications. Fishmeal, as an assumed source of rumen undegradable protein and iso-acids, was added to all diets (70 g/kg). Readily available energy was provided by spraying 35 g molasses per kg of feed on the diets during preparation. Urea was included in the wheat straw and maize cob leaf diets at a level of 2,79 g/kg. The lucerne, maize cob leaf and wheat straw diets contained 154, 100 and 101 g/kg crude protein and 467, 703, 654 g/kg NDF, respectively, after supplementation.

A suspension of purified corn starch in water, supplemented with urea (20 g/kg starch) was infused on a daily basis *per fistulam* (about 3 l/d). The amounts of starch infused varied between zero, during the adaptation period when only water was infused, and a maximum starch infusion when most animals showed symptoms of rumen acidosis. The amount of starch infused per animal was increased by increments of 20 g/d during the experimental period. The maximum amount of starch infused (before symptoms of rumen acidosis commenced) was 551 ± 19 g at 26,8 ± 0,3 days (mean ± standard error). The daily amount of starch was infused over either a 12- or a 24-h period for the sheep fed on each diet.

Experimental design and data analysis

The experiment was conducted over two experimental periods as an unbalanced change-over design (Patterson & Lucas, 1962, design No. 101). Only the first two periods were used.

Results were analysed by analysis of variance, Harvey's mixed model least-squares and maximum likelihood computer program (Harvey, 1988). All parameters measured were analysed in terms of response lines on starch infusion, where the amount of starch infused was taken to be independent, and the parameter under investigation the dependent variable. Experiments were concluded when sheep exhibited rumen acidosis problems and a cessation of feed intake.

The relationship between voluntary feed intake and starch infusion was tested for linearity by polynomial regression analysis and visual inspection of the plots. No consistent non-linear pattern could be identified between sheep, although results from individual sheep did show significant deviations from linearity in having significant, but dissimilar higher order polynomials. Since it was suspected that these inconsistent deviations from linearity were caused by autocorrelation (Fuller, 1976) induced by repeated measurements on a single sheep, auto-regression analysis (Fuller, 1976) was used to analyse each individual sheep's regression. When a term which accommodated the effect of the foregoing observation on the following observation was included, the data appeared to fit a linear regression. This supported the suspicion that the observed non-linearity was caused by autocorrelation. The slopes between all the measurements and the starch infused were calculated by finding the differences using the following formula:

$$b = (\Sigma\Delta x * \Delta y) / \Sigma x^2$$

where b = slope of the response line; Δx = an increment of infused starch; Δy = the change in the dependent variable during the change in x; and Σ = the sum of these values. When this formula is used to calculate b, the effect of autocorrelation is minimized (Fuller, 1976).

Actual values obtained at zero starch infusion, rather than values derived from regression analysis, were used as intercepts (reference values). Analysis of variance was done separately on the estimates of intercepts (values when no starch was infused) and on slopes (responses).

Although values for the slopes as such are not presented in these results, the values equivalent to the infusion of 600 g of starch are presented. Actual values for slopes may be obtained from the values given for 0 and 600 g starch infused.

Voluntary feed intake

Voluntary organic matter (OM) intake was determined on a daily basis. The daily allowance, which had to be 10% more than *ad libitum* intake, was calculated on a four-day moving average.

Rumen contents

Estimates of mean rumen digesta contents were obtained by manually emptying each sheep's rumen twice in a 24-h period, but not more than four times in seven days. The times selected to empty the rumen were 15h00 and 08h00, when minimum and maximum fill, respectively, were expected. Mean rumen fill was calculated for each point on the curve by averaging the corresponding minimum and maximum values for fill. The use of this method was justified by the fact that no negative effect on rumen anaerobiosis or voluntary feed intake could be detected and that *in vivo* methane production, which is very sensitive to aeration, was not inhibited (Hofmeyr, Slabbert & Pienaar, 1984; unpublished results). No effect on voluntary feed intake was observed in previous work where this schedule was followed, and this is confirmed by the fact that rumen anaerobiosis was not affected.

Mean retention time of water

Mean retention time (MRT) of water was determined by mixing a single dose of ^{51}Cr-EDTA into the rumen contents after emptying the rumen at 08h00. Samples of rumen liquor were taken by suction at 10h00 and 15h30 on the same day and at 08h00 on the following day. MRTs were calculated as described by Warner (1966). When results are expressed as MRTs the values are not in the additive form, thus all statistical calculations were done on the inverse of MRT. This was shown by plots of residuals against predicted values which showed a random distribution of points in the case of the inverse. These were then called 'rate constants' which are additive and have a normal error structure. The reason why this number of points was taken is that a straight line is estimated most accurately when measured over a longer time (24 h in this case instead of the usual 12 h) and when the extremes of the line are included.

MRTs for flow and fermentation of organic matter

The MRT for the outflow and fermentation of organic matter was calculated according to the method of Pienaar, Roux, Morgan & Grattarola (1980). However, the outflow was expressed in terms of MRT instead of rate constants, since the mathematical description of retention time holds true, regardless of the form of the outflow curves (Roux & Meissner, 1984). Detailed explanations of why MRTs, rather than rate constants, are preferrable for describing fermentation and outflow rates, were given by Pienaar, Roux & Cronjé (1989) and also by Pienaar & Roux (1989).

The potential OM digestibility of the diets and rumen contents was estimated by an *in vitro* method (Tilley & Terry, 1963), modified by using a 72-h incubation during the microbial phase. Solubility of OM was determined by suspending the feed for 30 min in a solution of Mc Dougals' artificial saliva (Tilley & Terry, 1963) before filtering according to the same *in vitro* procedure.

The pH of rumen contents

The pH of rumen contents was determined directly in the rumen digesta immediately after collection and thorough mixing. Since pH is measured on a log scale, it is not suitable as such for analysis of variance. Thus, analysis of variance on this measurement was done on the linear form, i.e. $[H^+]$ concentration.

Digestible OM intake

The amount of starch infused was excluded when digestible OM intake was calculated. This was possible since the type of starch used was totally digestible in the rumen. The value thus obtained would be digestible OM obtained from the roughage fraction at a particular level of starch infusion.

Results and Discussion

Voluntary feed intake

The effect of starch infusion on voluntary feed intake (see Table 1) was described by linear regression. Highly significant differences caused by diets, periods, and animals were observed between intakes at zero starch infusion (intercepts).

The slopes of regression indicate the responses in voluntary feed intake (substitution rate) on the different diets when starch was infused at different rates. A substitution rate of minus one (−1) would imply a 1 g decrease in intake for every 1 g increase in starch infused. A substitution rate that differs significantly from zero indicates that starch infusion had a significant effect on voluntary intake.

Both the 12-h and 24-h infusion rates had significant effects with the lucerne hay diet while neither had significant effects with the wheat straw diet. On the maize cob leaf diet a large and highly significant effect was obtained with the 12-h infusion rate and a small and statistically non-significant effect with the 24-h infusion rate.

Table 1 Voluntary roughage intakes (g/d) and substitution rates (g/g) during different starch infusion regimes

	Diet		
	Lucerne	Maize cob leaf	Wheat straw
Zero starch infusion			
OM intake	1293[a]	1160[b]	622[c]
SE	35	38	38
Starch infused over 12-h period			
OM[+] intake at 600 g starch	1025*	655**	689ns
Substitution rate	−0,45*	−0,84**	0,11ns
SE	0,18	0,21	0,21
Starch infused over 24-h period			
OM[+] intake at 600 g starch	1018*	1093ns	593ns
Substitution rate	−0,46*	−0,11ns	−0,05ns
SE	0,18	0,21	0,18

[a−c] Rows with different superscripts differ significantly ($P \leq 0,05$).

[+] Values calculated from regressions of starch infused on voluntary intake.

* Significant at $P = 0,05$; ** significant at $P = 0,01$.

SE: Standard error; ns: non-significant.

Table 2 Rumen OM content (g) and substitution rate (g/g) during starch infusion

	Diet		
	Lucerne	Maize cob leaves	Wheat straw
Zero starch infusion			
Rumen OM content	976[a]	973[a]	667[b]
SE	42,0	45,9	46,3
600 g Starch infused			
Rumen OM content[+]	882	924	725
Substitution rate	−0,156	−0,083	0,097
SE	0,153	0,177	0,165

[a,b] Rows with different superscripts differ significantly ($P \leq 0,05$).

[+] Values calculated from regressions of starch infusion on rumen OM.

SE: Standard error.

The fact that fermentation of starch in the rumen reduces voluntary feed intake and that different responses are obtained with different diets has already been reported (Golding, Moore, Franke & Ruelke, 1976; Jarrige, *et al.*, 1986). No report on the amount and rate of starch actually fermented in the rumen could be found. Differential responses of diets have often been ascribed to either a chemostatic or capacity control mechanism. The actual reason could, however, not be deduced from the previous studies since rumen fill without the complicating effects of the concentrate content of the rumen, could not be estimated.

It may be asked what caused the decrease in voluntary intake in this study. If rumen fill (OM contents) was not significantly influenced by starch infusion, it would imply that some kind of capacity control had influenced feed intake. If so, it would indicate that it was some kind of control other than reticulo-ruminal distention, e.g. fermentation in that organ.

Mass of OM in the rumen

The effect of starch infusion on the mass of OM in the rumen was also described by linear regression. The values obtained at zero starch infusion showed that the influence of diet (P = 0,005), period (P = 0,0273) and animals (P = 0,0102) was significant. The effects of diet are presented in Table 2. The slopes of the response curves showed that these were not significantly influenced by starch infusion (P = 0,873).

Sheep fed the wheat straw diet had a significantly lower OM content than sheep fed the other two diets (Table 2). This result is not unexpected, since Meissner, Pienaar, Liebenberg & Roux (1979) have shown significant differences in terms of mass of OM in the rumen at *ad libitum* intake, even though all protein, mineral and NPN deficiencies have been eliminated by supplementation.

The substitution rates observed in rumen OM content on all diets at both infusion rates were far from statistically significant ($P > 0,50$). Thus, the changes observed at a starch infusion rate of 600 g/d are also not statistically significant. This suggested that the significant effects of starch infusion on voluntary feed intake could not be associated with statistically significant changes in rumen fill. Some kind of capacity control (control due to reticulo-ruminal distension) (Grovum, 1986) can therefore be hypothesized.

Rumen ammonia concentration

Rumen ammonia concentration may have a significant influence on fibre digestion kinetics when supplied at rate-limiting concentrations (Satter & Slyter, 1974; Mehrez, Ørskov & MacDonald, 1977). This factor was studied to determine if the basal roughage diet as well as the starch supplement were correctly formulated so that rumen ammonia would not be the rate-limiting factor in rumen fermentation during all phases of the experiment. The results are presented in Table 3.

Table 3 Mean rumen ammonia concentrations (mmol/l) at three occasions

	Mean	± SD^a
Basal diet	10,0	± 3,2
Low starch	12,0	± 2,7
High starch	17,5	± 5,0

[a] Standard deviation.

It is clear that rumen ammonia concentration showed a tendency to increase with increased starch infusion. None of the values were close to the minimum requirement of 3,6 mmol/l (Satter & Slyter, 1974). The observed tendency for an increase in rumen ammonia concentration shows that the 20 g/kg urea infused together with the starch, was more than sufficient to meet the increased ammonia requirements in the rumen caused by starch fermentation. It is therefore unlikely that ammonia was the rate-limiting factor which limited voluntary roughage intake at any stage during the experiment.

The pH of the rumen contents

Two phases during the measurement of rumen pH need to be distinguished. The first concerns the observations obtained from the start of the experiment to nearly the end. During this stage, almost no change in pH was observed and these values were used to calculate the pH values presented in Table 4. The second phase concerns the last one or two observations where pH was measured before a sheep was removed from the experiment. At this stage a very rapid drop in pH occurred. Within two or three days the pH would decline from about 6 to 5 or even 4,5. This decline in pH was always associated with a cessation of feed intake. This rapid drop was probably induced because starch infusion continued even when an animal had stopped eating and ruminating. These low pH values were not included in the regression of the $[H^+]$ of rumen contents versus feed intake.

The effect of starch infusion on the $[H^+]$ of the rumen contents was described by linear regression. At zero starch infusion, there were significant differences between diets and periods. The differences between diets are presented in Table 4.

Ruminal pH on the maize cob leaf diet was considerably lower than the pH on the other two diets when measured at zero starch infusion, i.e. at the intercept. This difference was significant when tested as $[H^+]$. The infusion of starch did not significantly alter pH, as can be seen from the small pH difference when 0 g starch, compared with 600 g starch, was infused. It appears that the decreased voluntary intake

Table 4 The pH of rumen contents when starch was infused over 12 h or over 24 h

	Diets		
	Lucerne	Maize cob leaf	Wheat straw
pH when 0 g starch was infused and the 95% confidence interval	6,25[a] 6,09—6,51	5,97[b] 5,86—6,10	6,31[a] 6,12—6,66
pH^+ when 600 g starch was infused over 12 h or over 24 h	6,31 5,92	6,18 5,87	6,47 5,98

[a,b] Rows with different superscripts differ significantly ($P \leq 0,05$).

[+] Values calculated from regressions of starch infusion on $[H^+]$.

observed with starch infusion can not be directly associated with a lowering of rumen pH. However, the pH (5,97) measured on the maize cob leaf diet when no starch was infused, was below the value (pH 6) shown by Mould *et al.* (1983& 84) to be the 'cellulolysis-threshold'. Although this value should be considered as an indication rather than an absolute threshold value, it can be expected that a very small change in pH which could not be easily detected *in vivo*, could have had a large effect on microbial activity.

MRT of water

Although the MRT of water is not usually associated with animal production, a direct relationship between the MRT of water in the rumen and the efficiency of microbial protein production in the rumen has been shown (NRC, 1985). It is also interesting to see how the fermentation of starch influences this measurement.

At zero starch infusion, significant differences ($P < 0,05$) in the values were obtained between diets and periods, and in the slopes between diets and infusion rates (Table 5). At zero starch infusion, the lucerne diet differed from the other two diets. The t values calculated to show whether the slopes differed significantly from 0, were significant for the lucerne diet with starch infused over 12 h, and for the other two diets when starch was infused over 24 h.

A significant *decrease* in MRT was obtained with the diets which initially had the longest MRT, whereas a significant *increase* in MRT was obtained on lucerne with the shortest initial MRT. Possible reasons for the changes in two totally different directions for the different diets are unknown to the authors.

Table 5 also shows that infusing starch at the faster rate (12 h) tended to result in a longer MRT for water. This was true for all diets and may be observed in the differences between MRT values at the point where 600 g was infused at 12-h and 24-h infusion rates. The differences were significant only at $P = 0,068$. These results suggest that the dilution rate of water in the rumen (MRT) may be influenced by the kind of diet fed and also by starch fermentation in the rumen on all diets.

The MRT of water was increased on the lucerne diet and decreased on the maize cob leaf diet. This differs from the tendency observed with voluntary feed intake.

MRT for outflow of non-fermentable organic matter (NOM)

The passage of NOM from the rumen may be considered one of the rate-limiting steps which determines voluntary feed intake under conditions of capacity control. The outflow of NOM is presented as MRT in Table 6. The MRTs at 0 g starch infusion are presented together with their 95% confidence intervals, as well as the values calculated for MRTs when 600 g of starch was infused. At zero starch infusion, animals and periods had significant effects on the MRT of NOM, whereas the slopes were not significantly influenced by starch infusion in five out of six cases. Only the maize cob leaf diet was significantly influenced at the fast infusion rate.

The fact that passage of NOM (Table 6) was not significantly influenced by diets, confirms the original results of Pienaar *et al.* (1980). They also found that a common value for diets could be calculated for the passage of NOM. However, Pienaar *et al.* (1980) obtained a value of 48 h for MRT of NOM instead of the common value of 33,8 h with a 95% confidence interval of 32,8–34,9 obtained in this study. The probability that this difference was only a coincidence, is very small. In their study, the diets were ground through a coarser sieve than in the present study. Thus, differences in particle size as well as in age of animals could have caused the differences in MRT of NOM observed. It is interesting to note that, although significant differences ($P < 0,05$) existed between diets in MRT for water (Table 5), the differences observed in MRT of NOM (see Table 6) were not significant ($P = 0,134$).

The fact that starch infusion did not influence MRT of NOM significantly in five out of six cases, clearly shows that the kind of diet fed and the fermentation in the rumen have a relatively small effect on the passage of NOM. On the other hand, the effect of period ($P < 0,01$) and the significant differences observed between sheep, indicate that animal-

Table 5 MRT of water in the rumen (h) when starch was infused over 12 h or over 24 h

	Diet		
	Lucerne	Maize cob leaf	Wheat straw
MRT when 0 g starch was infused and the 95% confidence intervals	9,7[a]	13,5[b]	14,5[b]
	8,9—10,7	11,7—15,8	12,6—17,0
MRT[+] when 600 g starch was infused over 12 h or over 24 h	16,8*	12,8ns	13,1ns
	10,3ns	8,7*	8,8*

[a,b] Rows with different superscripts differ significantly ($P \leq 0,05$).

[+] Values calculated from regressions of starch infusion on MRT of water.

ns Non-significant slope; * significant ($P \leq 0,05$) slope.

Table 6 Mean retention time of non fermentable OM in 12 h or 24 h

	Diet		
	Lucerne	Maize cob leaves	Wheat straw
MRT (h) when 0 g starch was infused and the 95% confidence interval (h)	35,3	32,6	33,0
	33,3—37,7	30,7—34,7	31,1—35,3
MRT[+] (h) when 600 g starch was infused over 12 h or over 24 h	31,4ns	42,3*	32,3ns
	35,7ns	38,3ns	30,0ns

[+] Values calculated from regressions of starch infused on MRT of non-fermentable OM.

ns Non-significant slope; * significant ($P \leq 0,05$) slope.

associated factors are more important where the passage of NOM is concerned.

The effect of starch infusion on the MRT of NOM was only significant ($P < 0,05$) on the maize cob leaf diet at the 12-h infusion rate. This was the only indication of a significant effect of starch infusion on the MRT of NOM and was only observed with one diet on the fast infusion rate.

MRT for fermentation

MRT for fermentation can be considered one of the rate-limiting steps which determines voluntary feed intake under conditions of capacity control. It was calculated for insoluble fermentable OM (IFOM), both including and excluding infused starch.

MRT for fermentation including starch fermentation

At zero starch infusion, clear differences ($P < 0,01$) between diets and periods were found, as well as an interaction between diets and periods. Significant responses on starch infusion were also obtained. The results are presented in Table 7.

The lucerne diet (Table 7) fermented faster (smaller MRT) ($P < 0,01$) than the other two diets when no starch was infused. This is not surprising, since different forages normally ferment at different rates. Possible reasons for these differences are that lucerne has wider ratio between cell wall and cell contents, and also that particle size distributions and cell wall structure differ between forages. The fact that all MRTs were decreased by starch infusion shows that the addition of starch had net positive effects on the MRTs for fermentation of all diets, in spite of the negative effects of starch fermentation on roughage digestion.

MRT for fermentation excluding starch fermentation

The MRT for fermentation of IFOM excluding starch fermentation describes the fermentation of the 'roughage' only in the presence of starch. The MRTs calculated when no starch was infused, do not include the starch originally present in the basal diets. The values, when no starch was infused, differed ($P < 0,05$) between diets and periods and an interaction between diets and periods was also found. Only the differences between diets are presented in Table 8.

Table 7 MRT for fermentation including starch fermentation when starch was infused over 12 or 24 h

	Diet		
	Lucerne	Maize cob leaf	Wheat straw
MRT (h) when 0 g starch was infused and the 95% confidence interval (h)	9,03[a]	17,4[b]	20,1[b]
	7,1—12,3	14,3—22,5	16,2—26,5
MRT[+] (h) when 600 g starch was infused over 12 h	7,3ns	12,8ns	9,6**
or over 24 h	6,5**	11,4ns	10,5**

[a,b] Rows with different superscripts differ significantly ($P \leq 0,01$).
[+] Values calculated from regressions of starch infused on MRT for fermentation.
ns Non-significant slope; ** significant ($P \leq 0,01$) slope.

Table 8 The effect of starch infusion on the MRT for fermentation of IFOM excluding starch fermentation

	Diet		
	Lucerne	Maize cob leaf	Wheat straw
MRT (h) when 0 g starch was infused and the 95% confidence interval (h)	9,5[a]	17,9[b]	23,0[b]
	8,6—10,5	14,7—22,8	18,3—31,2
MRT[+] (h) when 600 g starch was infused over 12 h	17,3**	29,1ns	38,1ns
or over 24 h	16,9**	20,1ns	33,9ns

[a,b] Rows with different superscripts differ significantly ($P \leq 0,01$).
[+] Values calculated from regressions of starch infused on MRT of IFOM.
ns Non-significant slope; ** significant ($P \leq 0,01$) slope.

Table 8 shows differences ($P < 0,01$) between MRTs for fermentation when zero starch was infused . These differences occurred between the lucerne diet and the other two diets. Only the slopes obtained with the lucerne diet differed significantly from zero. Although the largest negative response on starch infusion was obtained with the lucerne diet, it still had the shortest MRT for fermentation at 600 g of starch infused. There was a tendency for the MRT for fermentation of all three diets to be influenced by starch infusion, although this tendency was only statistically significant on the lucerne diet. Thus, a reduction in MRT of roughage fermentation appeared to be a contributory factor where a reduced voluntary feed intake was observed.

Digestible OM intake

The effects of different diets and their reactions on starch

Table 9 Voluntary digestible OM intake (g) (excluding infused starch) when starch was infused over 12 or 24 h

	Diet		
	Lucerne	Maize cob leaf	Wheat straw
Intake when 0 g starch was infused	807[a]	815[a]	305[b]
Standard error	24	26	26
Intake[+] when 600 g starch was infused over 12 h	420**	306**	352ns
or over 24 h	634*	769ns	271ns

[a,b] Rows with different superscripts differ significantly ($P \leq 0,01$).
[+] Values calculated from regressions of starch infused on intake.
ns Non-significant slope.
* Significant ($P \leq 0,05$) slope; ** significant ($P \leq 0,01$) slope.

infusion in terms of the intake of digestible OM (excluding infused starch) are shown in Table 9. At zero starch infusion, digestible OM intake was significantly influenced by diets, periods and animals. Only the effects of diets are presented. The slopes were influenced ($P < 0,05$) by diets, periods and infusion rates.

Table 9 shows very similar intakes of digestible OM for the lucerne and maize cob leaf diets at zero starch infusion. Wheat straw had a lower ($P < 0,01$) intake of digestible OM than the other two diets at the intercept.

Infusing starch at a faster rate (12 h) had a larger negative effect ($P < 0,01$) than infusing at a slower rate (24 h). This tendency was apparent with both the lucerne and maize cob leaf diets. The slopes differed from zero for both infusion rates in the lucerne diet and also for the 12-h infusion rate in the maize cob leaf diet. The 24-h infusion rate on maize cob leaves as well as both infusion rates on the wheat straw diet showed no significant effect on digestible OM intake.

The effects of the different treatments are clearly shown when intakes are calculated from the regressions at the infusion of 600 g of starch per day. The large difference between the slopes of the two infusion rates within the maize cob leaf diet is striking. The two slopes obtained with the two infusion rates differed ($P < 0,01$) from each other on the maize cob leaf diet and tended to differ ($P < 0,10$) on the lucerne diet. This result emphasizes the beneficial effect of a constant infusion of starch, or conversely, a more homogeneous fermentation pattern for a starch supplement. The fact that the same large difference was not observed between the two infusion rates on the wheat straw diet suggests that an interaction exists between the substrate fermented and the supplement used. The interaction between roughage and concentrate digestion will therefore have to be determined separately for each roughage–concentrate combination.

Conclusions

The well known and well documented negative effect of starch (concentrate) addition on roughage digestion was observed in this study. The results agree in general with other work which reports on the effect of starch or concentrate supplementation on roughage digestion kinetics. An increased lag phase in roughage digestion was reported by most workers (Mertens & Loften, 1980; Miller & Muntifering, 1985; Aitchison, Gill, & Osbourn, 1986.) In this study a mean estimate, which includes both the lag and fermentation phases, was obtained. A reduced fermentation rate, which could also have been caused by an increased delay phase, was obtained on some diets.

The net effect of starch fermentation on voluntary roughage intake was demonstrated. In some cases, the maize cob leaf diet showed a possible advantage of more constant starch fermentation, since very small and statistically non-significant negative effects were observed even at high levels of starch, when evenly infused. Possible explanations for the negative effects observed in some treatments have been eliminated. The first of these is a possible deficiency in rumen ammonia for microbial fermentation. Since rumen ammonia was at no stage observed to be near any level where a negative effect on rumen fermentation could be expected, this possibility could be ruled out. A reduced roughage intake owing to a preferential selection for concentrates was also ruled out, since starch was infused and not eaten. Voluntary intake was reduced on some diets even though the mass of rumen OM contents was not significantly changed by starch infusion. Thus, a reduced roughage intake caused by some kind of rumen fill limitation can be hypothesized.

The effect of a reduced rumen pH could not be shown, since no significant reduction in pH could be detected during starch infusion. However, the values obtained in some cases were below the 'cellulolysis-threshold' mentioned by Mould *et al.* (1983&84). It would thus be dangerous to exclude the possibility that pH could have had an effect. Rate of passage of non-fermentable OM was one of the factors that was not consistently influenced by starch infusion. Rate of passage was significantly reduced only on the maize cob leaf diet (12-h infusion). A reduction in rate of passage was also observed by Miller & Muntifering (1985) on some of their extreme dietary treatments, but Aitchison *et al.* (1986) recorded no such effect. A possible effect of a low ph on rate of passage can also not be eliminated, since mild rumen acidosis results in diminished rumen contractions (Dirksen, 1970).

Although the MRT of water was significantly altered during starch fermentation on some diets, these changes were not consistent with the changes observed in voluntary feed intake. Thus, the decreased voluntary feed intake observed with starch infusion can not be directly related to its effects on the MRT of water.

Rate of passage of NOM could have contributed to the observed changes in voluntary intake on the maize cob leaf diet at the 12-h infusion rate. However, it was not an effect that was observed on all diets.

It appears that the most consistent negative effect of starch infusion, which could be related directly to feed intake, was observed in the MRT for fermentation of the non-starch components (IFOM). This parameter was altered in all cases, although not always significantly. It showed the same negative reaction on starch infusion that was observed in the intake of fermentable OM, and different diets responded differently to starch infusion in this measurement. However, the effect of infusion rate was not observed to be statistically significant in the MRT of fermentation, although infusion rate did have a statistically significant effect on the intake of non-starch fermentable OM. Thus, the effect of infusion rate can not be explained in terms of an altered rate of roughage digestion. There was a tendency to increase the MRT of IFOM on the wheat straw diets also, although there was no tendency to decrease voluntary feed intake in this diet. The tendency to increase the MRT of IFOM on this diet was counterbalanced by a tendency to decrease MRT of NOM and rumen OM content. In other words, a decreased rate of fermentation was counterbalanced by increases in rate of passage and rumen fill.

Although starch infusion seriously depressed the intake of non-starch fermentable OM in some diets, there was always a net gain in fermentable OM intake during starch infusion. This depression was the smallest with the low-quality wheat straw diets and the largest with maize cob leaf and lucerne diets. The two diets which showed the largest effects on starch infusion responded very differently to the different infusion rates. Infusing starch had the same effect on the lucerne diet, whether infused at a fast or a slow rate. The

maize cob leaf diet, however, was little influenced by a slow starch infusion but greatly by the fast infusion rate. Since these effects were not observed in the MRT for fermentation as well, these different responses can not be explained in terms of the MRT for fermentation. The measurement of intake may be more accurate than that of MRT for fermentation, since intake was measured continuously, whereas MRT for fermentation was measured only twice a week. In that case, the effects shown to be significant with intake would, in that case, be smaller than the ones shown to be significant in the MRT for fermentation. This would then explain the absence of significance in some intake-related parameters.

It is concluded that the negative associative effects observed between starch and roughage digestion can not be explained in terms of the effect of a single factor on rumen digestion kinetics. The negative associative effects were observed in most of the parameters on some diets, even though not all were statistically significant. The inconsistent response to the amounts and rates of starch infusion observed between and within roughages, shows that each roughage reacts differently to starch infusion. For accurate estimates associative effects will have to be assessed individually for each diet at the relevant rate of starch fermentation.

Acknowledgements

The authors gratefully acknowledge the skilled technical assistance of Jock Davie, Brenda Botha, Joan Behrens and Marie Mocke with the chemical analyses. We also appreciate the help of Messrs A.B. van Zyl and Charl Marais for other technical assistance and Messrs M. Bosoga, R.W. Sithole and F. Seshoka in taking care of the sheep. Our colleagues at Nutrition Section who helped with the preparation of the manuscript are also thanked.

Soli Deo Gloria

References

AITCHISON, E.M., GILL, M. & OSBOURN, D.F., 1986. The effect of supplementation with maize starch and level of intake of perennial ryegrass (*Lolium perenne* cv. Endura) hay on the removal of digesta from the rumen of sheep. *Brit. J. Nutr.* 56, 477.

DIRKSEN, G., 1970. Acidosis. In: Physiology of digestion and metabolism in the ruminant. Proceedings of the Third International Symposium Cambridge, England; August 1969. Ed. Phillipson, A.T., Oriel Press.

DIXON, R.M., 1986. Effects of dietary concentrates on rumen digestion of fibrous feedstuffs. *Anim. Feed. Sci. Technol.* 14, 193.

EADIE, J.M. & MANN, S.O., 1970. Development of the rumen microbial population. High starch diets and instability. In: Physiology of digestion and metabolism in the ruminant. Ed. Phillipson, A.T., Oriel Press.

FULLER, W.A., 1976. Introduction to statistical time series. Wiley, New York.

GOLDING, E.J., MOORE, J.E., FRANKE, D.E. & RUELKE, O.C., 1976. Formulation of hay-grain diets for ruminants. II. Depression in voluntary intake of different quality forages by limited grain in sheep. *J. Anim. Sci.* 42, 717.

GROVUM, W.L., 1986. A new look at what is controlling food intake. In: Proceedings of a symposium on feed intake by beef cattle, Oklahoma City, 20–22 November 1986. Eds. Owens, F.N., Gill, D.R. & Lusby, K.S., Animal Science Dept., Oklahoma State Univ., Stillwater, Oklahoma. 74078-0425.

HAMILTON, S.T., 1942. The effect of added glucose upon the digestibility of protein and of fibre in rations for sheep. *J. Nutr.* 23, 101.

HARVEY, W.R., 1988. Harvey's mixed model least-squares and maximum likelyhood computer program. Harvey's least squares, 4255 Mumford Dr, Columbus, Ohio, 43220, USA.

HENNING, P.A., VAN DER LINDEN, Y., MATTHEYSE, M.E., NAUHAUS, W.K., SCHWARTZ, H.M. & GILCHRIST, M.C., 1980. Factors affecting the intake and digestion of roughages by sheep fed maize straw supplemented with maize grain. *J. Agric. Sci. Camb.* 94, 565.

HOFMEYR, H.S., SLABBERT, N. & PIENAAR, J.P., 1984. Partitioning of methane production between ruminal and hind-gut fermentation sites in sheep. *Can. J. Anim. Sci.* (Suppl.), 171.

HUNGATE, R.E., 1966. The rumen and its microbes. Academic Press, New York.

JARRIGE, R., DEMARQUILLY, C., DULPHY, J.P., HODEN, A., ROBELIN, J., BERANGER, C., GEAY, Y., JOURNET, M., MALTERRE, M.D. & PETIT, M. 1986. The INRA 'Fill Unit' system for predicting the voluntary intake of forage-based diets in ruminants: A review. *J. Anim. Sci.* 63, 1737.

LIEBENBERG, L.H.P., MEISSNER, H.H. & PIENAAR, J.P., 1979. Disappearance of processed maize grain in the rumen. *S. Afr. J. Anim. Sci.* 9, 227.

MEHREZ, A.Z., ØRSKOV E.R. & McDONALD, I., 1977. Rates of rumen fermentation in relation to ammonia concentration. *Brit. J. Nutr.* 38, 437.

MEISSNER, H.H., PIENAAR, J.P., LIEBENBERG, L.H.P. & ROUX, C.Z., 1979. Rumen flow dynamics and voluntary intake in ruminants. *Ann. Rech. Vét.* 10, 219.

MERTENS, D.R. & LOFTEN, J.R., 1980. The effect of starch on forage fibre digestion kinetics *in vitro*. *J. Dairy Sci.* 63, 1437.

MILLER, B.G. & MUNTEFERING, R.B., 1985. Effect of forage: concentrate on kinetics of forage fiber digestion *in vivo*. *J. Dairy Sci.* 68, 40.

MOULD, F.L., ØRSKOV, E.R., 1983 & 84. Manipulation of rumen fluid pH and its influence on cellulolysis *in sacco*, dry matter degradation and the rumen micro-flora of sheep offered either hay or concentrate. *Anim. Feed Sci. Technol.* 10, 1.

MOULD, F.L., ØRSKOV, E.R. & GAULD, S.A., 1983 & 84. Associative effects of mixed feeds. II. The effect of dietary addition of bicarbonate salts on the voluntary intake and digestibility of diets containing various proportions of hay and barley. *Anim. Feed Sci. Technol.* 10, 31.

MOULD, F.L., ØRSKOV, E.R. & MANN, S.O., 1983 & 84. Associative effects of mixed feeds. 1. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis *in vivo* and dry matter digestion of various roughages. *Anim. Feed Sci. Technol.* 10, 15.

NRC, 1975. Nutrient requirements of domestic animals. No 5. Nutrient requirements of sheep. National Research Council. Washington, DC.

NRC, 1985. Ruminant nitrogen usage. Subcommittee on nitrogen usage in ruminants, Committee on animal nutrition, Board on Agriculture, National Research Council. National Academy Press. Washington, DC.

ØRSKOV, E.R. & FRASER, C., 1975. The effects of processing of barley-based supplements on rumen pH, rate of digestion and voluntary intake of dried grass in sheep. *Brit. J. Nutr.* 34, 493.

PATTERSON, H.D. & LUCAS, H.L., 1962. Change-over

designs. North Carolina Agric. Exp. Station & US Dept. of Agric. Technical Bulletin No. 147.

PIENAAR, J.P., ROUX, C.Z., MORGAN, P.J.K. & GRATTAROLA, L., 1980. Predicting voluntary intake on medium quality roughages. *S. Afr. J. Anim. Sci.* 10, 215.

PIENAAR, J.P., ROUX, C.Z. & CRONJÉ, P.B., 1989. Comparison of *in vivo* and *in sacco* methods to estimate the mean retention time of fermentable organic matter in the rumen. *S. Afr. J. Anim. Sci.* 19, 71.

PIENAAR, J.P. & ROUX, C.Z., 1989. Use of the gamma function in equations which describe ruminal fermentation and -outflow rates for the prediction of voluntary intake and protein degradation. *S. Afr. J. Anim. Sci.* 19, 99.

ROUX, C.Z. & MEISSNER, H.H., 1984. Growth and feed intake patterns. 1. The derived theory. In: Herbivore nutrition in the tropics and subtropics. Eds. Gilchrist, F.M.C. & Mackie, R.I. The Science Press. Craighall, South Africa. pp. 672–690.

SATTER, L.D. & SLYTER, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Brit. J. Nutr.* 32, 199.

TILLEY, J.M.A. & TERRY, R.A., 1963. A two-stage technique for the *in vitro* digestion of forage crops. *J. Br. Grassld. Soc.* 18, 104.

WARNER, A.C.I., 1966. Diurnal changes in the concentrations of micro-organisms in the rumens of sheep fed limited diets once daily. *J. Gen. Micro.* 45, 213.