FIBRE FERMENTATION KINETICS IN THE RUMEN OF SHEEP

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SUMMARY

Nylon bags containing 3 alkali treated and 3 untreated cereal straws were incubated in the rumen of sheep from 0 to 96 hours. Data calculated from this fitted a simple kinetic model involving an undegradable pool and a degradable pool where fermentation of potentially degradable fibre followed first order kinetics. Dry matter, neutral detergent fibre, acid detergent fibre, hemicellulose and cellulose had similar rates and extents of fermentation lending strong support to the two pool model. Substantial losses of lignin from the nylon bags were observed which was thought to be mainly due to solvation rather than true digestion.

INTRODUCTION

Microbial fermentation and the onward passage of undigested particulate matter are the two processes resulting in the disappearance of digesta from the reticulo rumen. Comparatively little is known about these two processes and the factors that may influence them. Mathematical models have been devised using current concepts of ruminant digestion to simulate processes occurring in the rumen and to fit experimental data. A total rumen model must embody several sub-models to express the processes of; passage of undigested materials, reduction in particle size and microbial fermentation. The role played by cell contents (CC) of forages in these sub systems relates primarily to the provision of nutrients to microbes since, as Van Soest (1967) has shown, this fraction is nearly 100 per cent digestible and is soluble. These models refer particularly to cell wall (CW) and its constituents.

Several models have been proposed to account for the disappearance of digesta from the reticulo rumen by microbial fermentation. The existence of a pool of relatively undegradable materials is now well established (Waldo 1969; Waldo et al. 1972; Dekker et al. 1972). Whether it is accompanied by one or more pools of degradable material is likely to be diet dependant. A simple first order kinetic model for the rate of fermentation of the potentially degradable fibre pool has been suggested (Gill et al. 1969; Smith et al. 1971, 1971a; Thiago et al. 1979; Poppi et al. 1981). Other workers have split the potentially degradable pool into two sub pools (Mehrez and Orskov 1977 for rolled barley; Mertens and Ely 1979, Dunlop and Kellaway 1980, for forages). The division of the fibre pool into these two degradable pools and an undegradable pool is supported by the tissue morphology studies of Akin and co-workers (1974 and 1975) who suggested that various plant tissues differ in the rate and extent to which they are degraded. Akin and Amos (1975) showed that the order of degradability of plant tissues by ruminal

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microbes (in order of decreasing ease and extent of degradation) was: Mesophyll and Phloem > Epidermis, Parenchyma bundle sheath > Sclerenchyma (largely undegraded), Lignified vascular tissue (non-degradable).

The objectives of this study were to determine which of these models best fitted fibre fermentation in low quality roughages and to assess whether chemically recognizable constituents of fibre were fermented at different rates and to determine how alkali treatment influenced the parameters of fermentation.

METHODS

Eight merino wethers (40 kg liveweight) were fitted with large ruminal cannulae (80 mm diameter) and kept in metabolism cages. Diets consisted of chopped cereal straws (two wheat straws and one oat straw) sprayed with a solution of urea and minerals (control diets) or sodium hydroxide and urea and minerals (alkali treated diets). The urea and mineral spray supplied (per kg of straw) 12g N, 3g P, 1.5g S, 8mg Cu and 0.05mg Co and the alkali supplied 50g NaOH per kg of straw. Diets were fed continuously at 95% of ad libitum intake.

Ten days after the animals had been introducedto their diets, nine nylon bags (Thiago 1979) filled with 6g of coarsely ground diet were placed in their rumens and allowed to incubate for 3, 6, 9, 12, 24, 48, 72 and 96 hours. Bags were soaked in water immediately before placing them in the rumen to imitate the effect of saliva on removal of soluble nutrients during ingestion and one such bag provided data for the zero duration of incubation. Once removed, bags were washed thoroughly under tap water, opened and dried at 60°C overnight and residues were weighed and analysed. Decay curves (residual proportion of fraction vs time) were then calculated for dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin, hemicelluloses (HC) and cellulose (C) for each of the diets. DM was analysed according to the methods of the Association of Official Agricultural Chemists (1963), NDF according to Van Soest and Wine (1967), and ADF and lignin by the method of Van Soest (1963). Hemicellulose was calculated as NDF-ADF and cellulose as ADF-lignin.

STATISTICAL ANALYSES

Individual data sets were fitted to a number of functions with up to 4 parameters. SAAM 25 (Simulation, Analysis and Modelling) was used to determine which functions best fitted the data (see Wadsworth 1983 for further information). This programme solves the set of simultaneous differential equations which form the model, then by an iterative process, yields least squares estimates of rate constants and pool sizes (Berman and Weiss 1967). Models of increasing complexity (Table 1) were fitted to a' few, representative sets of data and the most appropriate model selected with the aid of a variance ratio test of the improvement in residual sum of squares due to additional parameters. This model was then fitted to the remaining data sets using the simplex procedure of Nelder and Mead (1965), as described by O'Neill (1971), and a PRIME computer.

Treatment effects and interactions were tested by conventional procedures using the GENSTAT V, Version 4.03 computer program (Lawes Agricultural Trust, Rothamstead Experiment Station, U.K.).

TABLE 1 Mathematical models evaluated for simulation of disappearance of digesta by microbial fermentation, where qt is the proportion remaining at time t.

Number of parameters	Equation	Description of model
	Equation 1	
2	$qt = qe^{-kt} + (1-q)$	Single degradable pool with fermentation rate constant k and potential degradability, q.
	Equation 2	
4	$qt = q1e^{-k1t} + q2e^{-k2t} + (1 - (q_1 + q_2))$	2 potentially degradable pools (q_1 and q_2) with fermentation rates k1 and k2.
	Equation 3	
3	$qt = qe^{-kt\ (1-e^{-bt})} + (1-q)$	Potentially degradable pool (q) with fermentation rate constant (k) and lag constant (b, rate of onset of digestion).

RESULTS

Increasing the number of parameters in the equation improved its fit to observed data points, as evidenced by decreasing residual sum of squares. There were no significant improvements in goodness of fit when increasing the number of parameters beyond 3. It was concluded that model 3 (Table 1) best fitted the observed decay data.

The parameter estimates for q and k were well behaved but, in some cases the lag parameter, b, was unstable. This corresponded with data sets where lag effect was not prominent. Estimates of b ranged from 0.06 (showing significant lag) to 3.41 (showing minimal lag).

Equation 3 typically accounted for 99% of the variance about the mean for DM, NDF, ADF and HC, and for more than 98% for C.

The estimates of q, k and b for all data sets were then tested by analysis of variance for differences between chemical fractions, and effects of straw type and alkali treatment.

POTENTIAL DEGRADABILITY (q)

Potential degradabilities (q) of fibre and fibre fractions as influenced by straw type and alkali treatment are summarized in Table 2. There was a significant first order interaction between straw type and analytical fractions.

Potential degradabilities of all fractions excepting C were significantly higher in oat straw (OS) than corresponding fractions in wheat straw 1 (WS1) and wheat straw 2 (WS2). The C fraction of WS1 had the same potential degradibility as that in OS, both of which were significantly higher than C degradability in WS2. Potential degradabilities of HC were highest and of DM lowest and the degradibilities of NDF, ADF and C were not significantly different over all diets.

TABLE 2 - Potential degradability (q) of fibre fractions in oat straw (OS), wheat straw 1 (WS1) and wheat straw 2 (WS2)

Fractions of fibre	Straw			Mean
	OS	WS1	WS2	
DM	0.694	0.600	0.618	0.637
NDF	0.743	0.625	0.648	0.672
ADF	0.718	0.642	0.638	0.666
HC	0.766	0.693	0.690	0.717
C	0.698	0.671	0.614	0.661

Straw types x fibre fraction 5% LSD = 0.035
Between fibre fractions 5% LSD = 0.020

potential degradability (q) of dry matter (DM) and neutral detergent fibre (NDF) in each diet

Alkali	Straw						Mean	
	OS		WS1		WS2			
	DM	NDF	DM	NDF	DM	NDF	DM	NDF
Control	0.63	0.70	0.51	0.55	0.55	0.57	0.56	0.65
Alkali treated	0.76	0.79	0.69	0.70	0.69	0.73	0.67	0.74
Difference	+0.13	+0.09	+0.18	+0.15	+0.14	+0.16	+0.15	+0.13

Straw types x alkali treatments 5% LSD = 0.022
Between alkali treatments 5% LSD = 0.012

Alkali treatment increased the potential degradability of all fractions in all straws but a significant interaction between alkali treatment and base straw was displayed. Alkali treatment tended to increase the potential degradabilities of fractions in oat straw by less than it increased the degradabilities in the two wheat straws.

The degradability of HC was increased by 9% in oat straw compared with 11% and 15% in the two wheat straws, whereas the degradability of C was increased by 14% in oat straw and by 16% and 14% in the two wheat straws following alkali treatment.

FERMENTATION RATES (k)

Fermentation rates are reported on Table 3.

TABLE 3 Fermentation rates (k) of DM and HC in % per hour for each diet, and individual fibre fraction k values as influenced by alkali treatment

	Straw							
	Oat		Wheat 1		Wheat 2		Mean	
Treatment	DM	HC	DM	HC	DM	HC	DM	HC
Control	2.58	3.55	3.55	2.72	3.45	3.63	3.24	2.98
Alkali treated	5.98	6.52	5.30	5.57	5.05	4.85	5.44	5.65
Difference	+3.26	+2.94	+1.75	+2.85	+1.60	+1.22	+2.20	+2.67

Straw types x alkali treatments 5% LSD = 0.61
Between straws 5% LSD = 0.43
Between alkali treatments 5% LSD = 0.35

	DM	NDF	ADF	HC	C
Control	3.2	3.1	3.2	3.0	3.6
Alkali treated	5.4	5.5	5.3	5.6	5.3
Difference	+2.2	+2.4	+2.1	+2.6	+1.7

Fibre fractions x alkali treatments 5% LSD = 0.79
Between fibre fractions 5% LSD = 0.56

Alkali treatment increased fermentation rates of all fractions in all straws and an interaction between straw type and alkali treatment was evident. These effects were highly significant ($P < 0.005$). Fermentation rates in oat straw were increased by 3.3, 3.9 and 1.4%/hr for ADF, HC and C respectively whereas fermentation rates of these fractions were increased by only 2.0, 2.9 and 1.6%/hr in wheat straw 1, and 1.2, 1.2 and 2.0%/hr in wheat straw 2.

HC fermentation rates were increased most (2.6%/hr) and C fermentation rates least (1.7%/hr) following alkali treatment although the interaction between fibre fractions and alkali treatments was not significant.

Fermentation rates of individual fibre fractions averaged across all straws and treatments showed no significant differences, ranging from 4.27%/hr for ADF to 4.44%/hr for C.

LAG PARAMETER (b)

Significant interactions between fibre fraction and straw type, and straw type and alkali treatment were evident in the lag parameter, b, overriding any main effects (Table 4). Onset'of digestion was slow for all fibre fractions within the 2 untreated wheat straws and for C and ADF in OS, but was significantly faster for HC and, hence, NDF in OS. Alakali treatment depressed the high values of b for HC and NDF in OS and increased low valves for WS2 whereas for WSl alkali significantly increased b for C and ADF without altering b for HC and NDF.

TABLE 4 Lag constants (b) for each fibre fraction within each diet

Straw	OS		WSl		WS2		Mean	
Alkali	-	+	-	+	-	+	-	+
DM	0.16	0.15	0.17	0.23	0.20	0.36	0.18	0.25
NDF	1.35	0.11	0.11	0.18	0.16	0.50	0.54	0.26
ADF	0.14	0.13	0.16	1.23	0.14	0.37	0.15	0.58
H	1.43	0.12	0.15	0.18	0.15	1.13	0.57	0.48
C	0.06	0.22	0.64	3.41	0.11	0.18	0.27	1.27

Fibre fractions x straw types 5% LSD = 0.869
Straw types x alkali treatments 5% LSD = 0.549
Fibre fractions x straw types x alkali treatments 5% LSD = 1.228

DISAPPEARANCE OF LIGNIN

The disappearance of lignin from nylon bags (Table 5) in this experiment was unexpected.' Other workers have observed substantial degradation of lignin in vivo and in vitro.

TABLE 5 Acid detergent lignin (ADL) decay curve (percentage of ADL remaining at times 0 - 96 hrs)

Alkali	Time (hrs) 0	3	6	9	12	24	48	72	96
Control	100	104	97.3	98.6	97.8	87.7	80.5	77.8	80.5
Alkali treated	100	109.7	98.8	97.5	100.6	82.5	67.4	62.2	58.4

The percentages of lignin remaining after 96 hrs were 100.8%, 80.0% and 60.6% in untreated WSl, WS2 and OS respectively compared with 67.0%, 62.0% and 46.1% in the alkali treated straws.

DISCUSSION

The best model of fibre fermentation in these low quality roughages involved an undegradable pool (l-q) and a single potentially degradable pool (q) where the potentially degradable fibre was fermented at a fractional rate, k. No evidence supporting the two

potentially degradable pools suggested by Mertens and Ely (1978) and Dunlop and Kellaway (1980) was found. The latter workers used graphical curve peeling techniques as compared with the non-linear least squares techniques used here. Evidence of a lag was found in most data although this appeared to be dependent on the fraction analysed as well as straw type and alkali treatment. Lag constants varied markedly between animals within treatments. The occurence of delays in the onset of fibre digestion during in vitro studies similar to those found here suggests that nylon bags do not significantly impede access of microbes to the fibre contained in them. It is likely, therefore that the large differences in b between animals reflect differences in the microbial ecology of their rumens. Observations of bacteria during fermentation (Monson et al. 1972; Brazle et al. 1979; Cheng et al. 1980) indicate the sites of attack and penetration of bacteria into plant tissue is at exposed edges, ruptured surfaces,-and at stomata. Once phloem and mesophyll tissue are exposed to microbial action, degradation takes place rapidly (Brazle et al. 1979; Harbers and Thouvenille 1980). Because the material placed in the nylon bags in this experiment was coarsley ground, it is likely that the lag represents the process of microbial penetration and exposure of more degradable tissues which must occur for fermentation to attain its maximum rate.

The potential degradability of hemicellulose was highest and of DM lowest although fermentation rates of all fractions were similar.

Alkali treatment increased the potential degradability and rate of degradation of fibre as a whole. There was a trend for alkali treatment to increase cellulose degradability more than other fractions although there were differences between straws in which fractions exhibited the largest increase in degradability.

Thiago (1979) reported potential degradabilities for acid detergent lignin using nylon bags varying from 14% in lucerne to 69% in alkali treated wheatstraws, and fermentation rates of 0.22% to 1.75% per hour. Similarly, Dekker et al (1972) demonstrated apparent digestibilities in nylon bags approaching 50%. Lignin digestibilities in the whole tract of about 20% have been reported on forage diets (Jarrige et al. 1960; Waite et al. 1964) and other workers have reported that lignin is partly digested (Allinson and Osbourne 1970; Minson 1971, Grant et al. 1974; Fahey et al. 1979) although Thiago (1979) and Srikandarajah (1982) both reported faecal recoveries approaching 100%.

Muntifering and co-workers (1981) evaluated the differences in apparent lignin digestibilities due to roughage source and analytical method. They reported several negative digestibility coefficients indicating artifact lignin formation as well as apparent digestiblities of up to 46.8% in the whole tract. They suggested that apparent digestion of lignin may result from formation of soluble lignin-carbohydrate complexes which might not be measured in the fibrous residues of digesta by gravimetric methods and that chemical reagents may partially destroy these soluble carbohydrate esters of phenolic acids. Dekker et al (1972) suggested that as well as some true digestion of lignin, the disappearance of lignin from nylon bags is due to fragmentation of bundle sheaths as a result of the loss of

pectic substances which act as cementing materials. It is possible then for small solid particles produced by this process to pass through the terylene bag and so be apparently digested although not in fact dissolved. If lignin can disappear from nylon bags then it must be assumed that other components can also disappear without being truly digested. Caution must therefore be used in interpreting absolute values for potential degradabilities and fermentation rates as this technique may slightly overestimate both of these parameters.

CONCLUSION

It was concluded from this experiment that' a model with a potentially degradable pool and an undegradable pool best approximated fibre fermentation for cereal straws in the rumen of sheep. The potentially degradable pool followed first order kinetics being degraded at a constant fraction per hour, after a delay. The most appropriate equation to express the process of microbial fermentation for these low quality is equation 3 of Table 1.

Alkali treatment increases both potential degradability and fermentation rates of DM, NDF, ADF, HC and C. The fractions within each straw type responded differently to alkali treatment.

Substantial losses of lignin occurred, from nylon bags which may be the result of true digestion, or just the loss of very small particles of lignin which are not truly digested. This technique may therefore overestimate the potential degradability and fermentation rate of forages.

The fact that all fibre fractions behaved similarly lends strong support to the two pool model.

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