Onderstepoort J. Vet. Res. (1964) 31 (1) 69–76
Printed in the Republic of S. Afr. by the Government Printer, Pretoria

AN IMPROVED TECHNIQUE FOR MEASURING THE *IN VIVO* RATE OF CELLULOSE DIGESTION IN THE RUMEN

J. F. W. GROSSKOPF Veterinary Research Institute Onderstepoort

INTRODUCTION

Ruminant animals are dependent on the ability of their ruminal micro-organisms to break down cellulose to absorbable nutrients, such as short-chain fatty acids. The rate of cellulose digestion may be influenced by various other constituents in the feed and may even vary in the same animal on constant feed from day to day. As far as is known no simple accurate method for the daily determination of the rate of cellulose digestion in experimental animals has been described. Hoflund Quin & Clark (1948) described a simple method for the *in vivo* measurement of cellulose disintegration but it has the disadvantage of subjective interpretation and it cannot be used for daily determinations.

The method described in this paper is based on the above technique, but instead of estimating the breaking strength of the cotton threads after suspension in the rumen, the actual loss of weight of the cotton is determined.

PROCEDURE

Eight Merino sheep were fitted with permanent perspex rumen fistula tubes with an internal diameter of 18 mm. Four of these sheep (No. 1, 4, 5 and 8) were fed on lucerne hay *ad lib.*, two (No. 2 and 3) on teff hay *ad lib.* plus 15 gm of biuret daily, and the remaining two (No. 6 and 7) on teff hay *ad lib.* The food and water consumptions of each sheep were recorded daily. The lucerne hay had a protein content of about 15 per cent while that of the teff (*Eragrostis abyssinica*) was approximately 7·5 per cent.

The cellulose substrate used was a good grade of sewing cotton. A strand of approximately 100 cm of standard non-glazed six cord No. 10 white cotton was used as one unit. The strands were soaked in water, rinsed and then dried in an oven at 80° C for 24 hours. As it was intended to put as many as 18 of these units into one sheep's rumen simultaneously, all 18 strands had to be of equal weight.

The dried strands absorbed moisture too rapidly to allow of adjustment to the same dried weight. The weights were therefore corrected to within 0·1 mg at atmospheric humidity, and the dried weights, which were usually found not to vary more than 0·2 mg either way, checked later. The weight of each strand unit, weighed to the nearest 0·1 mg within 15 seconds after being removed from the drying oven, was regarded as its initial weight.

Received for publication on 29 August, 1963.—Editor

Each strand was then folded on itself three times to form a loop, approximately 12·5 cm long. This loop was attached by means of a strong silk thread to a rubber or plastic covered rod, approximately 20 cm long (Fig. 1). By inserting this rod through the fistula tube, the end carrying the cotton loop could be kept deeply plunged in the ruminal mass while the cork stopper simultaneously closed the fistula opening and held the rod in position.

FIG. 1.—A rod with cotton loop attached.

For the purpose of this experiment, 12 loops were attached individually to each rod intended for the lucerne-fed sheep and 18 loops for each of the sheep on the teff hay ration. A pilot experiment indicated that no measurable loss of weight occurred in the cotton threads during the first 13 or 14 hours in the rumen. In this experiment the first cotton loops were therefore removed 15 hours after insertion, simply by cutting off the silk thread by which they were suspended. Subsequently loops were removed hourly till the twentieth hour and thereafter two-hourly and later at three-hourly intervals.

After removal, the cotton loops were first rinsed under running water, then soaked in water for about an hour and subsequently carefully rolled between the fingers under water to remove all foreign particles and as much of the colouring matter as possible. They were then left in a drying oven at 80° C for 24 hours and weighed within 15 seconds after removal from the oven.

RESULTS

When the loss in weight of the cotton loops, expressed as a percentage of the initial weight, was plotted against time the graphs shown in Figures 2 and 3 were obtained.

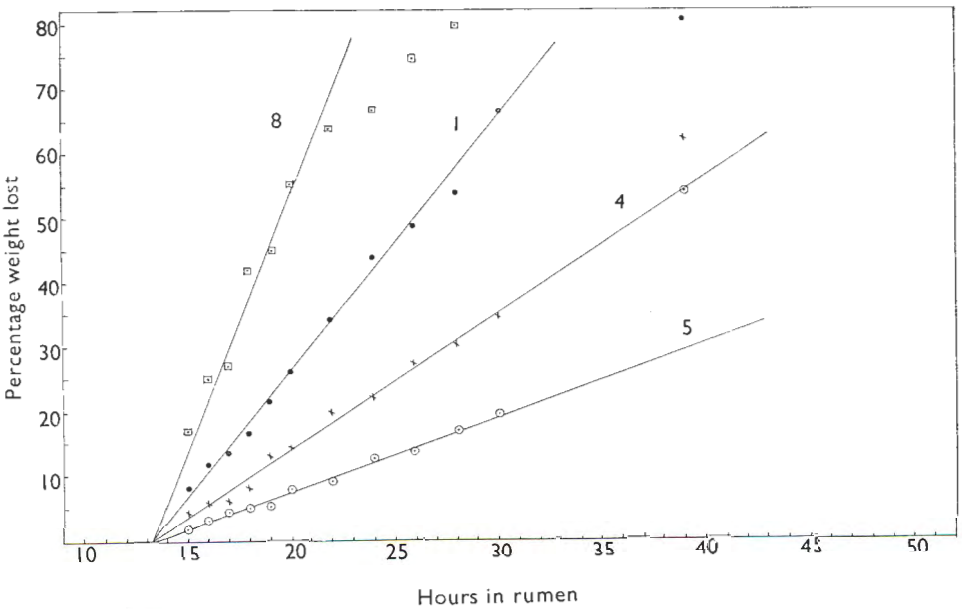

FIG. 2.—The rate of the loss of weight of cotton strands suspended in the rumens of sheep on lucerne hay ration.

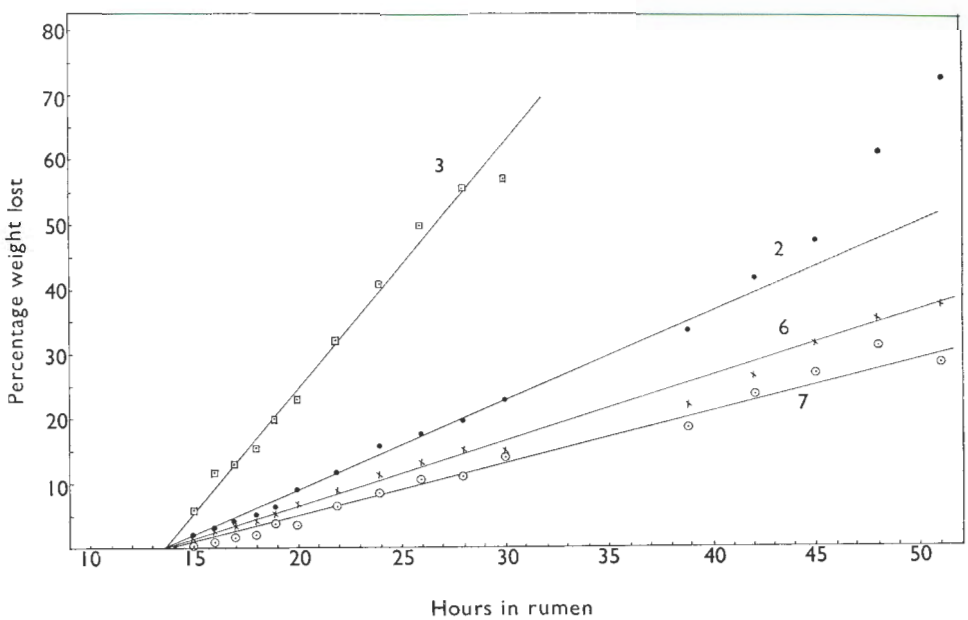

FIG. 3.—The rate of the loss of weight of cotton strands (expressed as a percentage of original weight) when suspended in the rumens of sheep on a teff hay ration. Sheep No. 2 and 3 received 15 gm of biuret, in addition, daily.

The actual weights of all the cotton loops are given in Table 1.

TABLE 1.—*The recorded weight losses of cotton loops in the rumens of sheep*

Hours in rumen	Sheep 1				Sheep 2				Sheep 3				Sheep 4			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	mg	mg	mg	%	mg	mg	mg	%	mg	mg	mg	%	mg	mg	mg	%
15	96·4	88·6	7·8	8·1	95·2	93·2	2·0	2·1	94·5	88·8	5·7	6·0	99·1	94·9	4·2	4·2
16	96·4	85·1	11·3	11·7	95·2	92·3	2·9	3·1	94·5	83·3	11·2	11·9	99·1	93·7	5·4	5·4
17	96·4	83·5	12·9	13·4	95·2	91·0	4·2	4·4	94·5	82·1	12·4	13·1	99·1	93·6	5·5	5·5
18	96·4	80·6	15·8	16·4	95·2	90·3	4·9	5·2	94·5	79·9	14·6	15·5	99·1	91·1	8·0	8·1
19	96·4	75·8	20·6	21·4	95·2	89·1	6·1	6·4	94·5	75·6	18·9	20·0	99·1	86·2	12·9	13·0
20	96·4	71·3	25·1	26·0	95·2	87·6	7·6	8·0	94·5	71·8	22·7	24·0	99·1	84·9	14·2	14·3
22	96·4	63·3	33·1	34·3	95·2	84·1	11·1	11·7	94·5	64·0	30·5	32·3	99·1	79·5	19·6	19·8
24	96·4	54·4	42·0	43·6	95·2	80·2	15·0	15·8	94·5	56·0	38·5	40·8	99·1	77·3	21·8	22·0
26	96·4	49·6	46·8	48·5	95·2	76·8	18·4	17·5	94·5	47·4	47·1	49·8	99·1	71·8	27·3	27·6
28	96·4	44·7	51·7	53·6	95·2	76·3	18·9	19·7	94·5	42·0	52·5	55·5	99·1	69·2	29·9	30·2
30	96·4	32·6	63·8	66·1	95·2	73·5	21·7	22·8	94·5	40·6	53·9	57·0	99·1	64·9	34·2	34·5
39	96·4	19·2	77·2	80·0	95·2	63·2	32·0	33·6	94·5	Free ends lost			99·1	37·9	61·2	61·7
42	—	—	—	—	95·2	55·7	39·5	41·5	94·5	Free ends lost			—	—	—	—
45	—	—	—	—	95·2	50·3	44·9	47·2	94·5	Free ends lost			—	—	—	—
48	—	—	—	—	95·2	37·3	57·9	60·8	94·5	Disintegrated			—	—	—	—
51	—	—	—	—	95·2	26·3	68·9	72·4	94·5	Disintegrated			—	—	—	—

A—Initial weight of cotton loops suspended in rumen (in mg)
B—Final weight of cotton loops removed from rumen (in mg)
C—Weight lost by cotton loops during suspension in rumen (i.e. A—B)
D—Weight lost as a percentage of initial weight

TABLE 1.—*The recorded weight losses of cotton loops in the rumens of sheep* (cont.)

Hours in rumen	Sheep 5				Sheep 6				Sheep 7				Sheep 8			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
	mg	mg	mg	%	mg	mg	mg	%	mg	mg	mg	%	mg	mg	mg	%
15	93·8	92·0	1·8	1·9	92·8	91·0	1·8	1·9	94·5	94·0	0·5	0·5	96·1	79·7	16·4	17·1
16	93·8	90·9	2·9	3·1	92·8	90·1	2·7	2·9	94·5	93·3	1·2	1·3	96·1	72·1	24·0	25·0
17	93·8	89·5	4·3	4·6	92·8	89·7	3·1	3·3	94·5	92·8	1·7	1·8	95·2	70·7	24·5	25·8
18	93·8	89·2	4·6	4·9	92·8	89·0	3·8	4·1	94·5	92·3	2·2	2·3	96·1	55·7	40·4	42·0
19	93·8	88·4	5·4	5·7	92·8	88·1	4·7	5·1	94·5	90·9	3·6	3·8	95·2	52·4	42·8	45·0
20	92·2	84·8	7·4	8·0	92·8	86·7	6·1	6·6	94·5	91·2	3·3	3·5	96·1	43·2	52·9	55·0
22	92·2	83·8	8·4	9·1	92·8	84·6	8·2	8·8	94·5	88·4	6·1	6·5	96·1	34·7	61·4	63·9
24	92·2	80·7	11·5	12·5	92·8	82·4	10·4	11·2	94·5	86·5	8·0	8·5	96·1	32·5	63·6	66·1
26	92·2	79·8	12·4	13·6	92·8	79·8	12·1	13·0	94·5	84·5	10·0	10·6	95·2	24·5	70·7	74·3
28	92·2	76·5	15·7	17·0	92·8	78·9	13·9	15·0	94·5	84·0	10·5	11·1	95·2	19·5	75·7	79·5
30	92·2	74·4	17·8	19·5	92·8	78·9	13·9	15·0	94·5	81·3	13·2	14·0	95·2	22·2	73·0	76·7
39	93·8	51·2	41·0	43·7	92·8	72·3	20·5	22·1	94·5	77·0	17·5	18·5	95·2	Disintegrated		
42	—	—	—	—	92·8	68·5	24·3	26·2	94·5	72·3	22·2	23·5	—	—	—	—
45	—	—	—	—	92·8	64·3	28·5	31·7	94·5	70·0	24·5	27·0	—	—	—	—
48	—	—	—	—	92·8	60·1	32·7	35·2	94·5	65·2	29·3	31·0	—	—	—	—
51	—	—	—	—	92·8	58·9	33·9	36·6	94·5	67·9	26·6	28·2	—	—	—	—

As can be seen, the points plotted for each sheep can be represented by a straight line. This linear relationship, however, does not hold after some 60 per cent weight loss has occurred, probably owing to breaking and loss of pieces of thread. Some results after more than 36 hours immersion were also found to be inconsistent. The errors as determined from the graphs within these limits are given in Table 2.

TABLE 2.—*Mean and maximum errors as determined from Figures* 1 *and* 2

Sheep No.	Mean error as per cent of initial weight	Maximum error as per cent of initial weight
1	1·25	4·25
2	0·50	1·20
3	1·43	5·50
4	0·80	1·75
5	0·41	1·00
6	0·45	1·50
7	0·39	1·25
8	2·75	4·75

Overall mean error 1·00 per cent.

From these and similar graphs the time taken for the cotton threads to lose half their initial weight can quite easily be estimated.

It has been found that the rate of cellulose breakdown may vary in the same sheep on a constant diet from time to time as can be seen from Table 3.

TABLE 3.—*Comparison between feed intake and digestion of cotton cellulose*

Sheep No.	Average daily hay consumption over seven days		Estimated time taken for cotton loops to lose 50 per cent of initial weight in rumen	
	22–28 April	29 Apr.–5 May	24–25 April	1–2 May
	gm	gm	hours	hours
4 (lucerne)	1,376	1,306	26·8	37·5
5 (lucerne)	1,046	964	28·0	57·0
6 (teff)	887	776	48·9	64·1
7 (teff)	701	830	112·6	73·6

DISCUSSION

When the percentage loss of weight of cotton loops suspended in the rumen of any particular sheep, is plotted against time, a straight line is found in every case. The slope of this line then represents the rate of breakdown of cotton cellulose in the rumen during that period. By using this method the rate of cellulose digestion in different sheep can be compared or studied in the same sheep from day to day.

As can be seen from Figures 2 and 3 and from Table 2, the results can be reasonably accurate provided the same brand of cotton (and preferably the same batch) is used throughout an experiment and provided the manipulations of the cotton loops are standardized. The accuracy of the determination was further tested by removing two and three cotton loops simultaneously from the same rumen after some degree of digestion had taken place. The percentage weight loss of the two loops removed together were 25·3 and 25·5 per cent respectively, while those of the three loops removed together were 39·2, 41·0 and 41·6 per cent respectively.

It is suggested that this rate of cellulose digestion be expressed as the estimated time taken for a cotton loop to lose half its weight during suspension in the rumen. The figures can be read from a graph. In those cases where extrapolation of the graph will be found necessary, the time will be so long that an error of even a few hours would be of little significance.

The similar lag periods encountered with the various rates of digestion were unexpected. At this stage no explanation for it can be given.

From this experiment it is obvious that the rate of cotton cellulose digestion is influenced by the type of ration.

The technique has also been successfully applied in a young bovine.

Summary

A practical method for the determination and expression of the rate of cellulose digestion in the rumen is described.

Acknowledgement

The author wishes to express his thanks to Prof. R. Clark for valuable advice during the preparation of this article.

Reference

HOFLUND, S., *QUIN*, J. I. & CLARK, R., 1948. Studies on the alimentary tract of Merino sheep in South Africa XV. The influence of different factors on the rate of cellulose digestion (*a*) in the rumen and (*b*) in ruminal ingesta as studied *in vitro*. *Onderstepoort J. Vet. Sci. Anim. Ind.* 23, 395–409.