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PRACTICAL IMPLICATIONS OF THE EFFECT OF DRYING AND TREATMENT ON NITROGEN CONTENT AND *IN VITRO* DIGESTIBILITY OF SAMPLES COLLECTED BY OESOPHAGEALLY FISTULATED ANIMALS

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(Sleutelwoorde: *Slukdermgefistuleerde diere, stikstofinhoud, verteerbaarheid, drogingsprosedure, monstervoorbereiding)*

OPSOMMING: PRAKTIESE IMPLIKASIES VAN DIE INVLOED VAN DROGING EN BEHANDELING OP STIKSTOFINHOUD EN *IN VITRO* VERTEERBAARHEID VAN MONSTERS VERSAMEL DEUR SLUKDERMGEFISTULEERDE DIERE

Slukdermgefistuleerde skape en beeste is gebruik om verteenwoordigende monsters van 7 groenvoere tydens verteringsproewe te versamel. Hierdie monsters is op verskillende maniere behandel en gedroog. In die geval van skape het behandeling van die monsters (totaal versamelde monsters vs. soliede fraksie van die monster) 'n statistiese betekenisvolle invloed ($P \leqslant 0,05$) op stikstofinhoud by slegs 2 van die 7 voere gehad. By beeste het die behandeling van monsters geen invloed op stikstofinhoud gehad nie alhoewel konstant hoër waardes in die totaal versamelde monsters voorgekom het. 'n Hoogsbetekenisvolle ($P \leqslant 0,01$) positiewe verwantskap tussen stikstofinhoud in die totaal versamelde monster en in die soliede fraksie is gevind. Droging van die totale monster versamel deur slukdermgefistuleerde diere by 'n temperatuur van 50°C, het 'n betekenisvolle ($P \leqslant 0,05$) neerdrukkende invloed op *in vitro* verteerbaarheid gehad. Regressievergelykings wat die verwantskap tussen *in vitro* verteerbaarheid van monsters versamel deur slukdermgefistuleerde diere en *in vivo* verteerbaarheid van die 7 voere beskryf, is bereken. Ten spyte van verskille ten opsigte van behandeling en droging van hierdie tipe monsters, is 'n goeie verwantskap tussen *in vitro* en *in vivo* verteerbaarheid deurgaans gevind. Verskillende regressievergelykings word vir skaap en bees aangetoon. Die implikasie van die resultate met verwysing na die gebruik van slukdermgefistuleerde diere en beskikbare drogingsfasiliteite in die laboratorium, word bespreek.

SUMMARY:

Oesophageally fistulated (OF) sheep and cattle were used to collect samples from 7 green herbages used in digestibility trials. These samples were treated and dried in different ways. In the case of sheep the treatment of OF-collected samples (total extrusa vs. solid fraction) had a significant effect ($P \leqslant 0,05$) on nitrogen content in only 2 of the 7 forages. With cattle, treatment of sample did not influence nitrogen content significantly ($P \leqslant 0,05$). However, a consistently higher nitrogen content was found in the total extrusa. A highly significant ($P \leqslant 0,01$) positive relationship between nitrogen content in total extrusa and the solid fraction of OF-collected samples was found. Drying of total extrusa at a temperature of 50°C had a significant ($P \leqslant 0,05$) depressing effect on *in vitro* digestibility. Regression equations describing the relationship between *in vitro* digestibility of OF-collected samples and *in vivo* digestibility, were derived. Irrespective of sample treatment or drying procedure, a close relationship between *in vitro* and *in vivo* digestibility, was found. Different equations for sheep and cattle are given. The implication of the results with regard to the use of OF-animals and availability of laboratory drying equipment, is discussed.

Oesophageally fistulated sheep and cattle are widely used to study the nutritive value of pastures. The samples collected by these animals are used for studying the chemical composition and digestibility of the herbage ingested by grazing animals. In this case the digestibility of the pasture is estimated by application of appropriate regression equations describing the relationship between *in vivo* and *in vitro* digestibility. When both digestibility and faeces output per unit of time are known, the feed intake of the grazing animal can be estimated. Therefore, it is evident that the *in vitro* digestibility of samples collected by oesophageally fistulated animals, is basic to feed-intake studies with the grazing animal.

It has been demonstrated that the drying of samples in ovens has a depressing effect on *in vitro* digestibility of plant material at temperatures normally prescribed for this purpose (Simkens & Baumgardt, 1963; Danley & Vetter, 1971; Cronje, 1973). Apparently this is caused by the condensation of carbohydrates and proteins via the non-enzymatic browning reaction (Hodge, 1953). According to Van Soest (1965) the presence of moisture will increase this reaction. Since samples collected by oesophageally fistulated animals are high in moisture content due to salivary contamination, decreased *in vitro* digestibilities as a result of oven drying, are to be expected.

This paper reports the results of an experiment in which a number of digestibility trials were carried out with sheep and cattle. Oesophageally fistulated animals were used to obtain samples representing the feed ingested by the animals in the digestibility trials. These extrusa samples were treated and dried in different ways. The object was to determine whether this would have an effect on nitrogen content and *in vitro* digestibility of these samples.

Experimental Procedure

Six Merino wethers and 4 Simmentaler steers were used to determine the digestibility of the following 7 green herbages: –

Sorghum bicolor (c. Haygrazer)	– flowering stage
Cenchrus ciliaris (c. Molopo)	– flowering stage
Medicago sativa (S.A. standard)	– young leafy, well before flowering
Cenchrus ciliaris (c. Molopo)	– mature and damaged by frost
Avena byzantina (c. Langgewens)	– soft dough stage
Medicago sativa (S.A. standard)	– young leafy, well before flowering
Sorghum bicolor (c. Trudan II)	– flowering stage

Each feed was fed to the animals for a period of 19 days – the last 9 days being used for measuring intake and total collection of faeces. All herbages were cut daily at 07h00 and chopped mechanically into approximately 2 cm lengths. An *ad lib.* level of feeding was used. The mass of the refusals of each animal was recorded and samples were taken daily and pooled for individual animals for the 9 day period.

Three Merino wethers and 3 Friesian steers fitted with oesophageal fisulae (OF), were used for the collection of samples representing the feed ingested by the animals in the digestibility trial. This collection took place in the morning when offering fresh feed to the animals after a fasting period of 2 hours. In order to avoid any loss of the liquid fraction polythene bags were fitted inside canvas bags designed for this sampling process. Samples containing regurgitated material or excessive quantities of saliva were discarded and the animal concerned was used immediately for the collection of another sample. Samples were also collected manually each day from the chopped feed to represent the conventional manner of sampling in digestibility trials.

During each digestibility trial the samples collected were treated and dried as follows:

(i) Samples collected manually were dried at either 50°C or 100°C in a force draught oven.

(ii) Samples collected by OF-animals (total extrusa) were either freeze dried or dried at 50°C in a force draught oven.

(iii) Samples collected by OF-animals were squeezed through 4 layers of cheese cloth, the liquid fraction discarded and the solid fraction was either freeze dried or dried at 50°C in a force draught oven.

The allocation of the 3 OF-animals of each species in the sampling procedure during the 9 day period, together with the treatment and drying procedure for each sample, was as follows:

	Total extrusa		Solid fraction of extrusa*	
Day	50°C	Freeze dried	50°C	Freeze dried
1	A	B	C	C
2	B	C	A	A
3	C	A	B	B
4	A	B	C	C
5	B	C	A	A
6	C	A	B	B
7	A	B	C	C
8	B	C	A	A
9	C	A	B	B

(A, B, C: identification symbols for the 3 different OF-animals; *Solid fraction of extrusa came from the same animal within a day, split into 2 subsamples and allocated to the 50°C or the freeze drying process).

All samples allocated to oven drying were dried immediately after sampling. The samples destined for freeze drying were kept at a temperature of –15°C before they were dried according to the capacity of the freeze drier.

Immediately after drying, all samples were milled through a Wiley mill with a 1 mm screen and stored in screw cap glass containers before being analyzed.

The two-stage technique of Tilley & Terry (1963) with slight modifications described by Engels & van der Merwe (1967) was used for the determination of *in vitro* digestibility. Nitrogen content was determined by means of the macro Kjeldahl method.

Multiple comparison procedures (Tukey's method) as described by Snedecor (1956) were adopted.

Results

The influence of sampling method, sample treatment and drying procedure on nitrogen content and in vitro *digestibility*

The nitrogen content of the feed samples collected, treated and dried in different ways, is presented in Table 1.

Table 1

The nitrogen content of feed samples collected, treated and dried in different ways

Feed	Feed samples collected by:										
	Oesophageally fistulated sheep				Oesophageally fistulated cattle				Hand		
	Total extrusa		Solid fraction		Total extrusa		Solid fraction				
	50°C	Freeze dried	50°C	Freeze dried	50°C	Freeze dried	50°C	Freeze dried	100°C	50°C	Freeze dried
	%	%	%	%	%	%	%	%	%	%	%
Sorghum (Hay-grazer)	1,73	1,64	1,63	1,67	1,14	1,18	1,06	1,07	0,94	1,07	1,08
C. ciliaris	1,16	1,12	0,85	0,89	0,93	1,01	0,81	0,81	0,86	0,90	0,88
Lucerne	4,50	4,23	3,79	3,86	3,81	3,76	3,49	3,44	3,22	3,61	3,36
C. ciliaris	0,95	0,83	0,85	0,80	0,75	0,68	0,67	0,66	0,67	0,66	0,66
Oats (*A. byzantina*)	2,10	2,02	1,57	1,57	1,84	1,82	1,50	1,49	1,75	1,78	1,72
Lucerne	4,20	4,45	3,75	3,77	3,76	3,71	3,39	3,42	3,43	3,69	3,36
Sorghum (Trudan II)	2,63	2,46	2,46	2,30	2,13	1,97	2,04	1,91	1,98	1,95	2,06

LSD – Tukey ($P \leqslant 0,05$) = 0,4978

Table 2

The in vitro *digestibility of organic matter (IVDOM) of feed samples collected, treated and dried in different ways*

Feed	Feed samples collected by:										
	Oesophageally fistulated sheep				Oesophageally fistulated cattle				Hand		
	Total extrusa		Solid fraction		Total extrusa		Solid fraction				
	50°C	Freeze dried	50°C	Freeze dried	50°C	Freeze dried	50°C	Freeze dried	100°C	50°C	Freeze dried
	%	%	%	%	%	%	%	%	%	%	%
Sorghum (Hay-grazer)	58,1	63,4	58,4	57,6	55,7	62,0	58,3	54,1	60,1	61,4	61,7
C. ciliaris	54,9	57,6	53,5	53,1	50,9	55,3	51,3	53,3	52,1	52,2	52,6
Lucerne	62,5	69,4	63,7	66,8	59,0	64,1	61,1	61,5	67,1	68,2	68,9
C. ciliaris	48,5	46,7	43,5	45,4	44,1	52,9	42,7	41,7	42,0	41,6	41,6
Oats (*A. byzantina*)	62,4	73,4	67,6	71,0	64,8	73,7	67,0	70,0	63,0	68,0	67,5
Lucerne	60,1	69,3	63,4	65,5	55,6	65,0	58,7	63,1	61,9	64,1	64,8
Sorghum (Trudan II)	63,6	68,2	65,9	66,8	63,9	67,0	65,0	65,7	64,1	65,0	65,6

LSD – Tukey ($P \leqslant 0,05$) = 5,9552

Table 3

Regression equations describing the relationship between nitrogen content of 7 feed samples collected, treated and dried in different ways

Dependent variable (Y)	Independent variable (X)	r	Regression equation	$S_{y.x}$
N-content of freeze dried OF-extrusa (sheep)	N-content of solid fraction of OF-extrusa (sheep) dried at 50°C	0,985	Y = 0,0170 + 1,1307 X ..... (1)	± 0,2441
N-content of freeze dried OF-extrusa (cattle)	N-content of solid fraction of OF-extrusa (cattle) dried at 50°C	0,990	Y = 0,0656 + 1,0559 X ..... (2)	± 0,1712
N-content of freeze dried OF-extrusa (sheep)	N-content of freeze dried OF-extrusa (cattle)	0,991	Y = 0,1053 + 1,1323 X ..... (3)	± 0,1931
N-content of freeze dried OF-extrusa (sheep)	N-content of feed samples collected manually and dried at 50°C	0,985	Y = 0,1881 + 1,1288 X .....(4)	± 0,2435
N-content of freeze dried OF-extrusa (cattle)	N-content of feed samples collected manually and dried at 50°C	0,983	Y = 0,0949 + 0,9858 X ..... (5)	± 0,2241
N-content of freeze dried feed samples collected manually	N-content of feed samples collected manually and dried at 50°C	0,993	Y = 0,1402 + 0,8884 X ..... (6)	± 0,1269

According to the results in Table 1, method of sampling had an effect on the nitrogen content of the samples. Although the nitrogen content of the samples collected by OF-sheep was consistently higher than those collected by cattle, the differences were not statistically significant ($P \leqslant 0,05$). The nitrogen content of the samples collected by the OF-cattle exceeded that of samples collected manually.

Treatment of samples (total extrusa vs. solid fraction) had a statistically significant ($P \leqslant 0,05$) effect on nitrogen content of the lucerne and oats collected by the OF-sheep only. Although the differences in nitrogen content of total extrusa and the solid fraction of cattle samples were not statistically significant, the values were consistently higher in the total extrusa.

Drying procedure (50°C vs. freeze drying) had an influence on the observed nitrogen content of the total extrusa collected by sheep. However, the differences were not statistically significant ($P \leqslant 0,05$). In all other samples, the influence of the drying procedure on nitrogen content was negligible.

The *in vitro* digestibility of organic matter (IVDOM) of the feed samples collected, treated and dried in different ways, are given in Table 2.

The differences in IVDOM as a result of method of sampling and sample treatment, were not statistically significant ($P \leqslant 0,05$). However, drying procedure had a significant effect on IVDOM in 3 of the 7 feeds where total extrusa was collected by OF-sheep. Statistically significant ($P \leqslant 0,05$) differences in IVDOM as a result of drying procedure, were found in total extrusa of both sheep and cattle. Drying of the total extrusa at a temperature of 50°C in comparison with freeze drying, had a pronounced depressing effect on IVDOM.

Freeze drying of OF-collected extrusa appears to be ideal since changes in chemical composition as a result of drying, are almost eliminated. However, freeze drying facilities are expensive and not always available. Therefore, conventional drying in ovens is a practical alternative. Drying of OF-collected extrusa in ovens, creates problems due to the high moisture content of green feeds, together with the saliva secreted during the pro-

Table 4

The average daily dry matter (DM) intake and digestibility of organic matter (OM) of the 7 different forages by sheep and cattle

Forage	Digestibility of OM (%)		DM intake (g/kg W)	
	Sheep	Cattle	Sheep	Cattle
Sorghum (Haygrazer)	63,1[a]	68,0[b]	23,6	20,9
C. ciliaris	57,9[a]	60,0[a]	22,6	21,6
Lucerne	67,6[a]	61,7[b]	27,2	25,2
C. ciliaris	38,2[a]	50,6[b]	17,4	18,8
Oats (*A. byzantina*)	67,9[a]	71,1[a]	27,8	23,6
Lucerne	66,3[a]	67,2[a]	29,3	27,8
Sorghum (Trudan II)	69,7[a]	75,3[b]	28,8	27,7

a, b: Means within the same line bearing the same superscript do not differ significantly ($P \leqslant 0,05$)

cess of sampling. The squeezing of OF-collected extrusa through cheese cloth will cause a sharp drop in moisture content, but some of the soluble nitrogen may be lost in the liquid fraction. Therefore, the possibility of a close relationship between the nitrogen content in freeze dried OF-collected extrusa and the nitrogen content of the solid fraction, was investigated. Some of the correlation coefficients and regression equations which could possibly be applied in practice, are presented in Table 3.

It is evident from Table 3 that a close relationship exists between the nitrogen content of freeze dried extrusa and the nitrogen content of both the solid fraction and manually collected samples dried at 50°C.

The influence of sampling method, sample treatment and drying procedure on the in vivo/in vitro *digestibility relationship*

The average daily voluntary feed intake and digestibility of organic matter by sheep and cattle are presented in Table 4.

The results in Table 4 indicate that in 3 of the forages, significantly higher ($P \leqslant 0,05$) digestibilities were obtained with cattle in comparison with sheep. However, the sheep digested one of the lucerne herbages significantly better ($P \leqslant 0,05$) than cattle. The differences between cattle and sheep, with regard to feed intake per unit body size, were not significant ($P \leqslant 0,05$).

The regression equations describing the relationship between IVDOM of feed samples collected, treated and dried in different ways and the *in vivo* digestibility of OM determined with sheep and cattle are presented in Table 5.

A close relationship between IVDOM of all feed samples collected, treated and dried differently and the *in vivo* digestibility of the different feeds, as determined with sheep and cattle, was found.

Discussion

The present study was aimed at quantifying the influence of method of sampling, sample treatment and drying procedure when using OF-collected samples for measuring nitrogen content and *in vitro* digestibility. Several studies reported that the differences in nitrogen content between prehended herbage and total extrusa, were not statistically significant (Lesperance, Bohman & Marble, 1960; Barth, Chandler, Fryer & Wang, 1970; Barth & Kazzal, 1971; Little, 1972; Wallace, Hyder & Van Dyne, 1972). Cohen (1979) found that the regression equation describing the relationship between nitrogen content in prehended herbage and total extrusa differed significantly from linearity when including herbage with a nitrogen content greater than 2,74 g N/100 g OM.

In the present study the nitrogen content of freeze dried total extrusa was used as a basis for reference. Method of sampling had an effect on the nitrogen content of samples (Table 1). Although these differences were not statistically significant ($P \leqslant 0,05$), the nitrogen content of the samples collected by OF-sheep was consistently higher than those collected by cattle. The nitrogen content of the samples collected by cattle exceeded those collected manually. Engels & Malan (1975) found highly significant differences between the nitrogen content of samples collected by OF-sheep and cattle and manually on native pasture. In the present study the possibility of selecting a diet with a higher nitrogen content was rather limited since the herbages were offered to the animals in a chopped form.

The nitrogen content of freeze dried total extrusa of sheep was significantly greater than that in the freeze dried solid fraction of samples originating from the one lucerne herbage only. All other differences in nitrogen content between freeze dried total extrusa and the solid fraction for both sheep and cattle were not significant. However, the nitrogen content in freeze dried total extrusa consistently exceeded that in the solid fraction. The relationship between nitrogen content in freeze dried total extrusa and nitrogen content of the solid fraction dried at 50°C, was found to be linear (Table 3). Therefore, the nitrogen content of total extrusa could be predicted accurately for both sheep and cattle from nitrogen content of the solid fraction. The appropriate regression equations are given in Table 3. Furthermore it is evident from Table 3 that the relationship between nitrogen content of manually collected samples and nitrogen content of the solid fraction of

Table 5

Regression equations describing the relationship between in vitro *digestibility of organic matter (IVDOM) of feed samples collected, treated and dried in different ways and the* in vivo *digestibility of organic matter determined with sheep and cattle*

Dependent variable (Y)	Independent variable (X)	r	Regression equation	$S_{y.x}$
In vivo digestibility of organic matter determined with sheep	IVDOM of OF-extrusa (sheep) dried at 50°C	0,875	Y = −53,4664 + 1,9640 X....(7) n = 7	±2,7278
do	IVDOM of freeze dried OF-extrusa (sheep)	0,956	Y = −11,9725 + 1,1483 X....(8) n = 7	±3,5511
do	IVDOM of solid fraction of OF-extrusa (sheep) dried at 50°C	0,965	Y = −12,8344 + 1,2513 X....(9) n = 7	±3,1463
do	IVDOM of the freeze dried solid fraction of OF-extrusa (sheep)	0,916	Y = −5,4214 + 1,0996 X..(10) n = 7	±4,8276
do	IVDOM of feed samples collected manually and dried at 50°C	0,961	Y = −3,4236 + 1,0755 X..(11) n = 7	±3,3319
do	IVDOM of feed samples collected manually and dried at 100°C	0,965	Y = −10,1754 + 1,2232 X..(12) n = 7	±3,1589
do	IVDOM of freeze dried feed samples collected manually	0,965	Y = −3,4209 + 1,0813 X..(13) n = 7	±3,1631
In vivo digestibility of organic matter determined with cattle	IVDOM of OF-extrusa (cattle) dried at 50°C	0,901	Y = 7,7169 + 1,0148 X..(14) n = 7	±3,8737
do	IVDOM of freeze dried OF-extrusa (cattle)	0,892	Y = 19,4645 + 0,7387 X..(15) n = 7	±4,0536
do	IVDOM of solid fraction of OF-extrusa (cattle) dried at 50°C	0,911	Y = 13,4471 + 0,8903 X..(16) n = 7	±3,6979
do	IVDOM of freeze dried solid fraction of OF-extrusa (cattle)	0,857	Y = 21,8325 + 0,7354 X..(17) n = 7	±4,6179
do	IVDOM of feed samples collected manually and dried at 50°C	0,814	Y = 24,0022 + 0,6799 X..(18) n = 7	±5,1926
do	IVDOM of feed samples collected manually and dried at 100°C	0,796	Y = 20,9658 + 0,7485 X..(19) n = 7	±5,4177
do	IVDOM of freeze dried feed samples collected manually	0,813	Y = 24,3595 + 0,6680 X..(20)	±5,1703

OF-collected samples dried at 50°C, was also linear (Equations 4 & 5, Table 3).

Drying procedure did not have a statistically significant effect on nitrogen content of the samples. This is in agreement with the results of Du Toit (unplublished) obtained at this Institute. However, it was found by Du Toit that the pepsin digestible fraction of OF-collected samples was adversely affected by oven drying at both 50°C and 100°C. The results of the present study showed that there was a very good relationship between the nitrogen content in total extrusa of OF-sheep and cattle (Table 3, Equation 3). However, the chopped herbages did not really allow the sheep to exploit their full preference for certain parts of plants. Therefore, it is doubtful whether this relationship would still exist under free grazing conditions on a mixed type of pasture. Under these conditions, sheep tend to graze more selectively than cattle, but unfortunately the superiority in this respect is inconsistent (Engels and Malan, 1975).

The drying of total extrusa at a temperature of 50°C in an oven, had a significant depressing effect on IVDOM. In the case of the solid fraction of OF-collected samples, freeze drying was no longer superior to oven drying at 50°C with regard to IVDOM. This is in complete agreement with the results of Simkins & Baumgardt (1963), Danley & Vetter (1971) and Cronje (1973). According to Hodge (1953) this depressed IVDOM is caused by an increase in the lignin content of the sample as a result of the condensation of the carbohydrate degradation products with protein and amino acids via the non-enzymatic browning reaction. Van Soest (1965) is of the opinion that the presence of moisture increases the rate of this reaction. Smith, Lesperance & Bohman (1967) found that the lignin content of plant material, with a high moisture content, such as green chopped herbages, rumen contents and faeces, increased by 11,5; 53,2 and 28,8%, respectively.

The digestive capacity of sheep and cattle is assumed to be similar (Forbes & Garrigus, 1950). Engels, Ferreira, Nieman & Swart (1978) found that differences between sheep and cattle with regard to digestive capacity were in fact significant with some forages. This finding is substantiated by the results in Table 4. Therefore it seems logical that different regression equations describing the *in vivo/in vitro* digestibility relationship will have to be established. Furthermore, the effect of drying of OF-collected samples will have to be accommodated in such regression equations.

The results in Table 5 indicate that the digestibility of a feed can be predicted fairly accurately from the IVDOM of both manually and OF-collected samples treated and dried in different ways. However, it must be stated that this does not mean that the digestibility of a mixed type of pasture could be predicted from the IVDOM of manually collected samples. It must be borne in mind that the chopped herbages used in this study were offered as mono diets. On a mixed type of native pasture the grazing animals prefer certain plants and even certain parts of plants. Therefore, it is virtually impossible to simulate the diet of the grazing animal by collecting plant material representative of the ingested material.

The regression equations that could have real practical application are those which describe the relationship between *in vivo* digestibility of OM of the herbages and IVDOM of OF-collected samples. Langlands (1975) found that if it is assumed that all organic matter in the liquid fraction of OF-collected samples is of feed origin, IVDOM will be overestimated by 1 or 2 percentage units. On the other hand if it is assumed that the liquid fraction is entirely saliva and is discarded, IVDOM will be underestimated by 3 to 4 percentage units (Langlands, 1975). Consequently, he derived equations for the prediction of IVDOM originating from the herbage. The approach in the present study, where the *in vivo* digestibilities of 7 herbages were determined with both sheep and cattle, with subsequent regression analyses to establish the relationship with IVDOM of samples collected by oesophageally fistulated animals in the same study, seems to be more acceptable and accurate.

Profound differences in corresponding regression equations for sheep and cattle were found. According to the results in Table 4, the cattle digested one of the *C. ciliaris* herbages substantially better than sheep. Although some of the other differences between sheep and cattle, regarding the digestibility of the herbages, were also significant, this exception was quite pronounced. Apparently the unexpectedly low digestibility obtained with sheep on this *C. ciliaris* was mainly responsible for the difference between the regression equations derived from the cattle and sheep data. Consequently, the digestibility of this *C. ciliaris* was omitted for sheep and the following 2 regression equations were derived:

$$Y = 16{,}4205 + 0{,}7892\ X\ (r = 0{,}962;\ S_{y.x} = \pm 1{,}3098; n = 6) \quad \ldots\ldots(21)$$

$$Y = 18{,}9124 + 0{,}6952\ X\ (r = 0{,}90;\ S_{y.x} = \pm 2{,}0930; n = 6) \quad \ldots\ldots(22)$$

In both equations Y = in vivo digestibility of OM. In Equation 21, X = IVDOM of the solid fraction of OF-collected samples dried at 50°C. In Equation 22, X = IVDOM of freeze dried total extrusa.

Obviously all the equations in Table 5 based on the sheep data, could be recalculated, but it is only Equations 21 and 22 that have real practical value. It must also be noted that these recalculated equations are in good agreement with the corresponding equations for cattle (Equations 15 and 16, Table 5). However, Equations 15, 16, 21 and 22 can by no means be applied in general for the prediction of the digestibility of a pasture from the IVDOM of OF-collected samples. They merely demonstrate the kind of relationship between *in vivo* digestibility and IVDOM of samples collected by OF-animals from the herbages tested. These samples are at present used as standards in each *in vitro* run where digestibility of a pasture is to be estimated from the IVDOM of OF-collected material at this Institute. Depending on the treatment of the OF-collected samples and the drying procedure applied, the standard samples are selected to correspond in treatment and drying procedure with those to be tested. After the *in vitro* run, the regression equation describing the relationship between *in vivo* and IVDOM of the standard samples is calculated and digestibility of the pasture is subsequently predicted. Engels (unpublished) found that the IVDOM of the standard samples varies from run to run. However, the slope of the regression (b-value) is not affected in spite of a rather large variation in the intercepts. This method enables the pasture scientist to make a direct comparison between the digestibility of samples collected and subjected to *in vitro* fermentation on different occasions.

At present all samples collected by OF-sheep and cattle at this Institute are squeezed through 4 layers of cheese cloth and dried at 50°C. Equations 1 and 2 are used for the prediction of nitrogen content for OF-sheep and cattle respectively. Digestibility is predicted by means of a regression equation relating *in vivo* digestibility to IVDOM of the standard samples treated and dried in the same way. The squeezing and drying of the resulting solid fraction at 50°C is preferred. Not only is the rate of drying a major consideration, but this renders expensive freeze drying equipment unnecessary.

It is concluded that research workers interested in the prediction of digestibility of pastures from IVDOM of OF-collected samples should develop their own regression equations. These equations must be based on an *in vivo*/IVDOM relationship of standard samples obtained, treated and dried according to type of pastures to be evaluated and availability of laboratory drying equipment.

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