USE OF COW FAECES AT DIFFERENT TIMES AFTER BEING VOIDED AS A SOURCE OF MICRO-ORGANISMS IN *IN VITRO* DIGESTIBILITY ASSAYS OF FORAGES

S. Akhter[1], E. Owen and M. M. Hossain[1]

University of Reading, Department of Agriculture, Earley Gate, P.O. Box 236, Reading RG6 2AT, U.K.

Summary

Experiments were conducted to determine the effect of time intervals between collecting and use of cattle faeces as a source of micro-organisms in *in vitro* digestibility assays of forages. The results suggested that temperature conservation capacity by faeces depended on the size of the sample. There was no significant difference ($p > 0.05$) between the first (T1 or 08:30 h) and second using time (T2 or 10:30 h). *In vitro* organic matter digestibility was significantly lower when faeces was used 5 h (T3 or 13:30 h) after collection. However, the organic matter digestibility determined at the second using time (T2) and third using time (T3) were highly correlated ($R^2 = 0.99$) with the first using time. It was concluded that faeces can be used as a source of micro-organisms for *in vitro* digestibility assays of forages even 5 h after being voided.

(**Key Words** : *In Vitro* Digestibility, Cow Faeces, Using Time)

Introduction

Current techniques for *in vitro* digestion of forages depend on the use of rumen fistulated animals to supply microbial suspensions in rumen liquor. Increasing awareness of animal welfare issues is discouraging the collection of rumen liquor from fistulated animals. To investigate the alternative procedure. El Shaer et al. (1987) and Omed et al. (1989) have revealed that sheep faeces could be used as the source of micro-organisms instead of rumen liquor in digestibility procedure. Akhter et al. (1994) using the two-stage *in vitro* procedure (Tilley and Terry, 1963) have also shown a high correlation between digestibilities of forages as determined using either sheep rumen liquor or cow faeces as the microbial inoculum. Getting fresh faeces from donor animals immediately after being voided could be a problem. Collection of fresh faeces might involve stimulation of the donor animals or a long wait for the animal to defaecate voluntarily on the day of the investigation. This dependency on collection of fresh faeces could make a delay in inoculation which ultimately would affect the routine work in a normal working day. Therefore the effect of different using time of faeces as a source of micro-organisms for *in vitro* digestibility was investigated.

A preliminary trial (Experiment 1) was conducted to observe the temperature conservation capacity of faeces and to select the inoculation time (using time) of faeces in Experiment 2. The effect of three using times of faeces as a source of micro-organisms for *in vitro* digestion of forages were compared in Experiment 2.

Materials and Methods

Experiment 1

Preliminary observation on temperature conservation

Faeces were collected from five Jersey cows (hay-fed) at 06:40 h. Faeces was preserved in a prewarmed (39℃) vacuum flask and brought to the University for laboratory use. Usually the rumen liquor of temperature 39℃ is used in inoculation, therefore it was assumed that faecal microbes may also be used at the same temperature. However, there was no publised information on this. Faeces was kept on a table in the laboratory to record the temperature conservation capacity at different times of the day. A laboratory thermometer was inserted into the centre of the faeces and temperature was recorded. Three using

[1] Address reprint requests to Dr. S. Akhter, Department of General Animal Sciences, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.

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times (08:30, 10:30 and 13:30 h) for Experiment 2 were selected on the basis of the results of Experiment 1.

Experiment 2

Forages

The 8 forages comprising of ryegrass, maize, barley and wheat were used as substrates in this experiment. The fresh forages were collected from the field and chopped (10 cm). The samples were dried at 60-65℃ for 72 h in a forced draught oven. All the samples were ground through a 1 mm dry mesh screen to perform *in vitro* studies. The substrates were chosen so as to represent a wide range of digestibilities (320-750 g/kg organic matter digestibility). The digestibilities were determined using Tilley and Terry (1963) technique. *In vitro* determinations were done in quadruplicate. The chemical composition of 8 forages were determined following the procedure of AOAC (1984) and NDF and ADF analyses were carried out according to the method of Goering and Van Soest (1970).

Collection and preparation of faeces

Freshly voided faeces was collected at 06:40 h from the rectum of a Jersey cow (hay-fed) from Old House Farm, University of Reading. The faeces was preserved in a prewarmed (39℃) vacuum flask until 08:30 h when used or allowed to cool. The faeces was removed from the flask at 08:30 h and split into three samples of 666 g each. One sample was immediately used for first inoculation at 08:30 h (T1). The other two samples were kept in an open polythene bag on a table, at ambient temperature, to await use in the second and third inoculations. The first inoculation time (08:30 h) was considered as an using time of freshly voided faeces after collection. The concentration of faeces used in making faecal liquor was 333 g faeces/l artificial saliva. The faeces inoculum was prepared by mixing 333 g fresh faeces and 333 ml CO_2 saturated artificial saliva (McDougall, 1948), straining the mixture through two layers of muslin, and adding a further 667 ml of CO_2 saturated artificial saliva to the strained liquor. Fifty ml of the inoculum was added to 0.55 g of forage for the *in vitro* fermentation which was for 48 h as in the Tilley and Terry technique. The second inoculation was performed 2 h after the first inoculation at 10:30 h (T2) and third inoculation was 5 h after the first inoculation at 13:30 h (T3). The core temperature of faeces at T1, T2 and T3 were 37, 30 and 22℃ respectively. The temperature of inoculum when added to substrates were 36.9, 33.8 and 31.6℃ at T1, T2 and T3.

Statistical analyses

The *in vitro* results obtained from Experiment 2 were analysed in a factorial design considering 8 forages and 3 using times using statistical package SAS (SAS, 1985). The regression relationships between OMD (g/kg) using freshly voided faeces and faeces using 2 h or 5 h after collection were also investigated.

Results and Discussion

The temperature of most of the faecal samples declined from 37℃ to ambient temperature within 3 h except one faecal samples (bigger than other samples) which returned to ambient temperature within 4.5 h. The results highlighted that the temperature conservation capacity by faecal samples depended on the size of the

TABLE 1. THE QUALITY OF DIFFERENT FORAGES IN TERMS OF DRY MATTER, ASH, NITROGEN AND FIBRE

Forages[1]	DM (g/kg)	Ash	N	ADF	NDF	Cellulose	Lignin
		(g/kg DM)					
BS4	932 ± 2.73	45 ± 0.09	10 ± 0.52	602 ± 6.70	838 ± 21.40	453 ± 3.35	149 ± 6.40
B4	941 ± 2.08	45 ± 0.23	14 ± 0.17	456 ± 19.00	649 ± 19.30	350 ± 6.05	86 ± 2.65
B3	930 ± 2.03	59 ± 0.30	18 ± 0.44	445 ± 4.70	725 ± 10.40	351 ± 0.10	89 ± 4.15
WS4	919 ± 1.73	72 ± 0.21	16 ± 0.05	383 ± 7.88	571 ± 14.80	308 ± 4.70	73 ± 7.10
RS4	930 ± 2.03	72 ± 0.25	20 ± 0.05	421 ± 3.25	695 ± 7.60	343 ± 3.80	81 ± 12.65
ML5	922 ± 2.40	95 ± 0.17	32 ± 0.005	341 ± 7.65	596 ± 9.05	251 ± 11.3	81 ± 12.65
RG3	922 ± 2.73	88 ± 0.27	24 ± 0.39	313 ± 5.15	516 ± 4.75	266 ± 3.75	48 ± 4.15
RG2	923 ± 2.73	89 ± 0.32	20 ± 0.15	302 ± 4.30	508 ± 9.40	258 ± 4.65	60 ± 3.03

[1]Age of the forages.

BS4 = Barley stem (70 days), B4 = Barley whole (70 days), B3 = Barley whole (60 days), WS4 = Wheat stem (70 days), RS4 = Ryegrass stem (60 days) ML5 = Maize leaf (80 days), RG3 = Ryegrass whole (50 days), RG2 = Ryegrass whole (40 days).

N.B: Sample analysed in triplicates (± values indicate the s.e. of determinations).

sample and how intact the faecal pellet was. The results suggested that more research is needed to define the effects of size of faeces samples and cooling rates, and cooling rates in relation to ambient temperature.

TABLE 2. *IN VITRO* OMD (g/kg) OF DIFFERENT FORAGES USING FAECES AT DIFFERENT TIMES AFTER BEING VOIDED

Forages	Using time of faeces (h)		
	T1 (08:30)	T2 (10:30)	T3 (13:30)
Barley stem (BS4)	163	158	143
Barley whole (B4)	201	201	186
Barley whole (B3)	252	249	232
Wheat stem (WS4)	389	384	351
Maize leaves (ML5)	413	391	408
Ryegrass stem (RS4)	430	396	408
Ryegrass whole (RG3)	564	555	532
Ryegrass whole (RG2)	622	616	553
s.e.	10.79	14.25	12.66

The quality of forages used in *in vitro* studies is presented in table 1. The main *in vitro* OMD obtained at different using times are presented in table 2. Statistical analyses showed that there was no time × forage interaction. Statistical analyses also revealed that there was no significant difference ($p > 0.05$) between first (T1) and second (T2) using times. *In vitro* results determined at the third using (T3) time was significantly lower than that determined at the first (T1) and second (T2) using time. It was expected that the inoculum preparation stage of *in vitro* digestion trials would influence digestibility because of the difficulty of meeting temperature criteria during the handling of inoculum before mixing it with the artificial saliva. The results of the present study indicate that a temperature drop to 30°C or a time delay of 2 h did not reduce digestibility, but a temperature drop to 22°C or a time delay of 5 h reduced digestibility. It is clear that faecal structure even cooling to ambient temperature, was favourable for micro-organisms' viability as the faecal microbes were capable of digesting forages.

There had been little data concerning the effect of inoculation time or temperature drop on rumen liquor activity. Daniel and Dan (1982) reported that a

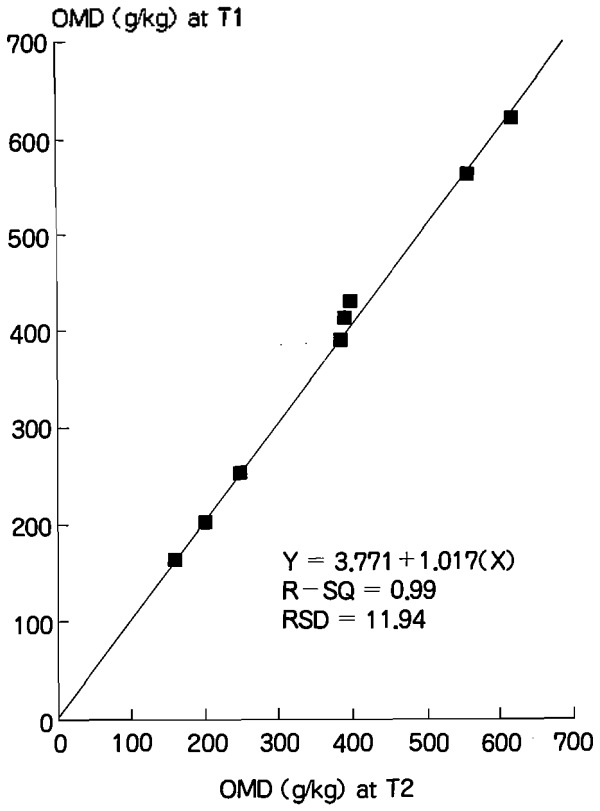

Figure 1. Linear relationship between OMD using faeces as a fresh (T1) and 2 h after collection (T2)

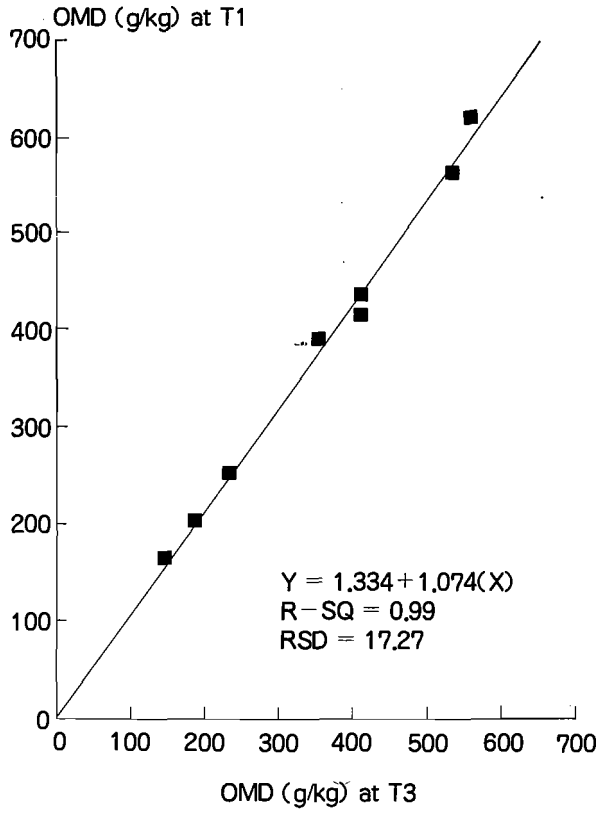

Figure 2. Linear relationship between OMD using faeces as a fresh (T1) and 5 h after collection (T3)

temperature drop of rumen liquor to 29℃ or a time delay of 2 h significantly reduced digestibility and the rate of reduction was different for different forages. The results obtained in Experiment 2 added some support to the conclusion reached by Daniel and Dan (1982) that time delay (at least 5 h) in the use of inoculum reduced digestibility and the rate of reduction was different for different forages.

However, the OMD (g/kg) determined at the second using time (T2) and the third using time (T3) were highly correlated ($R^2 = 0.99$) with first using time (figure 1 and 2). The relationships between the digestibilities were highly significant. These findings showed that researchers do not need to depend on fresh cow faeces for digestibility studies, faecal samples kept as long as 5 h after being voided could be used.

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