EFFLUENT FROM RUSITEC INOCULATED WITH RUMEN LIQUOR OR COW FAECES AS SOURCES OF MICRO-ORGANISMS FOR *IN VITRO* DIGESTION OF FORAGES

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Summary

The experiment investigated the possibility of using effluent from RUSITEC (rumen simulation technique) inoculated with rumen liquor or cow faeces as sources of micro-organisms for *in vitro* digestion of forages. Nine forages × 3 sources of inoculum were used in a factorial arrangement of treatments. Rumen liquor was collected from fistulated sheep and faeces was collected from cows. The RUSITEC apparatus consisted of 4 vessels, 2 vessels were charged with faecal liquor and 2 with rumen liquor. On the 8th day of the experiment RUSITEC effluent were collected to use in *in vitro* studies. *In vitro* OMD (g/kg) values using three sources of inoculum (fresh rumen liquor, RUSITEC effluent from rumen liquor or cow faeces) were statistically significant ($p < 0.001$). The regression relationships between OMD using fresh rumen liquor and RUSITEC effluent were highly significant ($R^2 > 0.90$). The results suggest that RUSITEC effluent either from rumen liquor or cow faeces can be used as a source of micro-organisms for *in vitro* digestion of forages.
(**Key Words** : RUSITEC, Inoculum, *In Vitro* Digestion, Forages)

Introduction

The development of reliable and acceptable laboratory methods for estimating forage quality is one of the problems in agricultural research today. The widely accepted Tilley and Terry (1963) procedure for *in vitro* digestibility determination of forage is less favoured, because it needs fistulated animals to supply fresh rumen liquor. Czerkawski and Breckenridge (1977 and 1979) developed a simple rumen simulation technique (RUSITEC) which can be used to minimize rumen fermentation for an extended time period. The problem is that RUSITEC needs a supply of fresh rumen liquor as does the Tilley and Terry (1963) method. Use of fresh rumen liquor from fistulated animals is becoming less favoured for various reasons such as animal welfare issue, management of fistulated animal in tropical countries. Concern has been raised to use alternative inoculum instead of rumen liquor. Different workers El-Shaer et al., 1987; Omed et al., 1989 and Akhter et al., 1994) demonstrated the use of faeces as a source of micro-organisms for *in vitro* digestibility studies. Owen et al. (1991) showed that the effluent from RUSITEC could be used as a source of micro-organisms for *in vitro* digestion assays instead of rumen liquor. So there was a question of whether RUSITEC could be inoculated with faecal liquor and the resultant RUSITEC liquor used as a source of micro-organisms for the Tilley and Terry (1963) technique. However, there is no information on the possibility of using RUSITEC effluent when RUSITEC is inoculated with faeces. On the basis of work with rumen liquor and faeces (Akhter et al. 1994) one could expect a difference between sources of inoculum when RUSITEC inoculated with faeces or rumen liquor.

Therefore the present study investigated the possibility of using the effluent from RUSITEC (inoculated with faeces or rumen liquor) as a source of micro-organisms using Tilley and Terry (1963) digestion technique.

Materials and Methods

Design and treatments

The study involved a 9 × 3 factorial arrangement of treatments. The factors were 9 forages and 3 sources of inoculum. Details of treatments are in table 1.

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TABLE 1. DETAILS OF TREATMENTS

Source of micro-organisms	RUSITEC : faeces inoculated (RF)	RUSITEC : rumen liquor inoculated (RR)	Fresh rumen liquor (FR)
Number of animals used to inoculate RUSITEC or fresh rumen liquor	3 cows	3 sheep	3 sheep
Date of inoculating the RUSITEC	05-08-1992	05-08-1992	–
Time of inoculating the RUSITEC (h)	08:30	08:30	–
Date of collecting RUSITEC effluent or rumen liquor	12-08-1992	12-08-1992	12-08-1992
Time of RUSITEC effluent or fresh liquor collection (h)	07:30	07:30	07:30
Time of inoculation for *in vitro* assay (h)	08:30	08:50	09:20
Ratio of effluent or fresh liquor to saliva	1:4	1:4	1:4
Forages digested	9	9	9

The RUSITEC apparatus

The RUSITEC vessel was located in the field laboratory at the Old House Farm, 3 miles from the Department of Agriculture, University of Reading, U.K. The RUSITEC apparatus consisted of four vessels (Czerkawski and Breckenridge, 1977). Each RUSITEC vessel was fed daily with 12.0 g hay and 3.0 g concentrate (both ground through a 3 mm screen).

RUSITEC liquor preparation

On the first day of the experiment 500 ml strained faecal liquor (333 g faeces/l artificial saliva) or strained rumen liquor were placed in each reaction vessel with 200 ml artificial saliva (McDougall, 1948) and 100 ml water. Two vessels were charged with faecal liquor and two with rumen liquor. Faeces was collected from 3 hay-fed Jersey cows. Rumen liquor was obtained from three fistulated sheep. Solid rumen digesta (80 g) or solid faecal contents obtained after squeezing through cheese cloth (80 g) were weighed into a nylon bag and one of these was placed inside the food container in each vessel together with a bag of food. The system was flushed through with CO_2 gas. The next morning the infusion was stopped and the RUSITEC food agitator was also stopped. The reaction vessels were removed from the water bath. The vessels were opened and the bags of solid inoculum were removed and replaced by new bags of food. The original solid inoculum was rejected. On subsequent days, bags that had spent 2 days in the vessels were removed and new bags were introduced. The bags that were removed from the vessels were placed inside polythene bags with 40 ml artificial saliva. The combined washing were poured back into the reaction vessels as described by Czerkawski and Breckenridge (1977 and 1979). The closing and flushing procedure was carried out as before and the infusion started. These procedures were repeated every morning.

RUSITEC liquor collection for *in vitro* procedure

On the 8th day of the experiment RUSITEC effluent was collected and preserved in a vacuum flask and transported to the laboratory at the Department of Agriculture for use in *in vitro* determination. On the same day of the *in vitro* digestion trial fresh rumen liquor was also collected from the sheep that had provided the RUSITEC rumen effluent. The fresh rumen liquor was also transported in a vacuum flask to the laboratory at the Department of Agriculture for use in *in vitro* digestibility determination.

Forages

The forages were collected from the Old House Farm, University of Reading. The 9 forages were chosen to represent a wide range of digestibilities and comprised of barley straw, hay and young ryegrass. Stem and leaf fractions of forages were also separated by hand. The dried forages were ground (1 mm screen) for use in the *in vitro* digestion technique. The different sources of inoculum were used to digest 9 forages to determine regression relationships between digestibilities using fresh rumen liquor or RUSITEC effluent either from rumen liquor or cow faeces.

In vitro digestibility procedure

The two-stage *in vitro* technique of Tilley and Terry (1963) was conducted to determine the digestibility of different forages. About 0.55 g sample was used for *in vitro* determination and each determination was done in quadruplicate on each forage. Fresh rumen liquor was strained through two layers of muslin cloth and mixed

with artificial saliva as in the Tilley and Terry procedure (1963). The effluent from 2 RUSITEC vessels charged with faeces were pooled but not strained before inoculum preparation. Similarly effluent from 2 RUSITEC vessels charged with rumen liquor were pooled but not strained before inoculum preparation. The RUSITEC effluent and fresh rumen liquor were mixed with artificial saliva as in the Tilley and Terry (1963) method. All sources of liquor were saturated with CO_2.

Statistical analyses

The data obtained were analysed in a 9 × 3 factorial form. Analysis of variance was made on the effect of source and forage on *in vitro* digestibility. The regression relationships (curvilinear) between OMD values using fresh rumen liquor (Y) and those obtained with RUSITEC effluent (X) were also investigated.

Results

Analysis of variance revealed that there was a highly significant interaction between forage and source. Inconsistency of some forage digestibilities with some sources was the probable cause of the forage × source interaction. The mean *in vitro* OMD values for each source and forage are presented in table 2. The relationships between OMD using different sources are presented in figures 1, 2 and 3. The relationships between OMD (g/kg) using fresh rumen liquor (Y) and OMD (g/kg) using RUSITEC effluent (X) were highly significant

TABLE 2. *IN VITRO* OMD (g/kg) OF DIFFERENT FORAGES USING MICRO-ORGANISMS FROM VARIOUS SOURCES

Forages[f]	Source[s] of micro-organisms			Forage means	s.e.d
	RF	RR	FR		
BW	145	150	388	228	15.83
BS	109	149	343	200	7.97
BL	234	490	570	431	15.05
HW	442	509	567	506	20.88
HS	292	439	531	420	16.77
HL	343	618	620	527	15.56
RW	738	749	781	756	18.59
RS	522	696	736	652	20.77
RL	818	831	800	816	19.42
Source means	405	515	593		
s.e.d.	15.64	19.68	15.90		

[f] BW = barley whole; BS = barley stem; BL = barley leaves; HW = hay whole; HS = hay stem; HL = hay leaves; RW = ryegrass whole; RS = ryegrass stem; RL = ryegrass leaves.
[s] RF = RUSITEC faeces liquor; RR = RUSITEC rumen liquor; FR = fresh rumen liquor.

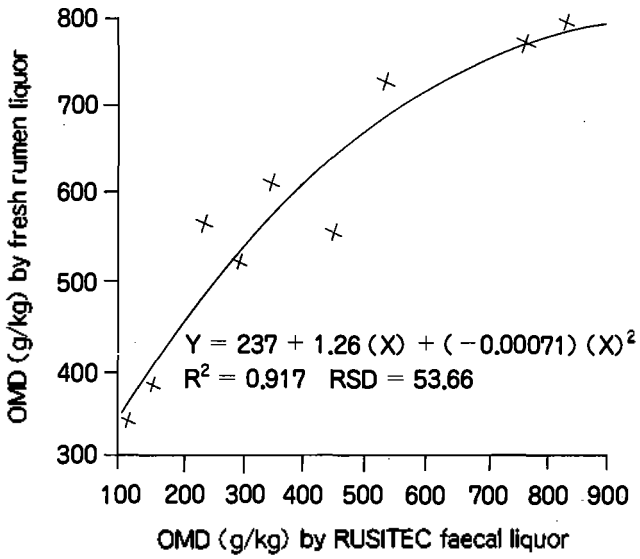

Figure 1. Relationship between OMD using fresh rumen liquor and RUSITEC faecal liquor.

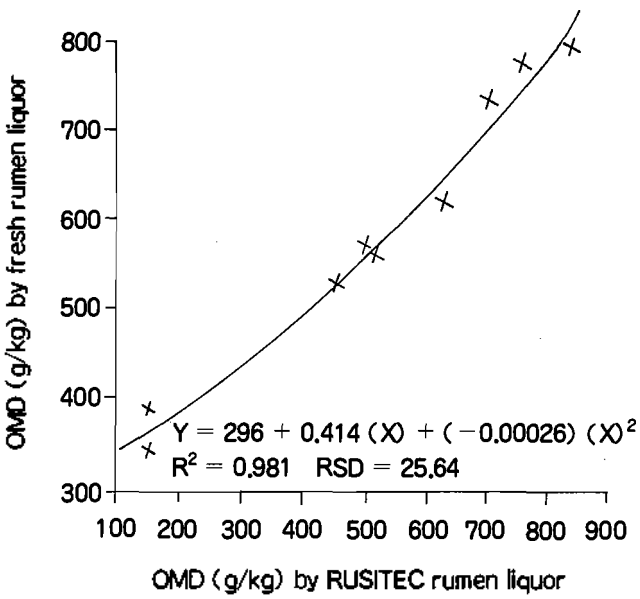

Figure 2. Relationship between OMD using fresh rumen liquor and RUSITEC rumen liquor.

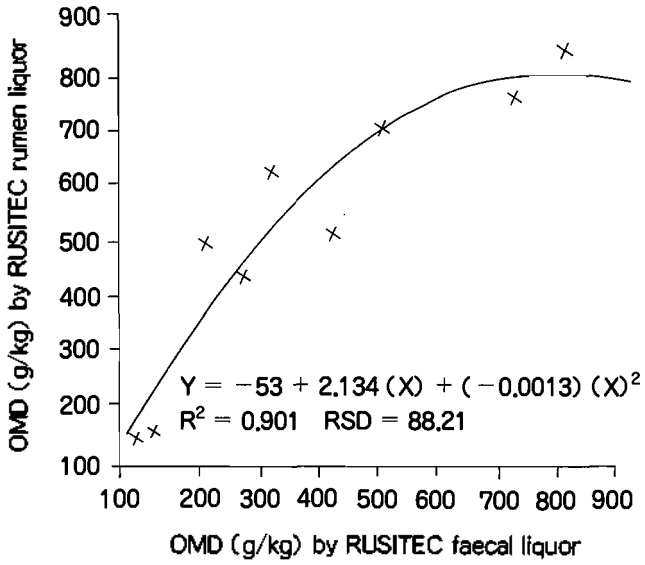

Figure 3. Relationship between OMD using RUSITEC rumen liquor and RUSITEC faecal liquor.

Discussion

The results show that RUSITEC effluent either from rumen liquor or cow faeces degraded the forage samples. It was expected that digestibility of forages would vary the source. The expectation was met (table 2). Some inconsistency of forage digestibilities with source probably caused the forage × source interaction.

The lower OMD values using RUSITEC faeces indicates that inoculum source have important effects on *in vitro* results. Bezeau (1965) also stated that the source of inoculum affects the *in vitro* (Tilley and Terry, 1963) digestibility. Owen et al. (1991) determined the digestibility of hay using either cow rumen liquor or RUSITEC effluent using various volumes of liquor and artificial saliva. They (Owen et al., 1991) found that DMD values determined with rumen liquor were 700 (g/kg) while those determined with RUSITEC effluent were 620 (g/kg). However, there is no information on the use of RUSITEC effluent when RUSITEC is inoculated with faeces.

In the present study, the curvilinear relationship (figures 1, 2 and 3) showed a significant ($p < 0.001$) correlation between OMD determined using rumen liquor and those obtained using RUSITEC effluent (inoculated with faeces or rumen liquor). The R^2 value exceeded 0.90. Owen et al. (1991) reported that there was a high correlation ($R^2 = 0.976$ and RSD = 2.43) between DMD using fresh cow rumen liquor and RUSITEC effluent (inoculated with rumen liquor). The present results also showed a high correlation ($R^2 = 0.981$, RSD = 2.56) between OMD using rumen liquor and RUSITEC effluent (inoculated with rumen liquor). As noted earlier, OMD using RUSITEC effluent (inoculated with faeces) resulted in lower OMD values (table 2), but the correlation with fresh rumen liquor was high.

The filtration of residues, especially the samples digested with RUSITEC faecal liquor, was a problem. The residue quickly blocked the pores of the sintered glass crucibles. Therefore, the residues were collected with lots of unwashed liquid and consequently crucibles and residues needed extra time to dry in the oven. Ten ml of RUSITEC faecal liquor per *in vitro* digestibility tube was used in the present study. It would be interesting to see if high digestibilities would occur if more liquor per tube would be used.

The results suggest that RUSITEC effluent either from rumen liquor or cow faeces can be used as a source of micro-organisms for *in vitro* digestion assays.

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