Uganda Journal of Agricultural Sciences, 2015, 16 (1): 93 - 98 ISSN 1026-0919
e-ISSN 2410-6909
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Comparison of *in vitro* digestibility using slaughtered and fistulated cattle as sources of inoculum

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Abstract

***In vitro* methods of feed evaluation utilise rumen liquour from fistulated cattle. Fistulated cattle are associated with high acquisition and maintenance costs in addition to its implications on animal welfare. An experiment was conducted to compare *in-vitro* dry matter (INVDMD) and organic matter (INVOMD) digestibility of four diets using two sources of inocula namely; (i) slaughtered cattle and (ii) fistulated steers. Four fistulated crossbred (Friesian × Ankole) steers were assembled and fed on four diets in a Latin Square change-over arrangement. The two-stage Tilley and Terry (1963) method of nutrient digestibility was used. The overall INVDMD significantly increased with dietary crude protein levels ($P < 0.05$). There was no difference in INVDMD obtained from slaughtered cattle or fistulated steers as inocula sources. Rumen liquour from slaughtered cattle can successfully replace use of fistulated cattle as the source of inoculum.**

Key words: Dry matter, organic matter

Introduction

Use of live animals in feed evaluation studies is limited by costs involved in acquiring and managing fistulated animals. Manipulation of live animals for *in vivo* methods of feed evaluation has implications on animal welfare, in addition to the high costs involved (Mohamed and Chaudhry, 2008). Mohamed and Chaudhry (2008) stressed the need for *in vitro* methods of digestibility evaluation that do not necessitate use of fistulated cattle. *In vitro* methods of digestibility evaluation utilise rumen liquour and thus require acquisition of fistulated cattle. Compared to faeces as the source of inoculum, rumen liquour gives dry matter digestibility values that significantly correlate with *in vivo* digestibility (Borba and Ramalho, 1996). However, Tufarelli *et al.* (2010) observed no difference in *in vitro* dry and

http://dx.doi.org/10.4314/ujas.v16i1.7

organic matter digestibility when using faeces as an alternative to rumen liquour. Fistulation has the advantage of allowing the conditioning of the rumen liquour, which is mainly done through dietary manipulation (Soder, 2005). Fistulating cattle requires experienced technicians and such animals are costly to acquire and maintain (Jones and Barnes, 1996). Rumen liquour from slaughtered cattle is a viable option where access to such animals is difficult or costly. Use of inoculum from the rumen content of cattle upon slaughter has the advantage of eliminating the need to canulate and reduces stress on animals (Mould *et al.*, 2005). Use of frozen rumen content as the inocula source, has been noted for yielding low degradability values compared with freshly prepared inocula. However, in the absence of fresh rumen content, frozen rumen content is a possible alternative (Mohamed *et al.*, 2002).

Inoculum obtained from the rumen content of slaughtered cattle and using the gas production technique resulted in organic matter digestibility that highly correlated with values obtained using the traditional Tilley and Terry method (Mutimura *et al.*, 2013). *In vitro* studies utilising inoculum from slaughtered cattle and sheep have showed potential of predicting *in vivo* dry matter digestibility (Denek *et al.*, 2006). Inter-laboratory comparisons by Rymer *et al.* (2005) indicated differences in values of parameters studied due to variations in apparatus, donor animals and laboratories. Thus, establishment of mathematical models is needed to cater for differences in laboratories and apparatus. A study was carried out to compare the *in vitro* dry matter digestibility (INVDMD) of four diets using rumen liquour from slaughtered cattle and fistulated cattle.

Materials and methods

Four fistulated crossbred (Friesian × Ankole) steers where assembled and fed on four experimental diets (Table 1), in a Latin Square Change-Over arrangement. The steers were fed on *Brachiaria molato* hay as the basal diet, supplemented with a 13% crude protein concentrate at 0% (diet 1), 10% (diet 2), 20% (diet 3) and 30% (diet 4) of dry matter intake. Feed samples were ground to pass through a 1 mm sieve using a hammer mill; and their DM was determined using the standard methods of AOAC (1990).

Rumen content was collected from cattle immediately after slaughter in the abattoir, packed in pre-warmed vacuum flasks and transported to the laboratory

Table 1. Change-over arrangement of steers and allocation of diets

	Treatment			
	0%Conc+Hay	10%Conc+Hay	20%Conc+Hay	30%Conc+Hay
Period one	Steer 1	Steer 2	Steer 3	Steer 4
Period two	Steer 4	Steer 1	Steer 2	Steer 3
Period three	Steer 3	Steer 4	Steer 1	Steer 2
Period four	Steer 2	Steer 3	Steer 4	Steer 1

within one hour. Rumen content from fistulated steers was collected before morning feeding (Mutimura *et al.*, 2013). The rumen content from each steer was collected separately into a pre-warmed flask. In the laboratory, while flushing with carbondioxide, rumen contents from fistulated and slaughtered cattle were separately squeezed through cheese cloth, into beakers placed in a water bath set at 39 °C. INVDMD of the four diets was determined as described by Tilley and Terry (1963). For each period, the four diets were digested individually with inocula obtained from a steer feeding on that particular diet.

Data collected were subjected to ANOVA using the General Linear Model procedure of SAS (2003). Treatment means were separated using the Least Significant Difference (LSD) at 5% probability level of significance. The model for the feeding trial was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

Y_{ij} = Response variable (INVDMD and INVOMD);

Table 2. Composition of 13 percent protein concentrate used in a digestibility evaluation using slaughtered cattle and fistulated steers in Uganda

Ingredient	g kg^{-1} (as fed basis)
Maize bran	75
Gliricidia	10
Caliandra	10
Cotton seed cake	5
Total	100

Animals had *ad-libitum* access to Vitamin-mineral block

μ = General mean;
T_{ij} = Effect of diet/liquour source; and
E_{ij} = Random error;

Results and discussion

The effect of supplementation and liquour source on INVDMD and INVOMD are summarised in Table 3. The mean INVDMD and INVOMD increased with dietary crude protein levels ($P<0.05$). Non-supplemented diet (diet 1) exhibited the lowest INVDMD (37.98 and 37.25%, respectively) while diet 4 attained the highest INVDMD (47.42 and 48.70%) for slaughtered cattle and fistulated steers as sources of inoculum, respectively. Chaudhry (2008) reported an increase in dry matter digestibility of high (25%) protein diet compared to a low (12%) protein diet. Soder (2005) also indicated that reducing dietary crude protein by 4% reduces INVDMD by approximately 10%. The INVDMD of diet 2 and 3 increased, though not significantly, when using both slaughtered cattle and fistulated steers as sources of inoculum.

Non-supplemented diet (diet 1) exhibited the lowest INVOMD (31.85 and 28.50%); while diet 4 attained the highest INVOMD (40.58 and 39.78%) for slaughtered cattle and fistulated steers as sources of inoculum, respectively. Addition of supplements to the basal diet increases *in vitro* digestibility (Nguyen, 2003; Zicarelli *et al*, 2008). Data from Zicarelli *et al.* (2008) showed an increase in organic matter digestibility when hay was supplemented with up to 30% concentrates.

The increase in digestibility coefficients in high protein diets is due to stimulated rumen microbial growth, which in turn degrades more diets compared to low protein or non-supplemented diets

(Chaudhry, 2008). The increase in INVDMD and INVOMD with increasing protein levels indicates the potential of improving feed efficiency of low quality forages (Bargo *et al.*, 2003). To minimise variations due to rumen liquor donor animals, it is recommended to have donor animals of similar characteristics under the same pre-slaughter treatment (Chaudhry, 2008).

The effect of liquour source on average INVDMD and INVOMD is summarised in Table 4. INVDMD and INVOMD values obtained when using liquour from fistulated steers or slaughtered cattle was not significantly different ($P>0.05$). This is in agreement with Denek *et al.* (2006), the author demonstrated that rumen liquor from slaughtered cattle can be used to predict *In vitro* dry matter digestibility of feeds. In contrast, Borba and Ramalho (1996) reported that rumen liquor from fistulated compared to slaughtered animals was more precise in determining dry matter digestibility of feeds. Borba *et al.* (2001) reported that digestibility of feeds is more accurately estimated using rumen liquour from slaughtered animals as compared to fistulated animals as sources of inoculum. However, the differences in digestibility reported by Borba and Ramalho (1996) and Borba *et al.* (2001) could have been due to species differences as fistulated sheep and slaughtered cattle where the donor animals of rumen liquor. Mutimura *et al.* (2013) concluded that rumen fluid from slaughtered cattle is an alternative source

Table 3. Effect of different inoculum source on INVDMD and INVOMD of supplemented diets

Diet	INVDMD (%)		INVOMD (%)	
	Slaughtered cattle	Fistulated steers	Slaughtered cattle	Fistulated steers
1	37.98^{c}	37.25^{c}	31.85^{c}	28.50^{c}
2	42.68^{b}	42.09^{b}	38.55^{b}	35.69^{b}
3	43.43^{b}	43.11^{b}	36.23^{b}	33.83^{b}
4	47.42^{a}	48.70^{a}	40.58^{a}	39.78^{a}
P-Value	<0.0001	<0.0001	<0.0001	<0.0001

Means in the same column with the same superscripts are not significantly different

Table 4. Comparison of fistulated steers and slaughtered cattle as sources of rumen liquour

	Liquour source		
	Fistulated steers	Slaughtered cattle	P-Value
Average INVDMD (%)	42.79	42.88	0.73
Average INVOMD (%)	34.36	36.88	

Means in the same row with the same superscripts are not significantly different

of microbial inoculum for feed evaluation studies in absence of fistulated cattle.

Conclusion

In vitro dry matter and organic matter digestibility of diets/feed increases with increase in crude protein levels. Rumen liquour from slaughtered cattle can successfully substitute for liquour from fistulated cattle as a source of inoculum for digestibility trials. The effect of feeding history, age and breed of slaughtered cattle on inoculum efficiency should also be investigated.

Acknowledgement

This study was supported by the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA). Dr. Rose Omaria (NaLIRRI-NARO) is acknowledged for co-coordinating the project.

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