# Fresh or frozen rumen contents from slaughtered cattle to estimate *in vitro* degradation of two contrasting feeds

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**ABSTRACT**: The present factorial experiment tested the thawed rumen fluid from frozen rumen contents against fresh rumen fluid from the same slaughtered cattle to estimate *in vitro* degradation of rapeseed meal (RSM) and grass nuts (GN) as two contrasting feeds at various times. Fresh rumen fluid showed higher ($P < 0.05$) *in vitro* dry matter (DMD) and crude protein (CPD) degradation than the thawed rumen fluid at most incubation times. Significant differences ($P < 0.05$) were found between fresh and differently prepared thawed rumen fluids for mean degradation constants (*a, b, c*) and effective degradability ($P_{0.02}$) of feeds. While the thawed rumen fluids had lower ($P < 0.01$) degradation than the fresh rumen fluid, their degradation values correlated very well ($R^2 \geq 0.95$) with those for the fresh rumen fluid. It appeared that the thawed rumen fluid can be used to predict *in vitro* degradation of the selected feeds when frequent access to slaughtered or fistulated animals is restricted. Nevertheless it would be vital to improve the activity of thawed rumen fluid prior to testing its suitability for numerous feeds before recommending its routine use to estimate degradation of ruminant feeds.

**Keywords**: frozen rumen fluid; slaughtered cattle; *in vitro* degradation; feeds

Information about the rumen degradation of various feed ingredients is needed before their use to formulate nutritionally balanced diets for ruminant livestock (AFRC, 1993; Thomas, 2004). The rumen degradation data for different feeds are traditionally obtained by using surgically modified animals for either *in sacco* studies or to obtain rumen fluid for *in vitro* studies (Chaudhry, 2007, 2008; Mohamed and Chaudhry, 2008; Chaudhry and Mohamed, 2011). Due to high management and welfare costs of maintaining rumen fistulated animals, the availability of such rumen fluid to commercial laboratories on regular basis is restricted. Although solubility, enzyme and faeces-based *in vitro* methods do not require the use of fistulated animals, they still need the *in sacco* or rumen fluid-based *in vitro* data for their further evaluation and validation (Mohamed and Chaudhry, 2008). Mohamed and Chaudhry (2008) have suggested the use of rumen fluid from slaughtered animals in the absence of fistulated animals to estimate *in vitro* degradation of feeds. However, it would be beneficial if rumen contents are preserved in sufficient quantities for their later use as a source of microbial inoculum when there is a need to estimate degradation of different ruminant feeds. Although slaughtered cattle may be used as an alternative source to obtain rumen fluid, obtaining rumen fluid from slaughtered animals needs transport, man-hours, and time adjustment between researchers and abattoirs (Chaudhry, 2008). Therefore, it would help if the rumen fluid of slaughtered animals could be preserved in sufficient quantity for its subsequent incubation with various feeds to estimate their *in vitro* degradation on a regular basis.

This study was designed to explore the suitability of using rumen fluid from thawed rumen contents to produce comparable degradation data for two contrasting feeds, rapeseed meal (RSM) and grass nuts (GN), to those of the fresh rumen fluid.

## MATERIAL AND METHODS

### Experimental design, feeds, and rumen contents

A factorial design (2 feeds × 4 slaughtered cattle × 4 inocula types × 8 incubation times), with two replicates per factor, was used to study the effect of freezing at –20°C on the activity of thawed inocula for the estimation of *in vitro* degradation of feeds. Duplicate samples of each buffered rumen fluid without substrate (blank) were also run simultaneously for each time of incubation to correct degradability estimates for potential microbial debris and residual fine particles.

Samples of RSM and GN were obtained from the University farm, dried, and ground to pass through 1 mm sieve before their incubations with rumen fluids of this study. Here GN samples were commercially available dried ryegrass based pellets. Rumen contents were obtained from four animals on four separate days but within 20 min from each slaughter at a local abattoir (Whitley Bay Meats Ltd, Newcastle, UK). Each week only one animal was used to collect the rumen contents. Relevant details about each animal's owner, breed, gender, and age were obtained from the corresponding animal passports that were available at the abattoir. However, information about each animal's diet prior to its slaughter was obtained either by telephone or in-writing from the owner farmer of that animal. The digestive organs were pulled out of each slaughtered animal from which the reticulo-rumen was cut open with a knife. The whole rumen contents (WRC), representing both liquids and solids, were then collected from at least 4 different locations of each rumen and pooled for each animal while transferring into pre-warmed 2 l insulated flasks. These flasks were immediately screw capped and transported to the laboratory within one hour of each collection.

### Preparation of rumen inocula

The WRC were used anaerobically under $CO_2$ to prepare four strained rumen fluids (RF) as inocula as illustrated in Figure 1. The WRC were divided into two parts where one part was immediately stored in an airtight container at –20°C for four weeks to represent stored WRC (SWRC). The second part of WRC was filtered through 4 layers of cheesecloth to obtain fresh RF (FRF) and the residual solids from this portion of WRC (RWRC) were stored in an airtight container at –20°C for 4 weeks. The RWRC were thawed for about 12 h in an oven at 39°C in an airtight container and squeezed through cheesecloth to obtain thawed residual RF (TRRF). The SWRC were also thawed for about 12 h and squeezed through a cheesecloth to obtain thawed RF (TRF), whereas the thawed residual SWRC (TRWRC) were stored in an air-tight container

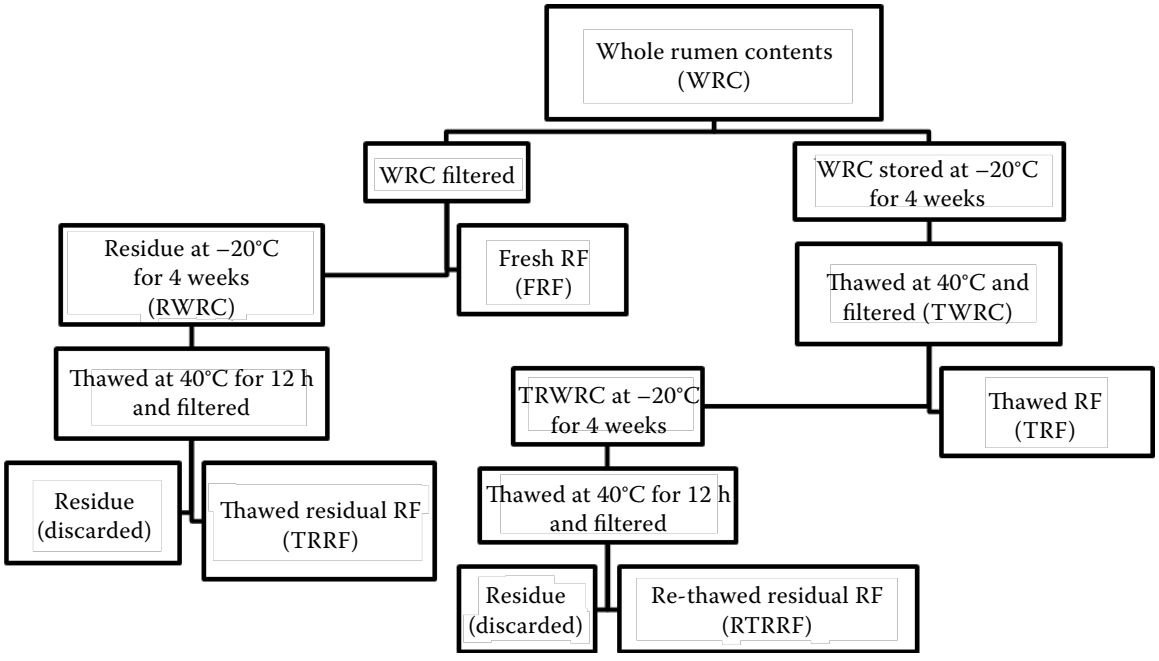


Figure 1. Illustration of the procedures used to prepare different strained rumen fluids (RF)
RWRC = residual WRC; TRWRC = thawed RWRC

at –20°C for further 4 weeks. The TRWRC were then re-thawed to obtain re-thawed residual RF (RTRRF). One part of each RF was mixed separately with 4 parts of a buffer (McDougall, 1948) to get the final RF to buffer ratio of 1 : 4. Each buffered RF was then transferred to a dark reagent bottle, gassed with oxygen-free $CO_2$, screw capped and kept at 39°C in a water bath before its use as an inoculum as described in the following section.

### Incubation of feeds and chemical analysis

About 0.4 g samples of RSM or GN were weighed separately into individual polypropylene tubes to which 40 ml of appropriate inocula were added, flushed with $CO_2$, and capped with a Bunsen valve. The tubes were incubated at 40°C in a water bath for 0, 3, 6, 12, 24, 48, 72, and 96 h, the contents of each tube were shaken 3 times daily to improve the contact between the incubated feed and rumen microorganisms. After each incubation time, 2 × 4 test tubes representing both feeds and 4 inoculum types and 2 blank tubes were immersed in ice to stop microbial activity. The tubes were then centrifuged at 3000 *g* to collect undegraded residues which were washed three times with about 40 ml of distilled water per wash. The residues were then dried in an oven at 60°C and weighed to estimate dry matter disappearance (DMD). The residues were also analysed for nitrogen to estimate CP (N × 6.25) degradation (CPD). Triplicate samples of each pre-incubated feed were also analysed for DM, OM, CP, EE, NDF, and ADF by using appropriate methods as previously described by Chaudhry (2007).

### Calculations and statistical analysis

The proportions of DMD and CPD from each feed in each tube at each incubation time were calculated as follows:

DMD or CPD (g/kg) = [(Initial DM or CP (g)– Final DM or CP (g))/Initial DM or CP (g)] × 1000

The data on DMD or CPD over various times from different inocula were separately fitted into the exponential model $P = a + b\,(1\{- e^{-ct}\})$ of Ørskov and McDonald (1979) to derive degradation constants (*a, b, c*). These degradation constants were used to calculate effective degradability ($P_{0.02}$) by using the equation:

$$P_{0.02} = a + \left(\frac{b \times c}{c + k}\right)$$

where:

$P_{0.02}$ = effective DMD (EDMD) or CPD (ECPD) at an outflow rate of 0.02/h

$a$ = constant; immediately soluble/degradable

$b$ = constant; slowly degradable

$c$ = fractional rate of degradation of constant $b$

$k$ = estimated rumen outflow rate of 0.02/h for animals consuming moderate quality of feed at maintenance

The data on degradation constants (*a, b, c*) and $P_{0.02}$ were statistically analysed by using GLM of SPSS to study the main effect of feed, inocula, and feed × inocula interaction on these parameters. The cattle effect was not studied for its significance as the cattle were used as replicates. Tukey's test was used to compare means of each studied parameter for their significance at $P < 0.05$. The simple linear regression was also performed separately for DMD and CPD (g/kg) to find correlations between fresh and thawed inocula by using degradation data for all but 0 h of incubation. Regression analysis was also used to derive prediction equations between CPD and DMD for each inocula. The strength of each relationship was reported by using the coefficient of determinations ($R^2$).

## RESULTS

### Test feeds and slaughtered cattle

Table 1 shows that the test feeds (RSM and GN) differed significantly in DM, OM ($P < 0.05$), CP ($P < 0.01$), NDF ($P < 0.01$), and ADF ($P < 0.01$). Table 2 presents the features of four animals that were used to obtain rumen fluid on four separate occasions. All cattle were females which consumed almost similar diets but represented two different breeds and two age groups.

### DMD constants and effective DMD (EDMD) of feeds

The mean degradation constants (*a, b, c*) and EDMD averaged over all four animals are shown in Table 3 whereas Table 4 presents the main effects of feed and inocula on DMD constants and EDMD. The main effects of feed and inocula were significant ($P < 0.01$) but not feed × inocula interaction ($P > 0.05$) for constant *a* where rapeseed meal had

Table 1. Chemical composition (g/kg DM) of two contrasting feeds

| Feeds | DM | OM | CP | EE | NDF | ADF |
|---|---|---|---|---|---|---|
| Rapeseed meal | 925 | 844 | 287 | 32 | 392 | 191 |
| Grass nuts | 955 | 869 | 153 | 36 | 606 | 295 |
| SEM | 5.8 | 7.2 | 38.9 | 1.3 | 61.8 | 30 |
| Significance | * | * | ** | NS | ** | ** |

DM = dry matter, OM = organic matter, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre, ADF = acid detergent fibre, SEM = standard error of means
*significant at $P < 0.05$, **significant at $P < 0.01$, NS = nonsignificant

Table 2. Features of freshly slaughtered cattle being used to obtain rumen contents

| Features | Cattle ID | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Breed | Charolais | Limousin X | Limousin X | Limousin X |
| Gender | female | female | female | female |
| Age in days | 840 | 658 | 662 | 906 |
| Pre-slaughter grazing | grass | grass+clover | grass+clover | grass |

higher ($P < 0.01$) values than grass nuts (Table 3). FRF had higher constants *a* and *b* than the thawed inocula (Table 4). While the main effect of inocula on constant *c* was not significant ($P > 0.05$), the main effect of feed was significant ($P < 0.01$) where rapeseed meal had higher ($P < 0.01$) values than the grass nuts (Table 4). The main effect of feed or inocula on EDMD was significant ($P < 0.01$) where FRF had higher ($P < 0.01$) EDMD than the thawed inocula (Table 4). FRF had, for each feed, the highest ($P < 0.01$) EDMD which were followed by RTRRF, TRRF, and TRF. Rapeseed meal had higher ($P < 0.01$) EDMD than the grass nuts (Table 4). RTRRF had higher EDMD for each feed than other thawed inocula, despite being frozen and thawed twice compared to TRF that was frozen and thawed only once. Feed × inocula interactions were not significant for any degradation constants and EDMD ($P > 0.05$).

## CPD constants and ECPD of feeds

Table 5 presents CPD constants (*a, b, c*) and ECPD for all combinations of feeds and inocula (feed × inocula). The mean values for the main effect of feed or inocula are shown in Table 6 which shows significant effect of feed and inocula on *a, b*, and ECPD ($P < 0.05$). While the main effect of feed

Table 3. Mean ($n = 8$) DM degradation (DMD) constants (*a, b*, and *c*) and effective DMD (EDMD) for feed × inocula interaction

| Inocula | Rapeseed meal | | | | Grass nuts | | | |
|---|---|---|---|---|---|---|---|---|
| | *a* (g/kg) | *b* (g/kg) | *c* ($h^{-1}$) | EDMD (g/kg) | *a* (g/kg) | *b* (g/kg) | *c* ($h^{-1}$) | EDMD (g/kg) |
| FRF | 278 | 593 | 0.030 | 612 | 267 | 638 | 0.019 | 569 |
| TRRF | 250 | 501 | 0.021 | 489 | 224 | 369 | 0.025 | 416 |
| TRF | 243 | 448 | 0.025 | 488 | 212 | 421 | 0.020 | 408 |
| RTRRF | 254 | 482 | 0.029 | 536 | 241 | 483 | 0.016 | 433 |
| SEM | 8.9 | 40.2 | 0.003 | 13.4 | | | | |

RF = rumen fluid, FRF = fresh RF, TRRF = thawed residual RF, TRF = thawed RF, RTRRF = re-thawed residual RF, SEM = standard error to compare means for feed × inocula interaction

Table 4. Main effects of feed and inocula on DM degradation (DMD) (*a*, *b*, and *c*) constants and effective DMD (EDMD)

| Main effects | *a* (g/kg) | *b* (g/kg) | *c* ($h^{-1}$) | EDMD (g/kg) |
|---|---|---|---|---|
| **Feed effect** (*n* = 32) | | | | |
| Rapeseed meal | 256[a] | 506 | 0.026[a] | 531[a] |
| Grass nuts | 236[b] | 478 | 0.020[b] | 457[b] |
| SEM | 4.4 | 20.1 | 0.001 | 6.7 |
| **Inocula effect** (*n* = 16) | | | | |
| FRF | 272[a] | 616[a] | 0.024 | 591[a] |
| TRRF | 236[b] | 435[b] | 0.023 | 452[bc] |
| TRF | 227[b] | 434[b] | 0.023 | 448[c] |
| RTRRF | 247[b] | 482[b] | 0.023 | 485[b] |
| SEM | 8.9 | 40.2 | 0.003 | 13.4 |

RF = rumen fluid, FRF = fresh RF, TRRF = thawed residual RF, TRF = thawed RF, RTRRF = re-thawed residual RF, SEM = standard error of means to compare feed or inocula for each constant
Means with different superscripts in the same column differ at $P < 0.01$

Table 5. Means (*n* = 8) for the CP degradation (CPD) constants and effective CPD (ECPD) (g/kg) for feed × inocula interaction

| Inocula | Rapeseed meal | | | | Grass nuts | | | |
|---|---|---|---|---|---|---|---|---|
| | *a* (g/kg) | *b* (g/kg) | *c* ($h^{-1}$) | EDMD (g/kg) | *a* (g/kg) | *b* (g/kg) | *c* ($h^{-1}$) | EDMD (g/kg) |
| FRF | 276 | 619 | 0.020 | 570 | 242 | 556 | 0.019 | 498 |
| TRRF | 219 | 495 | 0.021 | 453 | 193 | 276 | 0.037 | 341 |
| TRF | 232 | 475 | 0.017 | 451 | 145 | 330 | 0.025 | 319 |
| RTRRF | 230 | 641 | 0.015 | 500 | 215 | 332 | 0.028 | 356 |
| SEM | 24.9 | 48.1 | 0.01 | 33.1 | | | | |

RF = rumen fluid, FRF = fresh RF, TRRF = thawed residual RF, TRF= thawed RF, RTRRF = re-thawed residual RF, SEM = standard errors to compare means for the feed × inocula interaction

Table 6. Main effects of feed and inocula on mean CP degradation constants (*a*, *b*, and *c*) and ECPD

| Main effects | *a* (g/kg) | *b* (g/kg) | *c* ($h^{-1}$) | EDMD (g/kg) |
|---|---|---|---|---|
| **Feed effect** (*n* = 32) | | | | |
| Rapeseed | 239[a] | 558[a] | 0.018[a] | 493[a] |
| Grass nuts | 199[b] | 373[b] | 0.028[b] | 390[b] |
| SEM | 12.4 | 24.1 | 0.003 | 16.5 |
| **Inocula effect** (*n* = 16) | | | | |
| FRF | 259[a] | 587[a] | 0.019 | 534[a] |
| TRRF | 206[ab] | 385[b] | 0.029 | 397[b] |
| TRF | 189[b] | 403[b] | 0.021 | 390[b] |
| RTRRF | 223[ab] | 486[ab] | 0.022 | 430[b] |
| SEM | 24.9 | 48.1 | 0.009 | 33.1 |

RF = rumen fluid, FRF = fresh RF, TRRF = thawed residual RF, TRF= thawed RF, RTRRF = re-thawed residual RF, SEM = standard error to compare means for the feed or inocula effects
Means with different superscripts in the same column differ significantly at $P < 0.01$

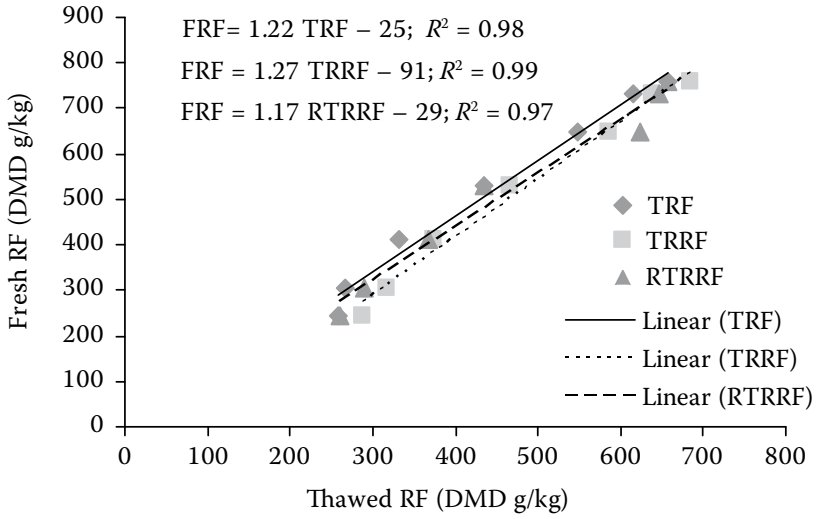


Figure 2. Relationship between fresh and different thawed rumen fluids (RF) for the *in vitro* DMD (g/kg)

FRF = fresh RF, TRF = thawed RF, TRRF = thawed residual RF, RTRRF = re-thawed residual RF

was significant ($P < 0.05$), the effects of inocula or feed × inocula were not significant ($P > 0.05$) for *c* (Tables 5–6). Rapeseed meal had higher ($P < 0.05$) *b* than grass nuts (Table 6). The main effects of feed or inocula were significant ($P < 0.001$) but not the feed × inocula ($P > 0.05$) for ECPD. As expected, rapeseed meal had higher ($P < 0.001$) ECPD than the grass nuts. FRF had the highest ($P < 0.001$) ECPD than all the thawed inocula (Table 6).

### Relationships for fresh vs. thawed inocula and CPD vs. DMD for each inocula

Figures 2 and 3 show excellent correlations ($R^2$ = 0.97 to 0.99) between the fresh inocula and each of the thawed inocula for DMD and CPD respectively. Figure 4 illustrates that CPD correlated very well with DMD for each corresponding inoculum. While the coefficient of determination for the relationship between CPD and DMD was very good ($R^2$ = 0.86) for TRF, it was excellent ($R^2$ = 0.99) for RTRRF.

## DISCUSSION

It appears from this study that the thawed rumen fluid could be used to estimate *in vitro* degradation of ruminant feeds over different times. This approach could be very helpful when access to fistulated or slaughtered animals as sources of fresh rumen fluid is not possible due to either ethical or management issues concerning the procurement of fresh rumen fluid. This study explored the possibility of using frozen rumen contents of four different slaughtered cattle as a source of rumen fluid to

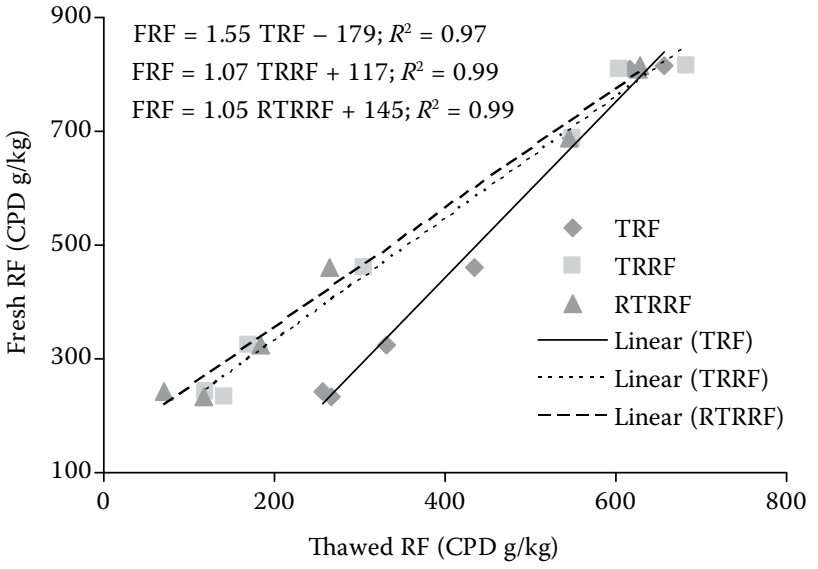


Figure 3. Relationship between fresh and different thawed rumen fluids (RF) for *in vitro* CPD (g/kg)

FRF = fresh RF, TRF = thawed RF, TRRF = thawed residual RF, RTRRF = re-thawed residual RF

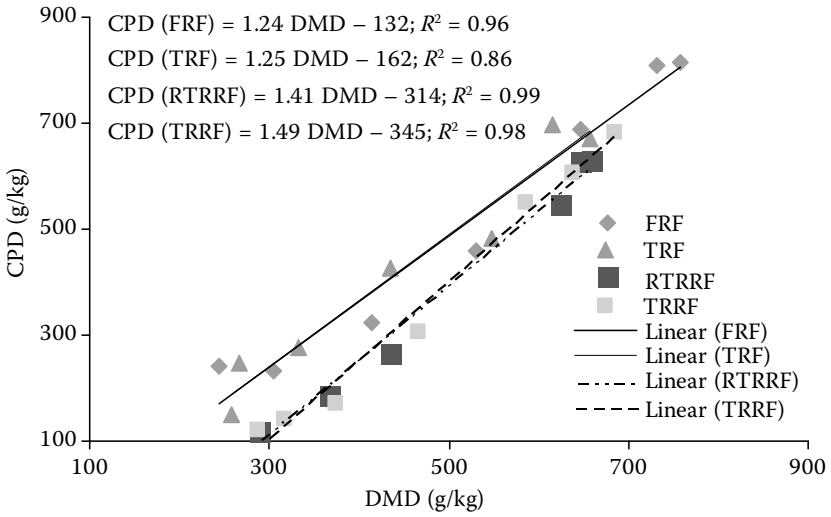


Figure 4. Relationship between DMD and CPD (g/kg) for each of the four inocula

FRF = fresh RF, TRF = thawed RF, TRRF = thawed residual RF, RTRRF = re-thawed residual RF

estimate degradation of only two contrasting feeds. The variability or similarity between slaughtered cattle was expected as the abattoir had little control on the day to day variations that existed between animal types and sizes that are presented to an abattoir for slaughter. The extent of variations between rapeseed meal and grass nuts for CP, EE, NDF, and ADF contents confirmed that these two feeds represented the contrasting composition for this purpose. In fact, the chemical components of these two feeds compared well with their published values (AFRC, 1993; Mohamed and Chaudhry, 2008).

The patterns of mean degradation with increasing time from 0 to 96 h for the fresh inocula also compared well with the published values of similar feeds that were tested *in sacco* in fistulated animals (AFRC, 1993; Chaudhry, 2007; Mohamed and Chaudhry, 2008). In fact the degradation of grass nuts in this study was similar to the earlier data for a different sample of the same batch of grass nuts (Chaudhry, 2008). The greater DM and CP degradation for the fresh than the thawed inocula at some or most hours indicated that the freezing followed by thawing did affect the feed degrading ability of rumen microbes in the thawed inocula. Likewise, the lower EDMD and ECPD for the thawed than the fresh inocula for each feed were expected due to the possible reduced or slower microbial activity in response to their unavoidable exposure to light and oxygen (Furchtenicht and Broderick, 1987) during the repeated processing of these inocula. The lower degradation values, in thawed RF following their storage at –20°C, could be because some microorganisms might not have survived or those that did survive had slower recovery from cold shocks. Although no microbial examination was not performed in this study, this assumption is supported by the findings of Furchtenicht and Broderick (1987) where chilled RF were lower in bacterial count and microbial dry weight. The effect of freezing on the rumen microbes can be attributed to the mechanical damage caused by the ice particles during freezing (Furchtenicht and Broderick, 1987) which can disrupt cell membranes and release intracellular contents as a result of heightened osmotic pressure (Scopes, 1988; Hristov et al., 2002).

Considering lower ECPD values for the thawed than the fresh inocula, it was possible that many nitrogen degrading microbes did not survive during the thawing process or the surviving microbes might have taken longer to recover during their *in vitro* incubations with these feeds. This assumption of late recovery of microbes in thawed inocula was evident towards the long hours of incubation where thawed inocula had increasing CP degradation in this study. Craig et al. (1984) have reported that inocula prepared from the whole rumen contents following chilling produced lower rates of protein degradation. The protozoa can deaminate amino acids in the rumen because they have more deaminating activity than bacteria (Hino and Russell, 1987) but due to their bigger size and surface area, the protozoa are most likely to be degraded during the thawing process. Scopes (1988) attributed the reduced deaminative activity of frozen rumen inoculum to enzyme inactivation that was not compensated even by the release of intracellular enzymes. Hristov et al. (2002) suggested that susceptibility of an inoculum to a preservation treatment depended on the principal enzyme be-

ing involved in the ruminal fermentation process. In contrast, rumen amylases are more resistant to freezing than cellulases or especially xylanases and higher amylase activity has been observed in previously frozen versus fresh samples from even fistulated cattle (Hristov et al., 2002). These apparently contradictory results may represent some compensation for the loss of enzyme activity by the release of intracellular enzymes (Hervas et al., 2005). Conversely, Dehority and Grubb (1980) reported no reduction in total viable bacterial counts after storing rumen contents at approximately 0°C for up to 8 h. Instead, total bacterial counts tended to increase which suggested that fermentative activity did not reduce during the chilling phase of these samples. In a comparable animal study, Cone et al. (2002) found that although substantial gas production occurred after storing RF for 24 h in a freezer, rate of gas production was considerably lower than in the fresh RF. In another comparable study, freezing reduced fermentative activity of RF for the *in vitro* gas production (Hervas et al., 2005). In general, gram-negative bacteria, which are predominant in the rumen, are considered to be particularly sensitive to freezing and thawing (Stewart et al., 1997). It is likely that the reduced activity in the thawed inocula of this study was a consequence of the decimation of gram-negative bacteria and protozoa during either freezing or thawing or a combination of both.

The greater EDMD or ECPD for rapeseed meal than the grass nuts were perhaps due to greater *b* and *c* values for rapeseed meal than the grass nuts in this study. In fact, rapeseed meal had a higher $P_{0.02}$ value with each inoculum than the grass nuts. The higher *b* value (slowly degradable fraction) for DM or CP for the fresh than the thawed inocula indicated that perhaps more microbial activity was present in fresh than the thawed inocula. However, the activity and the number or types of microorganisms in each inoculum were not investigated in this study. If we aim to increase the DMD values for the thawed as compared to the fresh RF, then the ways to increase the proportion of *b* value should be considered. This will require the use of procedures that could maintain the activity of microorganisms during the freezing of rumen fluid with a cryo-preservative to improve the feed degradation during the initial hours of incubation. It would also help to improve the freezing and thawing process by minimising the exposure of inocula to light and oxygen in order to maintain their microbial activity.

The higher *a* values for grass nuts than rapeseed meal were perhaps partly due to the fact that more fine particles were unavoidably discarded with the supernatants from grass nuts following the washing and centrifugation of undegraded residues. A great difficulty was experienced during the separation of the fine particles of residues from liquids in this study. This was especially true for those grass particles that were thinner but lighter and so they tended to float with the supernatant and did not settle with the residues despite centrifugation of tubes and so they were unavoidably removed with the supernatant. However, the results were comparable to earlier *in sacco* data (Madsen and Hvelplund, 1985) for even different samples of rapeseed meal and grass nuts. Fresh inoculum exhibited similar effective CP degradability values to those being previously reported for the *in sacco* or *in vitro* methods. The ECPD (g/kg) value of 546 for the *in sacco* method (Cone et al., 2002) compared well with the 480 being reported (Madsen and Hvelplund, 1985) for a different *in vitro* method for rapeseed. The ECPD obtained in this study for rapeseed meal and grass nuts were 493 and 379 respectively for the fresh inocula which indicated once again that more microbial activity was perhaps present in the fresh than the thawed inocula.

The high correlations between fresh inoculum and each of the thawed inocula (TRRF, TRF, and RTRRF) for DMD and CPD were very encouraging. These relationships as determined by $R^2$ values supported our hypothesis that the frozen rumen contents after their thawing can be used to predict *in vitro* DMD or CPD of different feeds. Likewise, it appeared that the *in vitro* DMD data can be used to predict CPD as well with a slight variation depending upon the type of thawed inocula. These results agreed well with Pereira and González (2004) who found that the *in sacco* DMD correlated well with the CPD values of different samples of dehydrated beet pulp and citrus pulp.

## CONCLUSION

Despite lower degradation of feeds for thawed than the fresh inocula, the strong correlations between these inocula suggest that the thawed inocula can be used to predict *in vitro* degradation of feeds when access to slaughtered or fistulated animals is restricted. However, it would be vital to test the suitability of thawed inocula for their routine use, under anaerobic conditions, to estimate degradation of numerous

feeds perhaps for their ranking and subsequent use to prepare nutritious diets for ruminant livestock.

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