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# Differences between the *in vitro* digestibility of extrusa collected from oesophageal fistulated steers and the forage consumed

*David B. Coates*[A,C] *and Robert J. Mayer*[B]

[A]Davies Laboratory, CSIRO Sustainable Ecosystems, PO, Aitkenvale, Townsville, Qld 4814, Australia.
[B]Department of Primary Industries and Fisheries, PO Box 1085, Townsville, Qld 4810, Australia.
[C]Corresponding author. Email: david.coates@csiro.au

**Abstract.** In a study that included $C_4$ tropical grasses, $C_3$ temperate grasses and $C_3$ pasture legumes, *in vitro* dry matter digestibility of extrusa, measured as *in vitro* dry matter loss (IVDML) during incubation, compared with that of the forage consumed, was greater for grass extrusa but not for legume extrusa. The increase in digestibility was not caused by mastication or by the freezing of extrusa samples during storage but by the action of saliva. Comparable increases in IVDML were achieved merely by mixing bovine saliva with ground forage samples. Differences were greater than could be explained by increases due to completely digestible salivary DM. There was no significant difference between animals in relation to the saliva effect on IVDML and, except for some minor differences, similar saliva effects on IVDML were measured using either the pepsin–cellulase or rumen fluid–pepsin *in vitro* techniques. For both $C_4$ and $C_3$ grasses the magnitude of the differences were inversely related to IVDML of the feed and there was little or no difference between extrusa and feed at high digestibilities (>70%) whereas differences of more than 10 percentage units were measured on low quality grass forages. The data did not suggest that the extrusa or saliva effect on digestibility was different for $C_3$ grasses than for $C_4$ grasses but data on $C_3$ grasses were limited to few species and to high digestibility samples. For legume forages there was no saliva effect when the pepsin–cellulase method was used but there was a small but significant positive effect using the rumen fluid–pepsin method. It was concluded that when samples of extrusa are analysed using *in vitro* techniques, predicted *in vivo* digestibility of the feed consumed will often be overestimated, especially for low quality grass diets. The implications of overestimating *in vivo* digestibility and suggestions for overcoming such errors are discussed.

## Introduction

Digestibility, a measure of energy value of feedstuff, is one of the basic quality attributes of feed. It is particularly important in determining other nutritional attributes such as intake, which is the main driver of productivity of ruminants and other herbivores. Voluntary intake in grazing animals has traditionally been estimated from digestibility and faecal output measurements (Minson 1990) though more recently intake has been measured by dosing cattle or sheep with alkanes (Dove 1994). In nutritional models such as GrazFeed, intake has been estimated as a function of digestibility and herbage mass (Freer *et al*. 1997). In turn, the intake of individual nutrients such as phosphorus (Hendricksen *et al*. 1994; McLean and Ternouth 1994; Ternouth and Coates 1997) is calculated from dry matter (DM) intake and the concentration of the nutrient in the feed. More recently, with the advent of near infrared spectroscopy (NIRS), laboratory measurements of digestibility are needed for the development of calibration equations for predicting digestibility of feeds and the diets of grazing ruminants. The ability to accurately measure or estimate the digestibility of feeds and diets is therefore of obvious importance. To this end *in vitro* techniques for measuring digestibility have been developed and continue to be widely used in forage and animal science. The two-stage rumen fluid–acid pepsin (RF-P) *in vitro* technique of Tilley and Terry (1963) and the two-stage pepsin–cellulase (P-C) procedure developed by Iowerth *et al.* (1975), together with their various modifications, continue to be used extensively. In forage science the *in vitro* analysis is carried out on ground samples of the forage but in grazing trials, *in vitro* digestibility determinations are usually made on samples of extrusa collected from oesophageal fistulated (OF) animals.

Reported differences between *in vitro* digestibility estimates of extrusa and the forage consumed vary but most reports indicate no difference or only small differences (Langlands 1966; Alder 1969; Barth and Kazzal 1971; Cohen 1979; Saul *et al*. 1986). However, the results of work conducted at the Lansdown Pasture Research Station south of Townsville and which involved *in vitro* digestibility determinations on samples of plucked grass and legume and of extrusa, could only be explained if the *in vitro* digestibility of extrusa differed from that of the feed. Because these results appeared to be contrary to the reports cited above there was an obvious and critical need to clarify the issue. On the basis of the initial results the

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following hypotheses were formulated: (i) the P-C *in vitro* digestibility of extrusa, collected from OF cattle grazing tropical pasture, is higher than that of the forage consumed (extrusa effect); and (ii) the extrusa effect is greater in grass forage than in legume forage.

This paper describes experiments that confirmed these hypotheses as well as experiments that investigated factors that may have contributed to the extrusa effect. The implications of the findings are also discussed.

## Materials and methods

### *In vitro* analytical procedures

The basic two-stage P-C *in vitro* digestibility procedure of Iowerth *et al.* (1975) was followed but with some modification. Duplicate 0.5-g DM samples of ground forage or extrusa were incubated at 40°C in 50-mL capped, sintered glass crucibles, first with acid pepsin for ~64 h and then with buffered Onozuka cellulase reagent for 48 h. The 64-h incubation in acid pepsin rather than the conventional 48 h was simply for convenience being from Friday afternoon to Monday morning. *In vitro* dry matter loss (hereafter referred to as IVDML) was calculated as:

$$\text{IVDML}\,\% = [1 - (\text{dry weight of undigested residue}/\text{dry weight sample})] * 100$$

Five standard samples of known *in vivo* dry matter digestibility (DMD) were included in each *in vitro* digestion run. These samples had previously been subjected to P-C *in vitro* digestion on 10 occasions and the mean IVDML was designated as the standardised IVDML for each of these samples. The inclusion of these standard samples in all subsequent P-C runs enabled calculated $\text{IVDML}_{\text{P-C}}$ values to be corrected for between-run differences.

A modified Tilley and Terry (1963) RF-P *in vitro* digestion procedure was conducted using an ANKOM Daisy Wheel incubator (Ankom Technology Corp., Macedon, NY, USA) where duplicate 0.5-g DM samples were weighed into dacron bags and incubated at 40°C, first in buffered rumen fluid for 72 h and then in acid pepsin for 48 h. The five standards used in the P-C runs were included in each of the four bottles in the Daisy Wheel in each run so that standardised IVDML values could be calculated by correcting for any between-bottle effect and any between-run effect ($\text{IVDML}_{\text{RF-P}}$).

No adjustment was made to convert IVDML to estimated *in vivo* DMD.

### *Processing of forage and extrusa samples*

Feed and extrusa were dried at 70°C in a forced draft oven before grinding in a laboratory hammer mill (Christy Turner, Ipswich, Suffolk, UK) to pass a 1-mm screen. Extrusa samples were dried on 200-mm-diameter enamel plates and the samples were spread across the surface of the plates and manually mixed and fluffed up at intervals of 2–3 h during the first day to hasten drying and prevent non-enzymic browning. Dry sample weights of feed or extrusa for *in vitro* analysis (~0.5 g) and of undigested residues following incubation were determined after overnight drying at 100°C. Samples were transferred from the oven into desiccators and allowed to cool to room temperature before weighing.

### *Experiment 1: P-C DM loss of feed and extrusa*

A collection of 22 different forage hays and two freshly cut forages were used to determine whether $\text{IVDML}_{\text{P-C}}$ of extrusa differed from that of the feed consumed. Seventeen hays and one fresh forage were $C_4$ grasses, two hays and one fresh forage were legumes, and three hays were $C_3$ grasses (Appendix 1). Approximately 0.5-kg subsamples of each hay, or the equivalent amount in DM of the fresh forages, were offered to four or more individually penned Droughtmaster OF steers following overnight fasting from feed and extrusa samples were collected in canvas collars lined with plastic bags. Collection continued until most or all of the offered feed was consumed. No more than three forages were fed on any one morning and where consumption of the offered feed was considered unsatisfactory such that the material collected might not be representative of the feed offered, the sample of extrusa was rejected. Retained samples of extrusa were handled in the same way as samples collected from grazed pastures. When the collars were removed, the contents were emptied into plastic basins and thoroughly mixed before subsampling into labelled, heavy duty, plastic bags which were then placed in an insulated container with cold bricks for transport back to the laboratory. For 11 of the $C_4$ grass hays and two of the $C_3$ grass hays duplicate subsamples were retained. At the laboratory, a sample of extrusa from each feed was transferred to a freezer for storage until processing (drying and grinding) and analysis. Where duplicate subsamples were retained, the second subsample was dried without freezing to determine whether freezing had any effect on $\text{IVDML}_{\text{P-C}}$. Samples of dried, ground extrusa were individually analysed in duplicate to determine $\text{IVDML}_{\text{P-C}}$. Representative samples of the feed offered were also retained for processing and *in vitro* analysis.

### *Experiment 2: effect of wetting, mastication and saliva on* in vitro *digestibility*

Having confirmed a positive extrusa effect on $\text{IVDML}_{\text{P-C}}$ for $C_4$ grass diets, a simple test was conducted to determine which of the processes during the collection of extrusa may have been responsible for the increase. Four $C_4$ grass hays that had been chaffed were subjected to the following three treatments: (i) simulated mastication and wetting with water: a subsample of each chaffed hay, together with a small amount of demineralised water, was put into a container and mechanically agitated with a manually operated food processing device for a period of a few minutes to try and simulate the physical fracturing of plant material that occurs during mastication; (ii) simulated mastication and wetting with saliva: treated as in (i) except that recently collected bovine saliva was used instead of demineralised water; and (iii) control – no simulated mastication and no wetting with either water or saliva.

The mechanically treated and wetted samples were dried in a similar manner to samples of extrusa and all samples were ground and analysed for $\text{IVDML}_{\text{P-C}}$.

### *Experiment 3: between-animal saliva effects on* in vitro *digestibility*

The results of experiment 2 (Table 1) indicated that the increase in $IVDML_{P\text{-}C}$ of extrusa compared with the feed ingested was due to the effect of saliva (saliva effect) and not the wetting and fracturing effect of mastication. An experiment was conducted to determine whether there are between-animal differences in saliva effect. In experiment 1 it was possible to determine whether there were any consistent between-animal differences in $IVDML_{P\text{-}C}$ of extrusa. If such differences occurred they may have been due to differences in the composition or potency of the saliva. However, they may have also arisen from differences in the recovery rate of ingested feed through the oesophageal fistula, differences that may have lead to differences between individual OF steers in the composition of extrusa. It was therefore necessary to test for between-animal differences in saliva effect unconfounded by other factors.

Saliva was collected from six OF steers, cooled for transport back to the laboratory, and then saliva from each of the six steers was mixed with 5–10-g samples of dry ground material from three $C_4$ grass hays and one $C_3$ grass hay. Sufficient saliva was added so that feed samples were completely moistened and a small amount of free saliva was apparent. These samples were left to stand on the laboratory bench for a minimum of 3 h, refrigerated overnight and then dried at 70°C in a forced draft oven. Each of the 28 samples, which included the four untreated hays, was analysed for $IVDML_{P\text{-}C}$.

### *Experiment 4:* in vitro *digestibility of feeds mixed with bovine saliva*

The results of experiment 3 indicated that the effect of bovine saliva on $IVDML_{P\text{-}C}$ was not influenced by the animal producing the saliva (see Results section and Table 2). Additional tests were conducted to determine more extensively the effect of saliva on *in vitro* digestion using a suite of hays studied in conventional *in vivo* digestibility trials (Coates 1998) where samples of the hays offered were set aside each day of an 8-day collection period, bulked and milled to provide standards of known *in vivo* digestibility. In the first of the tests (experiment 4A), *in vitro* analysis was conducted to determine $IVDML_{P\text{-}C}$ of the *in vivo* standards (24 $C_4$ grass hays, two $C_3$ grass hays, and nine pasture legume hays) with and without saliva treatment (Appendix 1). In the second test (experiment 4B), hay samples from the same hay batches used in the *in vivo* digestibility trials were milled through a 1-mm screen and mixed well. Because of within-batch variability in feed quality, $IVDML_{P\text{-}C}$ of these ground samples differed to a small extent from the *in vivo* standards described above. There were also two additional $C_4$ grass hays and two additional $C_3$ grass hays in experiment 4B (Appendix 2). A subsample from each of the ground samples was treated with freshly collected saliva as in experiment 4A. Treated and untreated subsamples were again analysed for $IVDML_{P\text{-}C}$. In addition, treated and untreated subsamples were also analysed using the RF-P technique to determine whether bovine saliva had similar effects on $IVDML_{RF\text{-}P}$ as it had on $IVDML_{P\text{-}C}$.

**Table 1. Dry matter loss (%) with pepsin–cellulase *in vitro* digestion (IVDML) of untreated forage samples, samples treated with demineralised water and simulated mastication, and samples treated with saliva and simulated mastication**

Forages are: *Urochloa mosambicensis*, *Panicum maximum* cv. Gatton, *Chloris gayana* cv. Katambora. Mean values followed by the same letter do not differ significantly ($P > 0.05$)

| Forage | IVDML (%) | | |
|---|---|---|---|
| | Untreated | Demineralised water | Saliva |
| Urochloa hay (Uro 1) | 41.7 | 39.7 | 48.8 |
| Gatton panic hay | 39.3 | 40.7 | 47.4 |
| Katambora Rhodes | 43.0 | 39.0 | 55.3 |
| Native grass hay | 29.2 | 29.5 | 39.1 |
| Mean | 38.3a | 37.2a | 47.7b |

### *Animal welfare*

Approval was granted by the Lansdown Research Station Animal Ethics committee for the conduct of the experiments using OF steers.

### *Statistical analysis*

Where comparisons were made on nine or more forages ($C_4$ grasses in experiments 1 and 4, pasture legumes in experiment 4, and frozen *v.* fresh samples in experiment 1) regression analysis was used to compare treatment effects on IVDMD. Where treatment comparisons involved only four or fewer forages, ANOVA was used to test for treatment differences. The latter occurred in experiments 2 (wetting, mastication and salivary effects) and 3 (between-animal salivary effect), and in sections of experiment 1 ($C_3$ grasses and legume species) and experiment 4 ($C_3$ grasses).

## Results

### *Experiment 1: P-C DM loss of feed and extrusa*

#### *$C_4$ grass forage*

The range in IVDML of the 18 feeds was 28.9–58.0% whereas that of the frozen extrusa was 38.0–63.4% (Appendix 1).

**Table 2. Dry matter loss (%) with pepsin–cellulase *in vitro* digestion (IVDML) of different grass forages (columns) when treated with saliva collected from different oesophageal fistulated steers (rows) and IVDML of the untreated forages**

Mean values followed by the same letter do not differ significantly ($P > 0.05$)

| Treatment | IVDML (%) | | | | |
|---|---|---|---|---|---|
| | Buffel grass (*Cenchrus ciliaris*) | Indian couch (*Bothriochloa pertusa*) | Purple pigeon (*Setaria incrassata*) | Wheat (*Triticum aestivum*) | Mean |
| Steer 21 | 46.7 | 45.7 | 53.4 | 65.5 | 52.8a |
| Steer 25 | 47.2 | 43.9 | 51.7 | 63.4 | 51.6a |
| Steer 38 | 46.2 | 42.7 | 52.5 | 66.1 | 51.9a |
| Steer 46 | 47.4 | 41.8 | 52.9 | 64.6 | 51.8a |
| Steer 58 | 47.4 | 43.3 | 53.2 | 66.0 | 52.5a |
| Steer 93 | 46.9 | 41.8 | 53.9 | 65.2 | 52.0a |
| No saliva | 40.5 | 34.5 | 47.6 | 65.1 | 46.9b |

The IVDML of extrusa was higher than that of the feed offered but the magnitude of the difference varied with feed IVDML, the differences being greater at low digestibilities such that the linear regression of IVDML of extrusa on IVDML of feed had a slope significantly different from one ($P < 0.05$). The regression equation of

$$IVDML_{P\text{-}C}\ \text{extrusa} = 0.7757(IVDML_{P\text{-}C}\ \text{feed}) + 17.83$$

indicated that differences between extrusa and feed were relatively small at high digestibilities (no difference at feed IVDML of 79%) increasing to >10 percentage units when IVDML of feed was lower than 35% (Fig. 1).

### $C_3$ grasses

There were only three $C_3$ grasses tested with a range in feed IVDML of 57.1–70.6% (Appendix 1). There were insufficient samples to test whether extrusa differed significantly from feed but the mean IVDML of extrusa was 4% higher than that of the feed and individual points lay on, or close to, the linear regression line for the $C_4$ grasses (Fig. 1).

### $C_3$ pasture legumes

Only three pasture legumes were tested (Appendix 1). The IVDML of the feed had a range of 47.3–66.8% and a mean of 54.6%. Although the extrusa mean was nearly 2% lower than the feed mean, this was due to a 6 percentage unit difference in fresh Leucaena (*Leucaena leucocephala*) (Fig. 1). Samples of extrusa from the fresh Leucaena were collected from five OF steers with IVDML values ranging from 58.3 to 63.3% compared with the feed value of 66.8%. It seems that the reduced IVDML of extrusa was likely due to a lesser recovery rate of cell contents compared with cell walls. For the other two pasture legumes (*Stylosanthes scabra* cv. Seca and *Neonotonia wightii*), IVDML of feed and extrusa were similar indicating no extrusa effect. Individual points for all three pasture legumes lay outside the 95% confidence limits of the linear regression developed for $C_4$ grasses.

### Frozen v. fresh extrusa

There were 35 paired samples of fresh and frozen extrusa representing the 11 $C_4$ grasses, two of the $C_3$ grasses, one of the pasture legumes, and different OF steers. The IVDML of fresh extrusa ranged from 45 to 75%. The regression of IVDML of frozen *v.* fresh extrusa did not differ significantly from the 1 : 1 line indicating that freezing did not have a significant effect on IVDML (Fig. 2).

### Experiment 2: effect of wetting, simulated mastication and saliva on in vitro digestibility

The IVDML of the four $C_4$ grass feeds treated with demineralised water and simulated mastication did not differ significantly from those of the untreated samples ($P > 0.05$, means of 37.2 and 38.3%, respectively), whereas IVDML of feed treated with saliva and simulated mastication (mean value of 47.7%) was significantly higher than both the untreated and demineralised water treatments ($P < 0.001$) (Table 1). Therefore, it can be concluded that, of the processes occurring to feed when extrusa is collected and stored, neither wetting *per se*, nor mastication, nor freezing, was responsible for the observed increase in IVDML. Rather, the increase can be attributed to the chemical action of saliva.

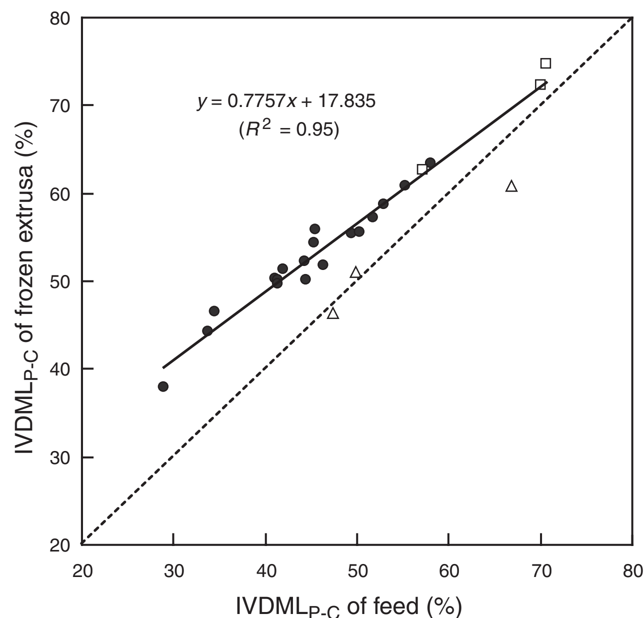


**Fig. 1.** Dry matter loss during pepsin–cellulase *in vitro* digestion of extrusa samples regressed against that of the feed consumed in experiment 1 (●, $C_4$ grasses; □, $C_3$ grasses; △, $C_3$ pasture legumes). The regression line for the $C_4$ grasses and the 1 : 1 line are shown.

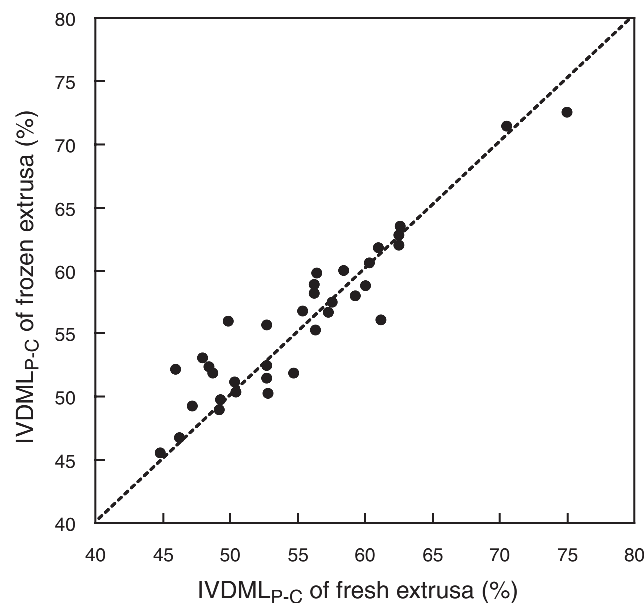


**Fig. 2.** Dry matter loss during pepsin–cellulase *in vitro* digestion ($IVDML_{P\text{-}C}$) of frozen extrusa plotted against $IVDML_{P\text{-}C}$ of fresh extrusa (experiment 1). The 1 : 1 line is shown.

### *Experiment 3: between-animal saliva effects on P-C* in vitro *digestibility*

Animal effect, with regard to the source of saliva and its influence on IVDML, was not significant ($P > 0.05$, Table 2). The extent of between-animal variation with respect to the effect of saliva was in most cases no larger than the variation between duplicate samples in routine *in vitro* analysis where the results from duplicates were classed as acceptable when within 2 percentage units of one another. Certainly the between-animal variation for IVDML of feed mixed with saliva was a lot less than the between-animal variation for IVDML of extrusa (data not shown). The higher variation observed with extrusa samples from the same feed was probably due largely to differences in the recovery rate and fractionation of ingested feed (see Discussion section).

### *Experiment 4:* in vitro *digestibility of feeds mixed with bovine saliva*

#### *$C_4$ grasses*

For P-C digestion, the relationship between IVDML of feed samples mixed with saliva and untreated samples was similar to that between extrusa and feed where the difference increased with decreasing digestibility of the feed. The results for experiments 4A and 4B (Appendix 2) followed the same pattern so that when $IVDML_{P\text{-}C}$ of feed mixed with saliva was regressed against that of untreated feed the slopes did not differ. However, there was a significant difference in displacement between the regressions for 4A and 4B such that, within the range in feed $IVDML_{P\text{-}C}$ of 30–60%, there was a bias of 3.6% between the two regression lines (Fig. 3). The regression line of IVDML of extrusa on IVDML of the feed consumed was parallel to the regressions for 4A and 4B and positioned approximately midway between the 4A and 4B lines (Fig. 3).

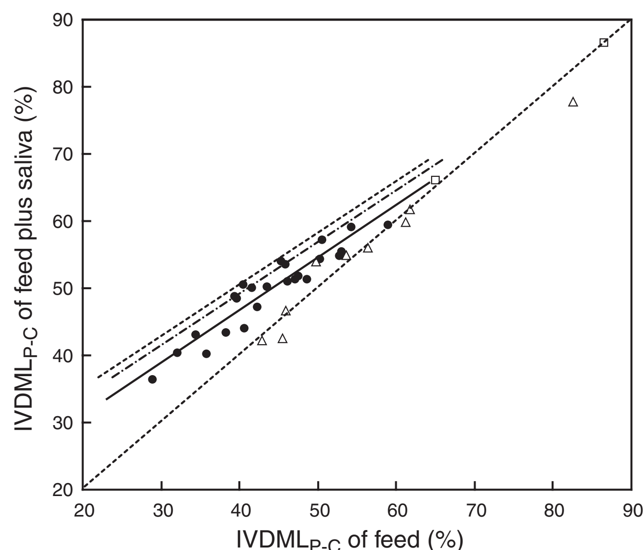


**Fig. 3.** Dry matter loss during pepsin–cellulase *in vitro* digestion of feed plus saliva plotted against that of the untreated feed in experiment 4A (●, $C_4$ grasses; □, $C_3$ grasses; △, $C_3$ pasture legumes). Regression lines for the $C_4$ grasses are shown for: experiment 4A (solid bold line); experiment 4B (dash line); experiment 1, extrusa on feed (dot-dash line).

Results using rumen fluid and pepsin (Appendix 2) followed the same pattern as the results for the P-C technique and the regression of $IVDML_{RF\text{-}P}$ for saliva treated on untreated feed was not significantly different from that using P-C in either slope or displacement ($P > 0.05$). However, the actual correlation was much weaker using the rumen fluid method ($R^2$ of 0.49 and 0.78 for rumen fluid and P-C methods respectively), so that differences between $IVDML_{RF\text{-}P}$ of treated and untreated feed for individual $C_4$ grass diets were often unexpectedly large, reaching 19.8 percentage units for one Rhodes grass sample.

#### *$C_3$ grasses*

In experiment 4A, $IVDML_{P\text{-}C}$ of the two saliva-treated $C_3$ grass samples were similar to those of the untreated samples (66.0 and 65.1% for wheaten hay, and 86.5 and 86.6% for oaten hay for treated and untreated samples respectively). In experiment 4B involving four $C_3$ grasses, there was a significant ($P < 0.05$) and positive saliva effect on IVDML for both the P-C and rumen fluid methods. Differences ranged from 1.6 to 8.5 percentage units averaging 5.6 for $IVDML_{P\text{-}C}$, and from 2.6 to 10.1 percentage units averaging 6.7 for $IVDML_{RF\text{-}P}$ (Appendix 2). The IVDML values for the $C_3$ grasses were outside the range of those for the $C_4$ grasses but regression slopes for IVDML of saliva treated on untreated feed did not differ significantly for either the P-C or rumen fluid methods. Moreover, a visual inspection of IVDML for feed plus saliva plotted against IVDML of untreated feed (data not shown) did not suggest the $C_3$ and $C_4$ grasses behaved differently with respect to saliva effect.

#### *$C_3$ pasture legumes*

The results are tabled in Appendix 2. The regression of P-C IVDML of saliva-treated legume on untreated feed did not differ from the 1 : 1 relationship in either experiment 4A or 4B, indicating no significant saliva effect on $IVDML_{P\text{-}C}$. In 4B the slope of the regression of $IVDML_{RF\text{-}P}$ of saliva treated on untreated feed samples did not differ significantly from 1 ($P > 0.05$) but the vertical displacement from the 1 : 1 line was significant ($P < 0.05$) and indicated a positive though small saliva effect on $IVDML_{RF\text{-}P}$ (mean ± s.d., 2.2 ± 1.54%).

#### *Rumen fluid* v. *P-C*

Differences between the rumen fluid and P-C IVDML averaged (±s.d.) 3.1 ± 2.6 percentage units (Fig. 4). For the $C_4$ grasses $IVDML_{RF\text{-}P}$ averaged 3.1 and 2.2 percentage units higher than $IVDML_{P\text{-}C}$ for the untreated and saliva-treated feeds, respectively, and the differences were significant ($P < 0.01$). For the $C_3$ grasses IVDML did not differ between the *in vitro* techniques for either untreated or saliva-treated samples. For untreated legume feeds there was no difference between the two *in vitro* methods but for saliva-treated legumes $IVDML_{RF\text{-}P}$ was significantly higher than $IVDML_{P\text{-}C}$ ($P < 0.01$, means of 57.4 and 55.5% respectively).

## Discussion

Published reports on the existence or otherwise of differences between the *in vitro* digestibility of extrusa and the forage

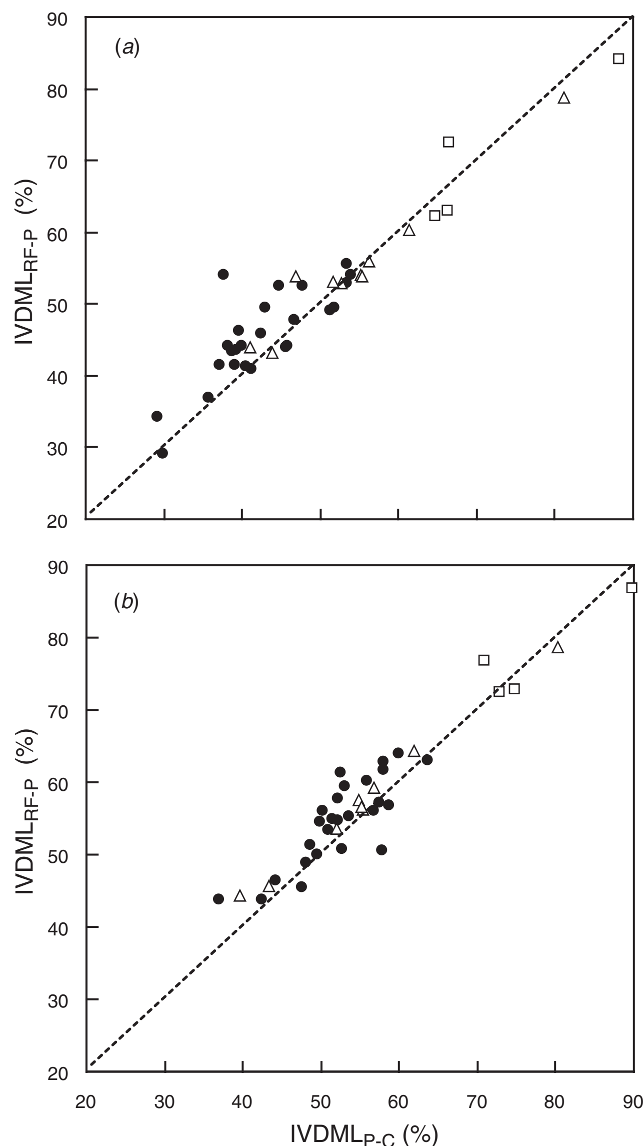


**Fig. 4.** Relationship between *in vitro* dry matter loss using either rumen fluid–pepsin or pepsin–cellulase for (*a*) feed samples without saliva and (*b*) feed samples treated with saliva in experiment 4B (●, $C_4$ grasses; □, $C_3$ grasses; △, $C_3$ pasture legumes).

consumed are somewhat equivocal. Alder (1969), Cohen (1979) and Saul *et al*. (1986) reported no difference between extrusa and forage. On the other hand, Langlands (1966) reported the organic matter digestibility (OMD) of the extrusa to be on average 1.6 percentage units higher than that of the feed 'when the complete (solid plus liquid fractions) sample was taken and 3.3 units lower when only the solid fraction was considered'. Barth *et al*. (1970) working with five legume and four temperate grass species reported significantly lower *in vitro* DMD for legume extrusa than for legume feed (average difference of 4 percentage units) but non-significantly higher DMD for grass extrusa than grass feed. Importantly, the difference (extrusa effect) for tall fescue (*Festuca arundinacea*) that had the lowest feed DMD of the grasses tested was 10–12 units. Barth and Kazzal (1971) again reported higher extrusa digestibility for tall fescue (mean difference of 2.8 units over five sampling occasions) but a non-significant difference of only 1 unit for orchard grass (*Dactylis glomerata*). Scales *et al*. (1974) reported variable effects when working with lucerne (*Medicago sativa*), blue grama (*Bouteloua gracilis*) and crested wheatgrass (*Agropyron desertorum*). In 1 year there was a small but non-significant increase in DMD of extrusa compared with feed for samples from all three species but in the previous year there was a significant interaction between forage species and extrusa effect with an increase in blue grama extrusa (4–5 units) and a decrease in lucerne extrusa (3 units). Apart from the extrusa effect on DMD reported by Barth *et al*. (1970) for tall fescue, any differences between extrusa and feed in the *in vitro* digestibility were small to moderate. Moreover, apart from the work of Cohen (1979) where subtropical $C_4$ grasses were included with various legume species, the forages tested in published reports were confined to $C_3$ species grown in cool climates.

The results from this study were clearly quite different from previous reports in that large IVDML differences between extrusa and feed were common. Moreover, the broad range in feed digestibility and the inclusion of three distinct classes of feeds ($C_4$ grasses, $C_3$ grasses and pasture legumes) provided some critical insights. Another important distinction between this study and previous studies was the use of IVDML determinations during *in vitro* incubation rather than predicted *in vivo* digestibility estimates. Although five *in vivo* DMD standards were included in every *in vitro* run, these were used primarily to remove between run variation in IVDML and not for the purpose of converting IVDML values to predicted *in vivo* DMD values, at least not in the first instance. IVDML values for both the P-C and RF-P techniques were substantially lower than *in vivo* DMD at low digestibility and substantially higher at high digestibility (data not shown). This meant that the magnitude of differences in IVDML were greater than differences in predicted *in vivo* DMD. In fact, an in-house regression for converting IVDML$_{P\text{-}C}$ values to estimated *in vivo* DMD had a slope of ~0.5 so that any difference in IVDML between samples or treatments is approximately twice the comparable difference in predicted *in vivo* DMD. Therefore, compared with published reports, the extrusa or saliva effect in this study was magnified by a factor of 2 but statistical significance was not affected by the magnification.

For $C_4$ (tropical) grasses, IVDML determinations made on samples of extrusa were higher than estimates made on the forage consumed and the magnitude of the difference was inversely related to digestibility level, being large at low digestibility and small or non-existent at high digestibility. Similar results were achieved with both the P-C and RF-P *in vitro* techniques. Although differences between extrusa and forage of the $C_3$ grasses tested were generally smaller than for the $C_4$ grasses, this was probably partly a consequence of their higher digestibility as the results for the $C_3$ grasses fitted the same pattern described for the $C_4$ grasses (Fig. 1). For the $C_3$ pasture legumes tested the estimates of IVDML did not differ for paired extrusa-feed samples when the P-C method was used and this remained true for samples of both

high and low digestibility. Although there was a significant and positive saliva effect with the RF-P method, the differences were small, averaging only 2.2 percentage units, and feed digestibility did not have a significant effect on the difference. In the study of Langlands (1966) that included temperate grasses and legumes, there was a wide range in predicted feed digestibility (49.5–83.5%) but the average increase in OMD of extrusa samples compared with feed was only 1.6 units. However, the feed samples containing grass were grass/legume mixtures and this may partly explain the absence of large differences between the digestibility of extrusa and feed samples.

In the study of Alder (1969), the overall conclusion was that 'the *in vitro* OMD of the grass offered and of the extrusa samples were identical'. However, the results were consistent with the present study in that feed digestibility was over 77% and at that digestibility level little or no difference would be expected. Saul *et al*. (1986) also reported no difference in digestible organic matter (DOM) between extrusa and feed from pen trials with OF sheep fed temperate grass hays, fresh grass, or fresh lucerne. Feed digestibility ranged from 55 to 85% DOM. Although the difference in DOM between extrusa and feed was not significant it was notable that DOM was slightly higher (1–6%) in extrusa for eight of nine sample pairs where feed DOM was under 70%. The remaining nine sample pairs had DOM higher than 75% where, according to the results of the present study, higher digestibility for extrusa would not be expected. The paper did not identify which samples were grass and which ones were lucerne.

The published results hardest to reconcile with the results of the present study were those of Cohen (1979) where one sample of lucerne hay and 14 mixtures of freshly plucked herbage from three legume species and five $C_4$ grass species were tested for extrusa effect on OMD using the Tilley and Terry (1963) method. Digestibility of the feed samples ranged from 38 to 85% and the regression of feed OMD on extrusa OMD had a slope that did not differ significantly from unity and an intercept that did not differ significantly from zero. Information on the grass/legume proportions of the different mixtures was not provided but it might be reasonably speculated that samples with feed digestibility below 50% ($n$ = 7) were probably dominated by $C_4$ grass and for only one such sample was extrusa OMD substantially higher than feed OMD (45.5 *v.* 34.1%). It is difficult to explain why extrusa digestibilities were not higher than the feed digestibilities for the poorer quality samples in the Cohen study considering the differences recorded in the present study. Had OMD rather than IVDML been measured in the present study smaller differences between feed and extrusa would be expected because of the small proportion of OM in salivary DM (Bailey and Balch 1961*a*, 1961*b*) but this would certainly not explain the major differences between the Cohen results and those of the present study. The drying temperature used by Cohen was 50°C compared with 70°C in this study. However, based on the results of Barth *et al*. (1970) higher drying temperature reduces rather than increases *in vitro* digestibility of extrusa. One possibility might be related to the climatic conditions under which the pasture species were grown: Cohen's pasture species were grown in northern New South Wales at latitude 29°41′S whereas all the forages used in the present study were grown at lower latitudes, many in the tropics. It is known that high temperature growth conditions reduces digestibility (Wilson *et al*. 1976; Ford *et al*. 1979). However, although digestibility of grasses may decline with high temperature growth conditions it does not necessarily follow that the extrusa effect on *in vitro* digestibility should become more pronounced when grasses are grown at high temperature.

The sequence of experiments conducted in the present study was designed to identify the agent or agents responsible for extrusa effect on *in vitro* digestibility. The mechanical effects of mastication and/or freezing of wet extrusa may have resulted in greater exposure of the plant material to the digestive enzymes compared with the feed samples. However, in the tests conducted, no such effect could be detected and the experiments where feed samples were treated with bovine saliva clearly identified saliva as the primary causative agent. One of the problems inherent in comparing analyses performed on paired extrusa-feed samples is the possible confounding effect of fractionation of the feed sample during the process of collecting extrusa. Fractionation results from different recovery rates of the feed components where recovery rate is defined as the DM proportion of feed component ingested that is recovered in the sample of extrusa. Recovery rate can be highly variable (Arnold *et al.* 1964; Little 1972) with between-animal, between-plant species and between-plant part effects (Arnold *et al.* 1964) but of particular concern is the potential influence of differential recovery rate of cell contents *v.* cell wall material on digestibility. In experiment 4 any confounding due to fractionation was eliminated by treating feed samples with saliva rather than collecting extrusa samples *per se*. The similarity between the results obtained from feed samples mixed with saliva in experiment 4 and extrusa samples in experiment 1 suggests that any confounding between extrusa effect and fractionation was probably minimal for most of the feeds.

Because salivary DM (~1% of fresh weight; Bailey and Balch 1961*a*, 1961*b*) is completely soluble, extrusa samples, or feed samples with added saliva, will have higher *in vitro* digestibility than the feed without saliva unless chemical reactions occur due to the presence of saliva (e.g. during drying) that render certain components of the feed less digestible. When Playne *et al*. (1978) investigated factors affecting the nylon bag technique for measuring rumen digestion of feed and extrusa samples, all DMD values were corrected for salivary DM. Extrusa samples were collected in screen bottomed bags and saliva accounted on average for ~4% of extrusa DM. Although the authors concluded that DM added from saliva therefore causes errors of around 4 percentage units in estimates of digestion if no correction is made, the error is in fact only 2% at feed DMD of 50%. The error is reduced at high feed digestibility and increased at lower feed digestibility. In the present study, salivary DM in milled feed samples treated with saliva in experiment 4 averaged 4.35% (range 2.2–8.0%, s.d. 1.2%). This would lead to feed IVDML of saliva-treated samples being

overestimated on average by 2.2 units when measured IVDML was 50%, by 1.3 units at 70% IVDML and by 2.6% at 40% IVDML. Therefore, the large increases in IVDML of saliva-treated samples in the present study could not be accounted for by the amount of salivary DM added to the samples. When measured *in vitro* digestibility of extrusa samples is lower than that of the feed ingested, one possible cause would be a lower recovery rate during sampling of the more digestible components of the feed compared with the less digestible components as discussed previously. In particular, a lower recovery rate of cell contents than for cell wall material could be expected and this is likely to be most prevalent in high quality green feeds. Barth *et al*. (1970) reported a positive extrusa effect on *in vitro* digestibility when fresh grass was fed to OF steers but a negative extrusa effect when fresh legume forage was fed. In that study extrusa samples were allowed to drain for 3–4 h after collection. Although the paper did not provide data on feed neutral detergent fibre levels, acid detergent fibre analyses indicated that recovery rate of cell contents was probably lower in the legume extrusa than in the grass extrusa and that increased digestibility due to the addition of salivary DM was insufficient to compensate for the loss of cell contents. Scales *et al*. (1974) also reported a significant negative extrusa effect on *in vitro* DMD when fresh alfalfa was fed on one occasion but no effect on another occasion. On the occasion of the significant negative effect, there was also a significant positive extrusa effect on acid detergent fibre indicating the possibility of less recovery of digestible components, probably cell contents, than of indigestible components. In the present study the most notable instance of a negative extrusa effect on *in vitro* IVDML was with freshly cut *L. leucocephala* in experiment 1 (Appendix 1). Fractionation due to the loss of cell contents during collection would be the most likely explanation. In the present study there was only one instance of a negative effect on digestibility when milled feed was treated with saliva. In this instance IVDML values of 82.6 and 77.8% were recorded for untreated and saliva-treated lucerne, respectively (experiment 4A). Obviously the negative effect in this instance had to be associated with chemical changes to the sample treated with saliva.

Clearly the legumes behaved differently to grasses regarding the salivary effect on IVDML. The small increase in rumen fluid IVDML of saliva-treated legume samples compared with the untreated samples (mean of 2.24 percentage units) was consistent with the expected effect of salivary DM on IVDML. However, the absence of any increase when using the P-C method suggested that there must have been some reaction between the added saliva and the legume feed that rendered the feed slightly less digestible to the two-stage P-C incubation and sufficient to counteract the addition of salivary DM. There is no obvious explanation why saliva should render legumes less digestible but grasses more digestible to P-C *in vitro* incubation but the results indicated that to be the case.

### Implications

The results from this study highlighted problems in making valid estimates of *in vivo* digestibility from samples of extrusa. The problems are caused by: (i) an effect of saliva on *in vitro* digestion; (ii) the effect being different for legumes than for grasses; and (iii) the magnitude of the effect on grasses being inversely related to digestibility level. When *in vitro* analyses are used to predict *in vivo* digestibility of feed samples, IVDML measurements are adjusted to estimated *in vivo* digestibility through the inclusion of standards of known *in vivo* digestibility and corrections using the appropriate regression equation relating the *in vitro* measurements to *in vivo* digestibility (McLeod and Minson 1978). When extrusa samples are analysed, regression equations relating feed IVDML to known *in vivo* digestibility will not be appropriate for calculating predicted *in vivo* digestibility except for legume samples or for grass samples of high digestibility where IVDML of feed and extrusa differ little. Logically there would be two ways to overcome this problem. The first would be to use extrusa samples of the *in vivo* standards, or the *in vivo* standards treated with saliva, for inclusion in the *in vitro* digestion runs rather than the untreated *in vivo* standards. An alternative would be to adjust the IVDML of the *in vivo* feed standards using regressions relating IVDML of extrusa to IVDML of the feed consumed. For example, the regression based on the grass data ($C_4$ and $C_3$ grasses combined) of experiment 1 in this study was:

$$\text{IVDML grass extrusa } \% = 0.800 \text{ (IVDML grass feed } \%) + 16.8 \; (R^2 = 0.98;\ \text{s.e.} = 1.35\%)$$

The high coefficient of determination indicated that IVDML of extrusa can be accurately calculated from feed IVDML provided saliva potency remains constant. Although the results of experiment 2 in this study indicated no significant between-animal effect on the magnitude of salivary effect, the potency of saliva collected at different times was not tested. The P-C $C_4$ grass results from experiments 4A and 4B suggest that salivary potency may vary (Fig. 3). The linear regression for IVDML of saliva-treated feed on untreated feed in 4A was parallel to the 4B regression line but the 4A regression line was significantly displaced from the 4B regression line (bias of 3.6 percentage units). Separate batches of saliva were used in 4A and 4B. If salivary potency varies it would be important to measure the salivary effect using saliva collected at the same time as extrusa samples were collected.

Further difficulties arise when extrusa samples are grass/legume mixtures. In tropical $C_4$ grass/$C_3$ legume mixtures the extrusa grass/legume proportions can be determined from the $^{13}C/^{12}C$ carbon isotope ratio (Jones 1981) and it is possible to calculate feed IVDML from extrusa IVDML (Appendix 3) provided a reasonable estimate of the difference between grass and legume IVDML is determined on plucked samples. This assumes that the difference between IVDML of the plucked samples will be the same as or similar to the difference between the grass and legume dietary fractions. Other methodologies such as microscopic examination (Hamilton and Hall 1975), alkane analysis (Dove and Mayes 1991; Dove 1994), or NIRS (Coates and Dixon 2008) can be used to estimate grass/legume proportions in extrusa.

Estimates of predicted *in vivo* digestibility are often made in conjunction with faecal output measurements to determine

nutrient intake (DM, metabolisable energy, protein or minerals) and any error in predicted *in vivo* digestibility will be carried through in the calculation of nutrient intake. For a given error in percentage units DMD, the resulting error in calculated DM intake will increase with increasing digestibility. However, because the extrusa effect on predicted digestibility is more pronounced at low digestibility the largest errors in predicted intake are likely to occur for low quality forages. Mathematically it can be shown that the percentage error in calculated intake is equal to the error in estimated DMD as a percentage of estimated indigestibility (100-DMD). Thus, if estimated DMD is 70% and actual DMD is 67%, there will be a 10% error in calculated DM intake. An overestimate of 6 percentage units DMD when actual DMD is 50% will translate into an intake error of 13.6%. These calculations demonstrate that the consequences of overestimating digestibility because of the extrusa effect are quite serious in relation to calculated nutrient intakes and efforts need to be made to overcome such errors.

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**Appendix 1. Feeds and *in vitro* dry matter losses during *in vitro* digestion with pepsin–cellulase for untreated feed (control), extrusa, and saliva-treated feed in experiment 4A**

| Feed species | Experiment 1 | | Experiment 4A | |
|---|---|---|---|---|
| | Control | Extrusa | Control | Saliva |
| *Cenchrus ciliaris* (1) | 45.5 | 55.9 | 48.7 | 51.2 |
| *C. ciliaris* (2) | 49.4 | 55.5 | 53.1 | 55.4 |
| *C. ciliaris* (3) | 50.3 | 55.6 | 50.3 | 54.3 |
| *C. ciliaris* (4) | – | – | 38.3 | 43.4 |
| *Urochloa mosambicensis* (1) | 44.3 | 52.2 | 46.2 | 51.0 |
| *U. mosambicensis* (2) | 41.1 | 50.3 | 42.3 | 47.2 |
| *U. mosambicensis* (3) | – | – | 40.6 | 50.4 |
| *U. mosambicensis* (4) | – | – | 43.6 | 50.1 |
| *Panicum maximum* cv. Gatton | 41.9 | 51.4 | 39.7 | 48.4 |
| *Bothriochloa pertusa* (1) | 51.7 | 57.3 | 52.8 | 54.8 |
| *B. pertusa* (2) | 33.7 | 44.3 | 34.5 | 43.0 |
| *Chloris gayana* cv. Katambora | 52.9 | 58.8 | 50.6 | 57.2 |
| *Heteropogon contortus* (1) | 46.3 | 51.8 | – | – |
| *Bothriochloa bladhii* | 44.4 | 50.2 | – | – |
| Forage sorghum | 58.0 | 63.4 | – | – |
| *Panicum maximum* (fresh) | 55.3 | 60.8 | – | – |
| Native grass hay (1) | 28.9 | 38.0 | 28.9 | 36.4 |
| Native grass hay (2) | – | – | 32.1 | 40.3 |
| *Chloris gayana* cv. Fine Cut (1) | 45.3 | 54.4 | 45.4 | 53.9 |
| *C. gayana* cv. Fine Cut (2) | – | – | 45.9 | 53.5 |
| *C. gayana* cv. Fine Cut (3) | – | – | 47.2 | 51.3 |
| *C. gayana* cv. Fine Cut (4) | – | – | 54.3 | 59.1 |
| *C. gayana* cv. Callide (1) | 41.3 | 50.1 | – | – |
| *C. gayana* cv. Callide (2) | 41.3 | 49.7 | – | – |
| *Astrebla* spp. | 34.5 | 46.6 | 40.7 | 43.9 |
| *Digitaria didactyla* | – | – | 41.7 | 50.0 |
| *Setaria incrassata* | – | – | 47.6 | 51.7 |
| *Pennisetum glaucum* | – | – | 59.0 | 59.4 |
| *Brachiaria humidicola* | – | – | 35.9 | 40.1 |
| *B. decumbens* | – | – | 39.4 | 48.7 |
| *Triticum aestivum* | – | – | 65.1 | 66.0 |
| *Lolium perenne* (1) | 70.6 | 74.7 | – | – |
| *Avena sativa* (1) | 70.1 | 72.3 | – | – |
| *A. sativa* (2) | 57.1 | 62.7 | – | – |
| *A. sativa* (3) | – | – | 86.6 | 86.5 |
| *Leucaena leucocephala* (fresh) | 66.8 | 60.8 | – | – |
| *Stylosanthes scabra* cv. Seca (1) | 47.3 | 46.4 | 45.5 | 42.5 |
| *S. scabra* cv. Seca (2) | – | – | 42.9 | 42.2 |
| *Neonotonia wightii* | 49.8 | 51.1 | 49.8 | 53.9 |
| *Stylosanthes hamata* cv. Verano (1) | – | – | 45.9 | 46.6 |
| *S. hamata* cv. Verano (2) | – | – | 53.6 | 55.0 |
| *Clitoria ternatea* | – | – | 61.2 | 59.9 |
| *Arachis hypogaea* | – | – | 56.4 | 56.0 |
| *Centrosema pascuorum* | – | – | 61.7 | 61.8 |
| *Medicago sativa* | – | – | 82.6 | 77.8 |

**Appendix 2. Feeds and *in vitro* dry matter losses during *in vitro* digestion with pepsin–cellulase or rumen fluid–pepsin for untreated feed (control) and saliva-treated feed in experiment 4B**

| Feed species | Pepsin–cellulase | | Rumen fluid–pepsin | |
|---|---|---|---|---|
| | Control | Saliva | Control | Saliva |
| *Cenchrus ciliaris* (1) | 45.7 | 52.7 | 44.1 | 50.8 |
| *Cenchrus ciliaris* (2) | 51.3 | 58.7 | 49.1 | 56.7 |
| *Cenchrus ciliaris* (3) | 47.8 | 57.7 | 52.6 | 50.5 |
| *C. ciliaris* (4) | 37.1 | 47.6 | 41.4 | 45.5 |
| *C. ciliaris* (5) | 51.9 | 57.4 | 49.5 | 57.2 |
| *C. ciliaris* (6) | 41.3 | 48.7 | 41.0 | 51.3 |
| *Urochloa mosambicensis* (1) | 39.7 | 51.4 | 46.3 | 54.9 |
| *U. mosambicensis* (2) | 38.2 | 48.1 | 44.1 | 48.9 |
| *U. mosambicensis* (3) | 39.1 | 49.6 | 41.5 | 50.0 |
| *U. mosambicensis* (4) | 43.0 | 49.9 | 49.5 | 54.6 |
| *Panicum maximum* cv. Gatton | 39.2 | 52.5 | 43.6 | 61.3 |
| *Bothriochloa pertusa* (2) | 53.4 | 58.0 | 53.0 | 62.9 |
| *Chloris gayana* cv. Katambora | 45.8 | 60.0 | 44.1 | 63.9 |
| *Heteropogon contortus* (2) | 46.9 | 50.3 | 53.8 | 56.0 |
| Native grass hay (2) | 29.2 | 37.0 | 34.3 | 43.8 |
| *Chloris gayana* cv. Fine Cut (1) | 44.7 | 55.8 | 52.5 | 60.1 |
| *C. gayana* cv. Fine Cut (3) | 46.6 | 56.8 | 47.8 | 56.1 |
| *C. gayana* cv. Fine Cut (4) | 53.9 | 63.6 | 54.1 | 62.9 |
| *C. gayana* cv. Callide (1) | 40.0 | 53.0 | 44.2 | 59.4 |
| *C. gayana* cv. Callide (2) | 37.6 | 53.5 | 54.1 | 55.3 |
| *Astrebla* spp. | 35.8 | 44.3 | 36.9 | 46.5 |
| *Digitaria didactyla* | 42.5 | 52.2 | 45.9 | 57.7 |
| *Pennisetum glaucum* | 53.4 | 58.1 | 55.5 | 61.7 |
| *Brachiaria humidicola* | 29.8 | 42.5 | 29.1 | 43.7 |
| *B. decumbens* (1) | 38.8 | 52.1 | 43.4 | 54.7 |
| *B. decumbens* (2) | 40.6 | 50.9 | 41.2 | 53.4 |
| *Triticum aestivum* | 66.6 | 70.9 | 72.6 | 76.8 |
| *Lolium perenne* (2) | 64.7 | 72.8 | 62.3 | 72.4 |
| *L. perenne* (3) | 66.3 | 74.8 | 63.1 | 72.9 |
| *Avena sativa* (3) | 88.2 | 89.8 | 84.2 | 86.8 |
| *Stylosanthes scabra* cv. Seca (1) | 43.9 | 43.4 | 43.3 | 45.7 |
| *S. scabra* cv. Seca (2) | 41.1 | 39.7 | 43.9 | 44.3 |
| *S. hamata* cv. Verano (2) | 56.3 | 56.8 | 56.0 | 59.2 |
| *S. hamata* cv. Verano (3) | 55.1 | 55.4 | 54.0 | 56.3 |
| *S. hamata* cv. Verano (4) | 51.7 | 55.1 | 53.2 | 56.7 |
| *Clitoria ternatea* | 55.3 | 54.8 | 53.8 | 57.6 |
| *Arachis hypogaea* | 52.8 | 52.0 | 52.9 | 53.7 |
| *Centrosema pascuorum* | 61.3 | 61.8 | 60.3 | 64.3 |
| *Medicago sativa* | 81.1 | 80.2 | 78.8 | 78.7 |

## Appendix 3. Calculation of feed *in vitro* dry matter loss (IVDML) from extrusa IVDML for grass legume mixtures

Let IVDML (%) of the grass/legume extrusa sample = $S_{ext}$
Let IVDML (%) of the grass in the extrusa = $G_{ext}$
Let IVDML (%) of the legume in the extrusa = $L_{ext}$
Let the feed IVDML (%) of the grass/legume sample = $S_{feed}$
Let the feed IVDML (%) of the grass in the mixed sample = $G_{feed}$
Let the feed IVDMD (%) of the legume in the mixed sample = $L_{feed}$
Now $L_{ext} = L_{feed}$ because there is no extrusa effect of digestibility of legumes
$G_{ext} = (0.8 \times G_{feed}) + 16.8$ from regression in paper
Let the difference between $G_{feed}$ and $L_{feed}$ = D
Therefore: $L_{ext} = L_{feed} = G_{feed} + D$
Let grass proportion (%/100) in the mixture = X
And therefore legume proportion in the mixture = 1 – X
Then: $S_{ext} = X(0.8 \times G_{feed} + 16.8) + (1 - X) \times (G_{feed} + D)$
As $G_{feed}$ in the above equation is the only unknown, then $G_{feed}$ can be calculated
and $G_{ext}$, $L_{feed}$ can be calculated from $G_{feed}$
And $S_{feed} = X(G_{feed}) + (1 - X) \times (L_{feed})$.

### Example

If $S_{ext}$ = 58%, and the proportion of grass (X) in the extrusa sample = 0.6, and the difference in IVDML between plucked grass and legume samples = 8%, then:

$$58 = 0.6(0.8 \times G_{feed} + 16.8) + 0.4(G_{feed} + 8)$$

Therefore: $58 = 0.48(G_{feed}) + 10.8 + 0.4(G_{feed}) + 3.2$
Therefore: $G_{feed} = 50.818$ and $L_{feed} = 58.818$
And $S_{feed} = 0.6(G_{feed}) + 0.4(L_{feed}) = 54.018$.