Estimation of *In vitro* Digestibility of Barley Straw by Using a Homogenized Rumen Fluid and Artificial Saliva Mixed with Nitrogen and Energy Sources

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**ABSTRACT:** A 2 × 2 × 4 factorial study was conducted to examine the possibility of improving estimates of *in vitro* digestibility, using untreated (UBS) and ammonia-treated (ABS) barley straw, through homogenization of rumen fluid (RF) and by additions of urea (U) and casein (C) as N sources and Xylose + Glucose (XG) as energy sources into artificial saliva. Digestibility of ABS was significantly greater than that of UBS (p < 0.001). There was a significant decrease in digestibility when additions (U, UC, UCXG) were compared with the control (p < 0.001). A 2-way interaction between RF and straw type was significant (p < 0.05) for dry matter digestibility (DMD). Homogenization of RF increased DMD of ABS (p < 0.05) whereas it decreased DMD of UBS (p > 0.05).

**INTRODUCTION**

*In vitro* methods are important in estimating digestibility of feeds in ruminants because of their speed, convenience and cost-effectiveness. However, their use to estimate digestibility of cereal straws may not be suitable because of nutrient deficiency in straws which may cause reduction in microbial activity and thus affect digestibility *in vitro*. Givens and Moss (1995) have reported good correlations ($R^2 = 0.6-0.8$) between *in vitro* and *in vivo* estimates for different straws. However, their *in vivo* estimates (0.41-0.48) for dry matter digestibility (DMD) were greater than those (0.33-0.37) observed *in vitro* for untreated wheat barley straw (UBS) by Chaudhry and Miller (1994a, 1994b). Likewise, *in vitro* DMD of ammonia-treated barley straw (ABS) were also lower than the *in vivo* digestibility reported by Givens and Moss (1995). The low estimates for *in vitro* DMD clearly demonstrated that some changes to the standard *in vitro* methods reported by Chaudhry and Miller (1994a) were required to improve estimation of digestibility in straws.

This study examined ways of improving the *in vitro* determination of digestibility primarily for straw and other low quality roughages. Tests were carried out to examine the effects of homogenizing rumen fluid (RF) and of adding urea and sugars to artificial saliva (McDougal, 1948) to enhance microbial growth during *in vitro* incubations. Chesson and Orskov (1984) reported that significantly greater cellulolytic and proteolytic activities were associated with particle adhering microbes. Other studies suggest that as much as 70-90% of microbial biomass is associated with feed particles in the rumen (Craig et al. 1984; Stewart et al. 1988). The dissociation of microbes from the particles may be helpful in improving the estimation of *in vitro* digestibility through perhaps increased microbial activity in the RF. Homogenization of RF was therefore included in this study to dissociate microbes from particles and by implication to improve the efficiency and validity of *in vitro* methods in estimating digestibility of a variety of feeds. Straws may provide inadequate amounts of readily-fermentable energy and growth factors such as N, S, P and branched chain fatty acids to sustain optimal microbial growth and reveal the potential digestible energy of the substrate.

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MATERIALS AND METHODS

Straws and sources of N and energy
Samples of commercially available UBS and ABS, prepared by standard oven method in the UK, were dried and ground through 1.4 mm sieve for use in this study. Urea (U) and casein (C) as N sources whereas xylose (X) and glucose (G) as energy sources were purchased from Sigma chemicals.

Experimental design
The experiment was conducted as $2 \times 2 \times 4$ factorial arrangement, in duplicate, having two straw types (UBS and ABS) incubating with RF (H or UH) and artificial saliva without any addition (control) or by adding U alone (U) or together with C (UC) or with X + G (UCXG).

RF, artificial saliva and in vitro DMD
RF was obtained from fistulated sheep, fed daily 1,200 g of grass cubes (CP 160 g/kg DM). One portion of RF was filtered through four layers of cheesecloth to represent UH. Another portion of RF was also filtered but after homogenization (H) in the presence of CO$_2$ at 1,000 rpm for 5 minutes with a laboratory homogenizer (Silverson Ltd, Chesham Bucks. England) to dissociate the cellulolytic microbes from particles. Samples (0.5 g) of ground straw were directly incubated with RF and artificial saliva (pH 7) using in vitro tubes kept at 38°C. Also artificial saliva was prepared by adding either U alone (40 mg N/g straw) or in combination at a ratio of 1: 1 with C (a total of 40 mg N/g straw, UC) or UC in combination with XG (XG at a N:C ratio of 1:1,100 mg/g straw, U CXG) for an increased microbial activity. In vitro DMD and cell wall digestibility (CWD) over 48 h were then determined by the method of Van Soest et al. (1966) as described by Chaudhry and Miller (1994a).

Chemical and statistical analysis
Neutral detergent fibre (NDF) representing total cell wall (CW) of the straws was determined as described by Van Soest et al. (1966). The data were analysed by Analysis of Variance using Genstat (Lawes Agricultural Trust, 1984). Treatment means were also compared by using orthogonal contrasts.

RESULTS
The mean DMD and CWD are given in table 1.

Table 1. Effect of homogenising rumen fluid (RF), and of adding N and energy sources to artificial saliva on the in vitro DMD and CWD of NH$_3$-treated (ABS) and untreated (UBS) barley straw (Means, g kg$^{-1}$, together with main effects, contrasts and interaction with s. e. d.) U, urea; C, casein; X, xylose; H, homogenized; UH, unhomogenized

| Straw type Rumen fluid (RF) | DMD | CWD |
|---------------------------|-----|-----|
|                           | ABS | UBS | ABS | UBS |
| Treatments                |     |     |     |     |
| Straw (Control)           | 478 | 510 | 341 | 362 |
| Straw + U                 | 438 | 457 | 331 | 314 |
| Straw + UC                | 440 | 468 | 294 | 283 |
| Straw + U CXG             | 483 | 487 | 339 | 270 |
| s. e. d                   | 20.7|     | 20.5|     |

Main effects, contrasts and interaction

| Straw type (ABS v UBS)    | (s. e. d., 7.3)$^{***}$ | (s. e. d., 7.2)$^{***}$ |
| RF (H v UH)              | (s. e. d., 7.3)$^{***}$ | (s. e. d., 7.2)$^{**}$  |
| Additions                | (s. e. d., 10.3)$^{***}$| (s. e. d., 10.3)$^{***}$|
| Control v others         | ***                      | ***                      |
| U v UC                   | n.s                      | n.s                      |
| UC v U CXG               | n.s                      | n.s                      |
| Interaction              | RF x Straw type          | (s. e. d., 10.3)$^{*}$   | (s. e. d., 10.3)$^{**}$ |

n.s., non-significant; *, p < 0.05; and $^{***}$, p < 0.001.
Digestibility of ABS was significantly greater (p < 0.001) than that of UBS. The mean DMD (averaged over all types of additions and rumen fluid; S. E. D. 7.3) for ABS and UBS were 470 and 317 g kg⁻¹ respectively, whereas the corresponding CWD (S. E. D. 7.3) were 386 and 212 g kg⁻¹ respectively.

Digestibility of straw was significantly reduced (p < 0.001) when additions were made to the rumen fluid. The mean DMD (averaged over all straw and rumen fluid types; S. E. D. 10.3) for Control, U, UC and UCXG were 423, 385, 372 and 395 g kg⁻¹ respectively, whereas the corresponding CWD (S. E. D. 10.3) were 329, 291, 272 and 304 g kg⁻¹ respectively. When UC was compared with U alone, there was a non-significant reduction (p > 0.05) in the DMD and CWD. The addition of UCXG showed less reduction in the DMD and CWD compared with those of UC addition. However, the effect (UC-UCXG) was non-significant (p > 0.05) for DMD whereas it was significant (p < 0.05) for CWD.

A 2-way interaction between type of rumen fluid and type of straw was significant (p < 0.05) for DMD. Homogenization of rumen fluid increased DMD of ABS by 21 g kg⁻¹ (S. E. D. 10.3; p < 0.05), whereas it decreased DMD of UBS by 20 g kg⁻¹ (S. E. D. 10.3; p > 0.05). None of the 2- or 3-way interactions were significant (p > 0.05) for CWD. The mean CWD (S. E. D. 7.3) were 301 and 297 g kg⁻¹ for homogenized and unhomogenized rumen fluid respectively.

**DISCUSSION**

The depressive effects of nitrogenous compounds (urea and casein) on the DMD and CWD values (table 1) are in agreement with those reported by McCullough (1979) where a sharp decline in red cedar (Juniperus virginiana) in vitro DMD occurred with the addition of 10 mg urea. In contrast, significantly increased in vitro DMD was observed (McCullough, 1979) when addition of 20 mg urea was tested for common juniper (J. communis), another forage. The experiment showed that nitrogen supplementation alone or in combination with energy sources was not better than the control, rather the reverse, an inhibition of digestion was produced by a combination of casein and urea. It was concluded that sufficient N and branched chain fatty acids were supplied in the inoculum from sheep fed high protein grass cubes. The results are in agreement to those reported by Zorrilla-Rios (1989), who found that high amounts of carbohydrates had detrimental effects on the in vitro digestion of roughages, but are contrary to those reported by McCullough (1979), where a combination of carbohydrates and urea increased in vitro DMD of 3 out of 7 forages, with varying levels of response dependent on the forage quality. Supplementation of forage with N and energy sources may only be effective if the forages are limiting in N and carbohydrates. Straw is a poor source of N and digestibility was expected to increase with N and sugar additions. However, the opposite response suggests that the provision of protein and soluble carbohydrate facilitated growth of non-cellulolytic bacteria at the expense of cellulolytic bacteria. The former bacteria are known to utilize amino acids and peptides whereas the cellulolytic bacteria utilize ammonia and branched chain volatile fatty acids (Bryant, 1973; Russell et al., 1992).

The inoculum from sheep fed high protein grass cubes appeared to supply sufficient N and branched chain volatile fatty acids to support the growth of cellulolytic bacteria during in vitro incubations. In contrast an interaction between effect of homogenization of rumen inoculum and straw type suggests a potential in homogenization to alter in vitro digestibility and perhaps deserves attention in future studies of this kind. Although the 2-way (RF × Additions) and the 3-way interaction were not significant, table 1 indicates that homogenization of inoculum tended to increase DMD and CWD for the controls and either have little consistent effect or to further depress DMD and CWD in the presence of U, UC and UCXG additions. The positive effect of homogenization on DMD and CWD for the controls is however contrary to that of Craig et al. (1984) who reported a reduced digestion of NDF when alfalfa hay was incubated with blended inoculum from a rumen-fistulated cow. The results are also contrary to those of Pell and Schofield (1993) where blended and unblended samples of rumen fluid did not differ in gas production in vitro. Homogenization using Silverson Homogenizer may have been more effective than the method of blending perhaps used by Craig et al. (1984) and Pell and Schofield (1993) in extracting particle-associated microbes and thus in improving digestibility. Homogenization, if applied correctly, may enhance the inoculum with cellulolytic microorganisms which can then be expressed under appropriate growth conditions.

It is concluded that the source of rumen fluid being used in this study was not limiting in nutrients. However, the most likely reason for low in vitro DMD estimates compared with the published estimates of in vitro digestibility was perhaps the incubation time of 48 h which was much shorter than the retention time being expected for such fibrous feeds during their in vivo evaluation.
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