day) were investigated in a 2×2 factorial design with 28-day periods. YC did not affect the total bacterial population in the rumen (3.09, 3.17 (s.e.d. 0.574) $\times 10^8$ per ml for silage and silage + YC, respectively, $P > 0.05$). As a result, microbial nitrogen synthesis, estimated from purine derivative excretion in the urine, was not affected (8.39, 8.41 (s.e.d. 0.846) g microbial nitrogen per day, respectively, $P > 0.05$). Thus while YC stimulated bacterial numbers in Rusitec when grass was the basal diet, YC failed to stimulate bacterial growth either in Rusitec or in the rumen of sheep when the basal diet was grass silage.

99. The dry matter and nitrogen degradation of whole crop wheat cut at three stages of crop maturity

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The experiment was designed to compare the effect of stage of growth, ensiling and urea treatment (40 kg/t dry matter (DM)) on the degradability of DM and nitrogen of wheat forages. Nine forages (var. Fortress) were prepared: fresh forage as harvested, ensiled or urea-treated at three maturities (growth stage 49, 71 and 89). Six mature sheep (mean live weight 76.9 kg) each fitted with a rumen cannula were fed a diet of hay and compound food (ratio 0.6:0.4) for 10 days prior to incubation of samples. Six replicates of 10 g DM of chopped forages were incubated in polyester bags using the complete exchange method for 0, 3, 8, 16, 24 and 48 h. All treatments were incubated separately to remove the possibility of bag to bag interaction. The subsequent data were fitted to the model $Y = a + b(1 - e^{-ct})$. The 'a' (g/kg), 'b' (g/kg) and 'c' (per h) values for DM for fresh, ensiled and urea-treated crops respectively at the three growth stages were: (GS49) 104.9, 560.8, 0.087; 76.2, 489.4, 0.068; 94.6, 568.1, 0.108; (GS71) 86.7, 491, 0.084; 54.3, 483.6, 0.092; 100.5, 585.2, 0.085; (GS87) -6.7, 707, 0.021; 16.3, 635.4, 0.02; 63, 726.5, 0.03; (s.e.d. 'a' 5.7 ($P < 0.01$), 'b' 23.0 ($P < 0.01$), 'c' 0.008 ($P < 0.01$)). The 'a' (g/kg), 'b' (g/kg) and 'c' (per h) values for N for fresh, ensiled and urea-treated crops respectively at the three growth stages were: (GS49) 242.8, 610.6, 0.091; 186.7, 658.3, 0.087; 310.5, 562.2, 0.067; (GS71) 231.2, 610.3, 0.093; 175.9, 628.9, 0.09; 316.5, 573.7, 0.053; (GS87) 101.1, 555.2, 0.104; 84.9, 556, 0.098; 238.4, 438, 0.065; (s.e.d. 'a' 7.8 ($P < 0.001$), 'b' 23.1 ($P < 0.01$), 'c' 0.009 ($P < 0.01$)). Increasing crop maturity had a negative effect on 'a', 'b' and 'c' values for DM and N whilst urea treatment maintained or increased these parameters for DM and N in relation to fresh forage. Ensiling reduced the 'a' and 'b' values for DM and increased the 'b' value of N.

100. Use of fresh or frozen cow faeces instead of sheep rumen liquor to provide micro-organisms for *in vitro* digestibility assays for forages

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The objective was to develop a non-invasive alternative to rumen liquor as a source of micro-organisms in the two-stage, *in vitro* digestion technique of Tilley and Terry (1963). Several preliminary trials with eight forages, varying widely in digestibility *in vitro*, showed high correlations ($R^2 > 0.9$) between *in vitro* digestibility determined using sheep rumen liquor and digestibility determined using freshly voided cow faeces used either immediately, or after freezing and thawing. In week 1 of the experiment to be reported, seven of the forages used in the preliminary trials were assayed, in triplicate, for *in vitro* organic matter digestibility (OMD) using rumen liquor pooled from three hay-plus-concentrate-fed sheep (RL) or freshly voided faeces pooled from three hay-fed cows (F).

A large sample of each of the rumen liquor (FRL) and faeces (FF) was frozen at -20°C . During week 3 of the experiment defrosted rumen liquor and defrosted faeces were used as sources of micro-organisms to determine the *in vitro* OMD of the seven forages used in week 1. There were highly significant correlations between OMD (g/kg) determined using RL and F, RL and FF or RL and FRL, viz: $RL = 172.7 + 0.984F$, $R^2 0.967$, residual s.d. 30.6; $RL = 172.0 + 0.972FF$, $R^2 0.974$, residual s.d. 27.0; $RL = 131.4 + 1.108FRL$, $R^2 0.996$, residual s.d. 10.1. The results show that freshly voided cow faeces, used immediately or after freezing and thawing, can substitute for rumen liquor in *in vitro* assays.

Tilley, J. M. A. and Terry, R. A. 1963. A two-stage technique for the *in vitro* digestion of forage crops. *Journal of the British Grassland Society* 18: 104-111.

101. Differentiation of yeast cultures in ruminant feedingstuffs using a rapid DNA fingerprinting technique

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Recent research has indicated that cells of certain yeast strains stimulate growth of key rumen microbial populations. Furthermore, such stimulation appears to be strain-specific with very similar yeast strains exerting their effect on totally different microbial populations. As such, future yeast culture supplements may contain mixtures of different strains designed to suit specific diets. This requires the development of rapid but sensitive methods to differentiate among taxonomically similar yeast strains in animal diets. Such a technique is termed the randomly amplified polymorphic DNA (RAPD) assay. This method is based upon the use of randomly designed short polynucleotide primers to amplify genetic sequences from the DNA of the desired yeast strain. When used under the correct conditions, the technique is sensitive enough to detect single base changes in a given sequence among yeast strains. Thus, strains which appear to be identical by classical taxonomic methods can be differentiated by this method. To prove this technique, three strains of *Saccharomyces cerevisiae* (1026, 2045, 2020) and *Candida utilis* 3001 were mixed into a standard cattle ration at a concentration of 10^6 colony forming units per g respectively. Following food extraction and colony isolation, a rapid (10 min) DNA isolation was performed on 30 randomly selected colonies. Amplification for 4 h under the conditions of the RAPD assay was followed by separation of the products by electrophoresis. Distinct products for each strain were detected which directly matched reference patterns generated previously. The technique represents a rapid (8 h) procedure for controlling addition of yeast to animal feedingstuffs.

102. Comparative digestibility of fresh herbage cut at two maturity stages by two breeds of sheep

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Two trials were conducted to study the effect of the maturity stage of fresh herbage on its digestibility by two breeds of sheep (Churra (CHR) v. Merino (MER)). The sward consisted of a mixture of grasses and clover, and was harvested either in early June (early cut, EC) or in late July (late cut, LC). In each trial, 12 mature (live weight (LW) 47 (s.e. 0.77) kg) sheep of each breed were used. The fresh herbage was offered to the animals at different levels of food allowance, ranging from 8.1 to 24.7 g dry matter (DM) per kg LW per day. Samples of dried herbage were incubated in the rumen of CHR and MER sheep to determine the DM degradability by the *in sacco* technique. EC herbage showed higher crude protein (116 v. 67 g/kg DM) and lower neutral detergent fibre (496 v. 667 g/kg DM) contents than the LC herbage. DM digestibility and rumen

potential degradability ($a + b$ value) of the herbage were significantly affected by its maturity stage, with observed mean values of 0.687 (s.e. 0.005) and 0.852 (s.e. 0.006) for the EC herbage, and of 0.463 (s.e. 0.007) and 0.676 (s.e. 0.010) for the LC herbage, respectively. Fractional degradation rates (c values) estimated when herbage samples were incubated in the rumen of CHR were significantly higher than in MER (0.046 (s.e. 0.003) v. 0.032 (s.e. 0.003)), with large differences between breeds in the DM disappearance rates at intermediate incubation times, and no significant differences between CHR and MER in the mean $a + b$ values. DM digestibility coefficients observed in CHR (0.694 (s.e. 0.028) with EC herbage and 0.474 (s.e. 0.028) with LC herbage) tended to be greater than those observed in MER (0.680 (s.e. 0.021) with EC herbage and 0.452 (s.e. 0.040) with LC herbage). With the EC herbage DM digestibility tended to decrease as the level of intake increased. These results suggest that there may be differences between breeds of sheep in their ability to digest fibrous diets.

103. Prediction of digestibility and intake of sheep fed Leguminosae or Graminae hays: comparison between the *in vitro* digestibility, kinetics of gas production or dry matter degradation

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The study was to compare the ability of the *in vitro* digestibility (two stages), the *in sacco* dry matter (DM) degradation (g/100 g DM) and gas production (ml/200 mg DM) techniques to predict daily intake (g DM per kg W^{0.75}) and DM digestibility (DMD) of nine leguminous and seven graminaceous hays fed *ad libitum* to four Merino male sheep. Gas production or DM degradation were calculated at 6, 12, 24, 48, 72 or 96 h and their kinetics were described using the equation $p = a + b(1 - e^{-ct})$. Intake and *in vivo* DMD of the hays were variable ($P < 0.01$) and poorly related ($P > 0.05$). *In vivo* DMD was related ($P < 0.05$) to *in vitro* digestibility ($r = 0.56$). Intake and *in vivo* DMD were better related to DM degradation ($r = 0.53$ to 0.79 ; $r = 0.56$ to 0.81) than to gas production ($r = 0.02$ to 0.63 ; $r = 0.24$ to 0.67). Multiple regression using separated kinetics of degradation resulted in the highest accuracy for predicting intake and digestibility from nylon bag degradability ($R = 0.84$; $R = 0.84$) and gas production ($R = 0.60$; $R = 0.71$). The low performance of the gas test was attributed to the higher production of gas per 200 mg DM from the graminaceous hays in comparison with the leguminous hays. This was probably due to the higher concentration of protein in the leguminous hays. When protein was included as an additional factor in the multiple regression, the prediction of intake and *in vivo* DMD from kinetics of gas production were improved ($R = 0.86$; $R = 0.78$). Therefore, for foods that are quite variable in protein content, it may be better if gas production is calculated per 200 mg DM of non-protein or cell wall substrate. Further investigations on the subject are required.

104. *In vitro* rumen microbial degradation of a selection of oilseeds and legume seeds under consecutive batch culture (CBC)

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An *in vitro* rumen degradability trial was conducted to measure dry matter (DM) loss and gas production of a selection of oilseeds (whole cottonseed, mustard seed, linseed, oil rape seed cv. Bienvenu, cv. Libravo and cv. Samourai) and legume seeds (narbon seed, mungbean, cowpeas cv. Banjo and cv. Caloona and *Lablab purpureus* seed). The DM losses (g/kg) and gas production (cm³) of the oilseeds were 378, 542, 482, 434, 468 (s.e. = 25.3, $P < 0.01$) and 1.5, 3.9, 3.1, 3.4, 3.1, 3.4 (s.e. = 0.16, $P < 0.01$),

respectively. While the DM loss (g/kg) and the gas production (cm³) of the legume seeds were 475, 511, 530, 495, 525 ($P > 0.05$) and 9.2, 8.4, 7.6, 7.9, 6.6 (s.e. = 0.46, $P < 0.01$). There were significant differences between the oilseeds in DM loss and gas production. Whole cottonseed (WCS) and oil rape seed cv. Bienvenu had lower values of DM loss and less gas production than observed in other oilseeds presumably due to the presence of antinutrient factors such as gossypol in WCS. However, there was no significant difference in DM loss and gas production between oil rape seed cv. Bienvenu which is known to have a high glucosinolate content and the other two cultivars known to contain low concentration of glucosinolate. There was no significant difference between the legume seeds in DM loss and gas production. Although some antinutrients such as lectins and protease inhibitors are known to be present in the legume seeds the results of this experiment suggest that the legume seeds had better nutritive value than the oilseeds.

105. Purine derivatives in urine and plasma of lactating cows given different levels of food intake

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We demonstrated in our previous work that the ratio of purine derivatives (PD) to creatinine (C) concentrations in spot urine can be used as a simple index of microbial protein supply in sheep. This study was carried out to examine the feasibility of using this approach in cattle. Twelve beef cows at a late stage of lactation were allocated into three groups, which were fed twice daily with a pelleted straw/concentrate based diet (400 g sodium hydroxide-treated straw, 240 g barley, 100 g soya, 250 g molassed sugar-beet pulp, and 10 g urea per kg fresh weight) at three daily intakes of 6.15, 8.78 and 11.42 kg dry matter. Milk yield was indirectly measured by calf weights before and after suckling. Daily urinary excretion of PD was measured for 1 week. Two-hourly collections of urine were also made during the same period. Plasma PD concentration was measured on the last day of urine collection. Daily excretion of PD (107 (s.e. 7), 149 (s.e. 17) and 166 (s.e. 27) mmol/day for the three intakes) were positively correlated ($r^2 = 0.71$) with digestible organic matter intake (DOMI). Milk yield 8.9 to 13.2 l/day was quadratically correlated ($r^2 = 0.61$) with the estimated daily supply of microbial N (53 to 133 g/day). There were little diurnal or between-day variations in the PD:C ratio of spot urine. The PD:C ratio was correlated with daily PD excretion ($r^2 = 0.47$) but the correlation coefficient was lower than previously found in sheep ($r^2 = 0.84$). Plasma PD concentration was not correlated with daily PD excretion since the glomerular filtration rate (GFR) varied between levels of DOMI ($r^2 = 0.78$). The PD:C ratio in spot urine samples could be used as an index to distinguish between large differences in microbial N supply in cattle. Plasma PD concentration was however not a suitable index.

106. Effect of hindgut fermentation on urinary excretion of purine derivatives

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The effect of hindgut fermentation on the urinary excretion of purine derivatives (PD) was investigated on four ewes (35 (s.e. 0.4) kg live weight) fitted with duodenal and caecal catheters, consuming 700 g dry matter per day of alfalfa hay. In order to induce variations in microbial fermentation and PD concentration in the hindgut, animals were infused either 3 g/day RNA, 100 g/day starch, 200 g/day cellulose or a saline solution as control treatment, in a complete randomized cross-over design. Infusates were given via caecum except cellulose that was infused into the duodenum due to the frequent clogging of the caecal catheter. After 9 days of adaptation, faeces and urine were collected during 5 days. Urine samples were analysed by high performance liquid