Animal (2012), **6:10**, pp 1642–1647 © The Animal Consortium 2012
doi:10.1017/S175173111200050X

animal

Rumen digestion of rice straw structural polysaccharides: effect of ammonia treatment and lucerne extract supplementation *in vitro*

A. Agbagla-Dohnani[1], A. Cornu[1] and L. P. Broudiscou[2,3†]

[1]*INRA, UMR1213 Herbivores, F-63122 St Genès Champanelle, France;* [2]*INRA, UMR791 Modélisation Systémique Appliquée aux Ruminants, F-75005 Paris, France;* [3]*AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants, F-75005 Paris, France*

(Received 8 July 2011; Accepted 27 January 2012; First published online 28 February 2012)

*The combined effects of lucerne (*Medicago sativa *L.) extract supplementation and ammonia treatment of rice straw (*Oryza sativa*, variety Thaibonnet) on the ruminal digestion of cell wall components were investigated in six continuous culture systems using a randomised complete block design. Data were fitted to second-order polynomial models. Untreated rice straw had higher contents of ash-free cell wall residues (CWR; 763* v. *687 g/kg dry matter (DM)) and non-cellulosic sugars (191* v. *166 g/kg DM) than treated rice straw. Ammoniation preferentially removed xylose, which resulted in a lower xylose-to-arabinose ratio (5.1* v. *5.8). In absence of lucerne supplementation and ammoniation, degradability coefficients were 0.54, 0.46, 0.58, 0.54, 0.42 and 0.60 for cellulose–glucose, xylose, arabinose, galactose, mannose and uronic acids, respectively. Both factors had significant effects on the microbial degradation of structural polysaccharides. With lucerne extract at an optimal level, ammonia treatment increased ash-free cell wall degradation by more than 10%. The degradability coefficients were increased by ammoniation without any significant interaction with lucerne extract, except for glucose, whose degradability was mostly influenced by lucerne extract in a curvilinear way. The comparison of regression coefficients in cell wall and CWR models suggested that ammoniation improved the degradabilities of xylose, galactose and mannose by partly solubilising the corresponding hemicelluloses and by improving the susceptibility of the remaining fraction to microbial attack, whereas it increased the degradability of arabinose only by favouring microbial attack.*

Keywords: rice straw, cell wall, rumen digestion, ammoniation, lucerne

Implications

In Asia and Africa, the straw harvested from rice cultivation areas is the main roughage available to ruminants for a significant part of the year. However, it is poorly degraded in the rumen because of its low crude protein and high lignin and silica contents. In the present study, the effects of ammonia pretreatment and lucerne extract supplementation on the digestion of cell wall monosaccharides and uronic acids were assessed. The simultaneous use of treatment and supplementation is expected to improve the feeding value of rice straw for ruminants.

Introduction

In Asia and, to a lesser extent, in Africa, the straw harvested from rice cultivation areas is the main roughage available to cattle and small ruminants for a significant part of the year. Like other cereal residues, rice straw contains large amounts of polysaccharides that are a potential source of energy for the rumen microbiota. Its low nutritive value, linked to low crude protein (CP) and high lignin and silica contents, has prompted the use of various strategies to improve its utilisation by microbes (Van Soest, 2006). Polysaccharide solubilising processes calling upon alkali-based agents have increased cell wall and sugar degradabilities in rice straws by facilitating microbial access (Schiere and de Wit, 1995; Harada *et al.*, 1999; Nguyen *et al.*, 2010). By-products from local gardening and the agro-industry have been supplied to provide rumen microbes with limiting nutrients in semi-intensive production systems in Africa and in Asia. The nutritional values of local leguminous trees and vegetables are well documented in this regard (Kaitho *et al.*, 1998; El-Nor and El-Sayed, 2000; Orden *et al.*, 2000; Pamo *et al.*, 2007).

† E-mail: laurent.broudiscou@agroparistech.fr

In an *in vitro* experiment on the effect of rice straw ammoniation and supply of fresh lucerne extract – a model of legume supplementation – on rumen metabolism, we observed that at low input levels of lucerne extract both ash-free NDF (NDFom) and ash-free ADF (ADFom) degradabilities tended to be lower with treated rice straw (e.g. 0.30 *v.* 0.46 for ADF), whereas at higher input levels they were not modified by straw treatment (Broudiscou *et al.*, 2003). The present work was aimed at addressing the origin and extent of this interaction with respect to cell wall constituents. We studied the effects of ammonia pretreatment and lucerne extract supplementation on the digestion of cell wall neutral monosaccharides and uronic acids (UAs) for a better understanding of how cell wall monomers were made available to microbial fermentation by these two factors.

Material and methods

Experimental design

The chemical compositions of lucerne (*Medicago sativa* L.) and African legumes, such as *Vigna unguiculata*, consolidated in phytochemical databases are similar, especially for β-carotene, niacin, riboflavin, thiamine and mineral concentrations in their aerial parts (Duke, 1992). These data only indicated one major difference with respect to the concentration of ascorbic acid in *V. unguiculata*, one-third of that found in lucerne. Lucerne extract was thus used as a model in response to the lack of fresh material from *V. unguiculata* in France.

Two types of rice straw (*Oryza sativa*, variety Thaïbonnet), either native (untreated rice straw (URS)) or ammonia-treated rice straw (TRS), were used. Lucerne extract was introduced into continuous cultures at three levels: 0, 0.227 and 0.454 ml/g straw dry matter (DM). This corresponded to an incorporation level of whole lucerne in the diet up to 100 g/kg DM. The six combinations of experimental treatments were randomly assigned to six fermentors (working volume of 1.1 l) for two consecutive periods of 7 days. This randomised complete block design allowed the estimation of all main effects, interaction and quadratic effect of lucerne supply. Second-order polynomials have been shown to correctly describe the action of lucerne extract on rumen microbial metabolism (Broudiscou *et al.*, 2001).

Experimental feeds and incubation procedure

Rice straw was harvested from the Camargue region of France. A batch of straw was treated with ammonia as described in Broudiscou *et al.* (2003). The TRS and a batch of URS were both ground through a hammer mill (screen aperture of 8 mm) and pelleted (Unité de Préparation des Aliments Expérimentaux, INRA Jouy-en-Josas, France). Before the experiment, a batch of lucerne at the beginning of flowering (first growth) was coarsely chopped and divided into portions of 200 g, immediately stored at −20°C in air-tight plastic bags until use. On each day of incubation, a portion was thawed and extracted as described in Broudiscou *et al.* (2003). The soluble sugars and CP contents of the extract were 12.0 and 15.93 g/l, respectively. When lucerne extract was introduced at 0.454 ml/g straw DM, it supplemented the fermentor with 0.12 g/day soluble sugars and 0.159 g/day CP.

Three wethers fitted with rumen cannula and fed 1200 g/day rice straw and 100 g/day soybean meal served as rumen fluid donors to inoculate fermentors at the beginning of each period. Animal care and use procedures were approved by the French Ministry of Agriculture in agreement with French regulations for animal experimentation (guideline 19/04/1988). The fermentor design and incubation procedure is detailed in Broudiscou *et al.* (2003). The dilution rates of particle and liquid phases were set at 0.03 and 0.06/h. Because 65% to 75% of the nitrogen (N) fixed on roughage through ammonia treatment was readily available to microbes (Dulphy *et al.*, 1984), the amounts of available N were equalised among diets by pipeting into URS fermentors 5 ml of a 30.57 g/l NH_4Cl solution simultaneously with the supply of substrate every 12 h.

Chemical analysis

After a 5-day adaptation period, the displaced and filtered effluents were collected during 2 days, pooled and freeze-dried. Feeds and effluents were ground before analysis in a Culatti grinder (Zurich, Switzerland) with a screen of 0.8 mm aperture. They were analysed for DM, organic matter, NDF (assayed without sodium sulphite and with alpha amylase), ADF and ADL (Robertson and Van Soest, 1981). NDFom and ADFom were calculated from the determination of their ash content (550°C, 5 h). Hemicellulose contents were calculated from NDFom and ADFom. Lucerne extract did not interfere with the measurement of straw cell wall constituent degradabilities, as its contribution to the inflow of cell wall constituents was below the determination level of the method. Total N was determined by the Dumas technique (Sweeney and Rexroad, 1987) and CP was calculated as N × 6.25.

The data used in the calculation of cell wall component degradabilities were collected as follows. Cell wall residues (CWR) from URS, TRS and effluents were obtained by extraction in ethanol and ethanol/toluene (Jarrige, 1961) after a thorough washing with deionised water at 40°C. This method was preferred to the more widely used NDF method because the neutral detergent dissolves a fraction of cell wall polysaccharides, especially pectic substances (Jarrige, 1980). Before quantitative determination of monosaccharides, CWR were finely ground in a ball mill. UA concentration was determined colorimetrically (Blumenkrantz and Asboe-Hansen, 1973) after a sulphuric acid hydrolysis step (Englyst *et al.*, 1982). Cell wall neutral monosaccharides were analysed by gas chromatography of alditol acetates (Englyst and Cummings, 1984). All analyses were in triplicate.

Calculations and statistical analysis

For both straws, the degradability of a given cell wall component d_T was calculated using the following formula:

$$d_T = 1 - \frac{O}{I_T} \quad (1)$$

where O being the component daily outflow and I_T its total daily inflow, that is, the amount supplied by URS. In this way,

the calculation of d_T included the TRS cell walls already solubilised by ammoniation and it accounted for the effects of our experimental factors on the degradability of the entire cell wall fraction. In the following text, CWRt refers to the CWR degradability calculated using formula (1).

The degradability of a given CWR component d_R was calculated by using as the denominator its daily inflow I_R calculated for each type of straw:

$$d_R = 1 - \frac{O}{I_R} \quad (2)$$

The variation of d_R in function of experimental factors would specifically highlight differences in CWR nutritional properties such as the accessibility to microbial attack.

Results were subjected to multiple linear regression using a MINITAB procedure (Minitab, 1998). The variables were straw ammoniation (S) and lucerne extract supplementation (L). Period (P) was included as a randomising block. The variable S was set to -1 for URS and $+1$ for TRS. The variable A was related to the actual amount of lucerne supplied L' (in ml/g straw DM) by the equation:

$$L = L' / 0.227 - 1 \quad (3)$$

Thus, for values of L' of 0, 0.227 and 0.454 ml/g straw DM L was -1, 0 and 1, respectively. Data were fitted to the following second-order polynomial model:

$$Y = b_0 + b_1 S + b_2 L + b_3 P + b_4 L^2 + b_5 S \times L \quad (4)$$

where Y was the response; S, L and P the three coded variables described above; and b_0, b_1, b_2, b_3, b_4 and b_5 the six regression coefficients to be estimated. As an illustration, when fermentors were supplied with TRS and lucerne extract at maximal level, the variables S and L were set to $+1$ and, according to the regression coefficients, the predicted CWR degradability equalled:

$$d_T\,CWR = 0.541 + 0.044 + 0.052 - 0.121 + 0.015 = 0.531 \quad (5)$$

When fermentors were supplied with URS and no lucerne extract, the predicted CWRt degradability was

$$d_T\,CWR = 0.541 - 0.044 - 0.052 - 0.121 + 0.015 = 0.339 \quad (6)$$

The regression coefficient estimates were compared to zero by a student t-test.

Results

The compositions of URS and TRS are given in Table 1. Ash contents were similar in both straws (112 and 121 g/kg DM). Ammonia treatment decreased ash-free NDF content of rice straw by 68 g/kg DM and increased ash-free ADF and ADL contents by 59 and 35 g/kg DM, respectively, along with an expected increase in CP. Concentrations of monomers in URS and TRS are given in Table 2. URS exhibited higher contents of ash-free CWR (763 *v.* 687 g/kg DM) and non-cellulosic sugars (191 *v.* 166 g/kg DM). Ammonia treatment preferentially removed xylose, which led to a lower xylose-to-arabinose ratio in TRS (5.1 *v.* 5.8).

Table 1 *Composition of rice straw with (TRS) or without (URS) ammonia treatment (g/kg DM)*

	URS	TRS
OM	888 ± 0.8	879 ± 0.5
CP	36.9 ± 0.64	102.6 ± 0.53
NDFom	733 ± 11.6	665 ± 7.8
ADFom	428 ± 5.3	487 ± 2.2
ADL	65 ± 1.0	100 ± 1.2

TRS = ammonia-treated rice straw; URS = untreated rice straw; DM = dry matter; OM = organic matter; CP = crude protein; NDFom = ash-free NDF; ADFom = ash-free ADF.
The s.e.m. ($n = 4$) is given for each determination.

Table 2 *Concentrations of neutral monosaccharides and UAs in URS and TRS (g/kg DM)*

	URS	TRS
CWR	842 ± 9.0	767 ± 11.5
Glucose	291 ± 1.9	304 ± 2.9
Xylose	152 ± 1.5	131 ± 0.9
Arabinose	26.4 ± 0.21	25.6 ± 0.55
Galactose	10.1 ± 0.21	7.3 ± 0.07
Mannose	2.1 ± 0.05	1.8 ± 0.03
UAs	11.0 ± 0.78	7.1 ± 0.53
CWR ash	78.8 ± 0.08	80.1 ± 0.08

UA = uronic acid; URS = untreated rice straw; TRS = ammonia-treated rice straw; DM = dry matter.
CWR: cell wall residue according to Jarrige (1961).
The s.e.m. ($n = 3$) is given for each determination.

The effects of ammonia treatment and lucerne extract on the degradabilities of cell wall components are given in Table 3. The most degraded compounds were galactose (d_T from 0.57 to 0.83), arabinose (d_T from 0.59 to 0.77) and UAs (d_T from 0.59 to 0.80). Glucose and xylose degradabilities were much lower, from 0.46 to 0.64 and from 0.36 to 0.63, respectively, whereas mannose d_T was intermediate, from 0.49 to 0.74. The model fitted well to all data except for arabinose. Ammonia treatment had a positive impact on all degradabilities without any significant $S \times A$ interaction term, with the noticeable exception of glucose. CWRt, ash-free CWRt and mannose degradabilities were increased mainly by ammonia treatment. As an example, for an intermediate lucerne extract supply of 0.227 ml/g straw DM, CWRt degradability shifted from 0.497 to 0.585 (+18%) following ammonia treatment. CWRt, ash-free CWRt and mannose degradabilities were also increased by lucerne extract in a curvilinear way, the optimal value of lucerne extract input being estimated at 0.28 ml/kg straw DM. In contrast, this

Table 3 *Effects of ammonia treatment and lucerne supplementation on the degradability* d_T *of cell wall components*

Response	CWRt	Ash-free CWRt	Glucose	Xylose	Arabinose	Galactose	Mannose	Uronic acids
$P > F$	0.034	0.014	0.009	0.023	0.20	0.006	0.005	0.001
Adjusted R^2	0.72	0.81	0.84	0.77	0.38	0.86	0.87	0.92
r.s.d.	0.048	0.038	0.035	0.061	0.081	0.043	0.047	0.030
Terms	Regression coefficients							
Intercept	0.541	0.579	0.615	0.573	0.694	0.734	0.650	0.750
S	0.044 (0.03)	0.048 (0.01)	0.008 (0.50)	0.084 (0.008)	0.075 (0.03)	0.090 (0.001)	0.082 (0.003)	0.045 (0.005)
L	0.052 (0.04)	0.052 (0.02)	0.022 (0.18)	−0.012 (0.65)	0.009 (0.79)	0.015 (0.43)	0.066 (0.02)	0.009 (0.47)
L^2	−0.121 (0.01)	−0.108 (0.007)	−0.088 (0.01)	−0.071 (0.13)	−0.021 (0.70)	−0.090 (0.02)	−0.116 (0.01)	−0.112 (0.002)
$S \times L$	0.015 (0.47)	0.018 (0.29)	0.041 (0.03)	0.031 (0.27)	−0.006 (0.86)	0.002 (0.90)	0.035 (0.13)	0.017 (0.20)

CWRt: the reference value is the URS cell wall residue according to Jarrige (1961).
$P > F$: *P*-value associated with the *F*-statistic.
Coded variables: *S*, straw ammoniation; *L*, lucerne extract supplementation.
For each regression coefficient, *P*-value for the null hypothesis is in brackets.

Table 4 *Effects of ammonia treatment and lucerne supplementation on the degradability* d_R *of CWR components*

Response	CWR	Ash-free CWR	Glucose	Xylose	Arabinose	Galactose	Mannose	Uronic acids
$P > F$	0.054	0.029	0.008	0.055	0.24	0.056	0.015	0.014
Adjusted R^2	0.66	0.74	0.85	0.66	0.32	0.65	0.80	0.81
r.s.d.	0.053	0.044	0.033	0.068	0.083	0.067	0.059	0.065
Terms	Regression coefficients							
Intercept	0.521	0.560	0.622	0.543	0.689	0.703	0.628	0.695
S	0.020 (0.28)	0.024 (0.14)	0.017 (0.17)	0.053 (0.05)	0.072 (0.04)	0.050 (0.06)	0.054 (0.03)	−0.025 (0.27)
L	0.056 (0.05)	0.057 (0.02)	0.020 (0.20)	−0.008 (0.78)	0.009 (0.79)	0.023 (0.42)	0.078 (0.02)	0.026 (0.36)
L^2	−0.130 (0.01)	−0.117 (0.009)	−0.085 (0.01)	−0.075 (0.15)	−0.021 (0.71)	−0.114 (0.04)	−0.131 (0.02)	−0.154 (0.01)
$S \times L$	0.017 (0.46)	0.021 (0.29)	0.040 (0.03)	0.030 (0.32)	−0.006 (0.86)	0.001 (0.99)	0.040 (0.15)	0.015 (0.59)

CWR: cell wall residue according to Jarrige (1961).
$P > F$: *P*-value associated with the *F*-statistic.
Coded variables: *S*, straw ammoniation; *L*, lucerne extract supplementation.
For each regression coefficient, *P*-value for the null hypothesis is in brackets.

factor had no effect on xylose, arabinose and galactose degradabilities. Glucose degradation showed a particular pattern of variation as it was mainly influenced by the level of lucerne extract in significant interaction with the type of straw. This curvilinear effect, overall, was more favourable with TRS, shifting the optimal level of lucerne input from 0.20 to 0.31 ml/g straw DM.

The effects of ammonia treatment and lucerne extract on the degradabilities of CWR components are given in Table 4. CWR degradability varied from 0.33 to 0.56. Among monomers, glucose (d_R between 0.48 and 0.66) and xylose (d_R between 0.36 and 0.57) were the least degraded, followed by mannose (d_R from 0.42 to 0.70) and UAs (d_R varying from 0.47 to 0.70). The highest degradabilities were found for galactose (d_R from 0.57 to 0.77) and arabinose (d_R from 0.59 to 0.76). The polynomial model explained most of the variability in sugar degradabilities except for arabinose. CWR and ash-free CWR degradabilities were only modified by the lucerne extract in a curvilinear way. Glucose degradability in CWR followed the same trend as in the entire cell wall fraction, that is, linear effect of lucerne extract supplementation and a significant interaction with straw treatment. Non-cellulosic sugars had d_R influenced mainly by straw treatment. However, the positive effect of ammoniation on xylose, galactose and mannose degradation was 40% lower than that when the entire cell wall fraction was considered.

Discussion

The decrease in NDFom content following ammoniation was consistent with the existing data (Bae *et al.*, 1997). As expected, the most important effects of ammonia treatment were on cell wall structure. Hemicelluloses accounted for 42% ash-free NDF in URS and only 27% in TRS. Comparable decreases in concentrations of hemicelluloses and arabinose residues after ammonia or urea treatment have also been reported in maize stover (Sewalt *et al.*, 1996) and barley straw (Caneque *et al.*, 1998). During ammoniation, ammonia

cleaves ester linkages, such as diferulic bridges between arabinoxylan chains, but does not release the degrafted polymers, which are subsequently washed away during the first step of cell wall analysis procedure with water or neutral detergent. In our conditions, about 9% CWR and ash-free NDF were alkali labile, a ratio similar to those previously found in wheat, barley and oat straws (Mason *et al.*, 1988). We took this alkali-labile fraction into account in the calculation of d_T in order to differentiate these cell wall polymers from actual cell contents.

The major neutral sugars and the ash-free cell wall fractions were degraded to a similar extent and in agreement with published data. Glucose and xylose basal degradabilities, from URS with no lucerne extract supplementation, equalled 0.54 and 0.46, respectively, close to the values of 0.61 and 0.50 previously reported in continuous fermentors receiving URS (Karunanandaa and Varga, 1996). The other monomers, either xylan substituents (UAs and arabinose) or constitutive of other hemicelluloses, had much greater degradabilities. A similar ranking of cell wall monosaccharides on the basis of ruminal digestibilities was reported in steers fed wheat or barley straw supplemented with flaked maize and various N sources, after correction for microbial contribution (McAllan and Smith, 1976; McAllan, 1991). Similarly, in dairy cows fed ear and husk meal maize silage and grass hay, xylose had the lowest rumen apparent digestibility (0.46 to 0.66), followed by glucose (0.61 to 0.72) then arabinose (0.75 to 0.85), galactose (0.81 to 0.87) and mannose (0.80 to 0.84; Südekum *et al.*, 1992).

Our empirical modelling of the degradation extent of structural monosaccharides provided new insights into the origin of the interaction specific to cell wall fractions, because cellulose and hemicelluloses were clearly differentiated on the mode of action of our experimental factors. In the cell wall and in CWR, glucose was the only monomer whose degradability was affected by straw pretreatment through the interaction with lucerne extract, suggesting a role for microbial interspecies competition under the control of cellulose availability. In continuous or batch culture, *Ruminococcus flavefaciens* predominated in coculture with either *Fibrobacter succinogenes* or *Ruminococcus albus* under cellulose limitation, whereas coexistence was observed in cellulose-excess conditions (Shi *et al.*, 1997). These species differ slightly in their nutritional requirements (Hungate, 1966) and a shift in their relative populations caused by ammoniation through an increased cell wall accessibility might explain the differential impact of lucerne extract on cellulose digestion. Cytoplasmic contents other than nitrogenous compounds or macrominerals may be involved in these effects on cellulolytic bacteria. In the present study, a similar amount of N was available to microorganisms whatever the continuous culture and it met the requirement for microbial growth set in the INRA feeding system (Vérité and Peyraud, 1988) at 145 g CP/kg fermented organic matter (FOM). This ratio averaged 191 and 145 g CP/kg FOM with TRS and URS, respectively. Besides, phosphorus and sulphur inputs from artificial saliva provided microbial metabolic requirements (Durand and Kawashima, 1980). The curvilinear influence of lucerne supplementation has been observed on other rumen variables such as microbial protein synthesis efficiency and microbial biomass production (Broudiscou *et al.*, 2001). Similar effects of lucerne hay supplementation of a roughage-based diet have also been reported *in vivo* (Wang *et al.*, 2008).

Ammonia treatment had a positive effect on the degradation of hemicellulosic sugars. The positive link between hemicellulose solubilisation and fibre degradability is well documented in barley straw (Caneque *et al.*, 1998) and in lucerne (Ballet *et al.*, 1997). When regression coefficients were compared in cell wall and CWR models for a given monomer, it suggested that ammoniation improved the degradabilities of xylose, galactose and mannose by solubilising a hemicellulose fraction and by improving the susceptibility of the remaining fraction to microbial colonisation and enzymatic attack. In contrast, ammoniation appeared to affect each xylose substituent in a single way. It increased arabinose degradability by favouring microbial attack, whereas it acted on UAs mostly by solubilising the related polymers. This differential effect of ammonia treatment on monomeric compounds might be related to the cell wall structural heterogeneity at the molecular level and the type and number of ester linkages differing between hemicelluloses (Cornu *et al.*, 1994). It might also stem from differences in chemical composition and accessibility between plant tissues and parts (Bourquin and Fahey, 1994). It has been observed that alkali treatment damages the silicified cuticular layer of leaf blades (Wang *et al.*, 2007) and lowers the physical strength of rice straw particles (Selim *et al.*, 2004), allowing microbial access to inner tissues.

Conclusion

The analysis of cell wall degradation at a monomer level allowed the drawing of inferences about the respective roles of ammoniation and legume supplementation on rice straw rumen digestion in relation with the nature of the structural polysaccharide degraded.

References

Bae HD, McAllister TA, Kokko EG, Leggett FL, Yanke LJ, Jakober KD, Ha JK, Shin HT and Cheng K-J 1997. Effect of silica on the colonization of rice straw by ruminal bacteria. Animal Feed Science and Technology 65, 165–181.

Ballet N, Besle JM and Demarquilly C 1997. Effect of ammonia and urea treatments on digestibility and nitrogen content of dehydrated lucerne. Animal Feed Science and Technology 67, 69–82.

Blumenkrantz N and Asboe-Hansen G 1973. New method for quantitative determination of uronic acids. Analytical Biochemistry 54, 484–489.

Bourquin LD and Fahey GC 1994. Ruminal digestion and glycosyl linkage patterns of cell-wall components from leaf and stem fractions of alfalfa, orchardgrass, and wheat-straw. Journal of Animal Science 72, 1362–1374.

Broudiscou LP, Agbagla-Dohnani A, Papon Y, Cornu A, Grenet E and Broudiscou A 2001. Quantitative effects of alfalfa extract supply on rice straw degradation, fermentation and biomass synthesis by rumen microorganisms in vitro. Animal Research 50, 429–440.

Broudiscou LP, Agbagla-Dohnani A, Papon Y, Cornu A, Grenet E and Broudiscou AF 2003. Rice straw degradation and biomass synthesis by rumen micro-organisms in continuous culture in response to ammonia treatment and legume extract supplementation. Animal Feed Science and Technology 105, 95–108.

Caneque V, Velasco S, Sancha JL, Manzanares C and Souza O 1998. Effect of moisture and temperature on the degradability of fiber and on nitrogen fractions in barley straw treated with urea. Animal Feed Science and Technology 74, 241–258.

Cornu A, Besle JM, Mosoni P and Grenet E 1994. Lignin–carbohydrate complexes in forages: structure and consequences in the ruminal degradation of cell-wall carbohydrates. Reproduction Nutrition Development 34, 385–398.

Duke JA 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. CRC Press, Boca Raton, CA, USA.

Dulphy JP, Komar A and Zwaenepoel P 1984. Effets comparés des traitements à l'ammoniac et à la soude sur la valeur alimentaire des fourrages pauvres. Annales de Zootechnie 33, 321–342.

Durand M and Kawashima R 1980. Influence of minerals in rumen microbial digestion. In Digestive physiology and metabolism in ruminants (ed. Y Ruckebusch and P Thivend), pp. 375–408. MTP Press Limited, Lancaster, UK.

El-Nor SAHA and El-Sayed HM 2000. *Lecuaena leucocephala* and dried poultry litter in rice straw-based diets for lactating goats. Egyptian Journal of Dairy Science 28, 97–107.

Englyst HN and Cummings JH 1984. Simplified method for the measurement of total non-starch polysaccharides by gas–liquid chromatography of constituent sugars as alditol acetates. Analyst 109, 937–942.

Englyst HN, Wiggings JH and Cummings JH 1982. Determination of the non-starch polysaccharides in plant foods by gas–liquid chromatography of constituent sugars as alditol acetates. Analyst 107, 307–318.

Harada C, Nakamura Y and Minato H 1999. Effect of sodium hydroxide treatment of rice straw on cell wall composition and digestibility of dry matter. Animal Science Journal 70, 61–66.

Hungate RE 1966. The rumen and its microbes. Academic Press, New York, NY, USA.

Jarrige R 1961. Analyse des constituants glucidiques des plantes fourragères. II. La lignocellulose: composition, dosage et comparaison avec la cellulose brute. Annales de Biologie Animale, Biochimie et Biophysique 1, 421–447.

Jarrige R 1980. Chemical methods for predicting the energy and protein value of forages. Annales de Zootechnie 29, 299–323.

Kaitho RJ, Umunna NN, Nsahlai IV, Tamminga S and Van Bruchem J 1998. Effect of feeding graded levels of *Leucaena leucocephala*, *Leucaena pallida*, *Sesbania sesban* and *Chamaecytisus palmensis* supplements to teff straw given to Ethiopan highland sheep. Animal Feed Science and Technology 72, 355–366.

Karunanandaa K and Varga GA 1996. Colonization of rice straw by white-rot fungi (*Cyathus stercoreus*): effect on ruminal fermentation pattern, nitrogen metabolism, and fiber utilization during continuous culture. Animal Feed Science and Technology 61, 1–16.

Mason VC, Roy D, Hartley RD, Keene AS and Cobby JM 1988. The effect of ammoniation on the nutritive value of wheat, barley and oat straws. I. Changes in chemical composition in relation to digestibility in vitro and cell wall degradability. Animal Feed Science and Technology 19, 159–171.

McAllan AB 1991. Carbohydrate and nitrogen metabolism in the forestomach of steers given untreated or ammonia treated barley straw diets supplemented with urea or urea plus fishmeal. Animal Feed Science and Technology 33, 195–208.

McAllan AB and Smith RH 1976. Effect of dietary nitrogen source on carbohydrate metabolism in the rumen of the young steer. British Journal of Nutrition 36, 511–522.

Minitab 1998. User's guide 2: data analysis and quality tools. Minitab Inc., State College, PA, USA.

Nguyen TAD, Kim KR, Han SJ, Cho HY, Kim JW, Park SM, Park JC and Sim SJ 2010. Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. Bioresource Technology 101, 7432–7438.

Orden EA, Abdulrazak SA, Cruz EM, Orden MEM, Ichinohe T and Fujihara T 2000. *Leucaena leucocephala* and *gliricidia sepium* supplementation in sheep fed with ammonia treated rice straw: effects on intake, digestibility, microbial protein yield and live-weight changes. Asian-Australasian Journal of Animal Sciences 13, 1659–1666.

Pamo ET, Boukila B, Fonteh FA, Tendonkeng F, Kana JR and Nanda AS 2007. Nutritive value of some grasses and leguminous tree leaves of the central region of Africa. Animal Feed Science and Technology 135, 273–282.

Robertson JB and Van Soest PJ 1981. The detergent system of analysis and its application to human foods. In The analysis of dietary fibre in food (ed. WPT James and O Theander), pp. 123–158. Marcel Dekker, New York, NY, USA.

Schiere JB and de Wit J 1995. Feeding of urea ammonia treated rice straw in the tropics. II. Assumptions on nutritive value and their validity for least cost ration formulation. Animal Feed Science and Technology 51, 45–63.

Selim ASM, Pan J, Takano T, Suzuki T, Koike S, Kobayashi Y and Tanaka K 2004. Effect of ammonia treatment on physical strength of rice straw, distribution of straw particles and particle-associated bacteria in sheep rumen. Animal Feed Science and Technology 115, 117–128.

Sewalt VJH, Fontenot JP, Allen VG and Glasser WG 1996. Fiber composition and in vitro digestibility of corn stover fractions in response to ammonia treatment. Journal of Agricultural and Food Chemistry 44, 3136–3142.

Shi Y, Odt CL and Weimer PJ 1997. Competition for cellulose among three predominant ruminal cellulolytic bacteria under substrate-excess and substrate-limited conditions. Applied and Environmental Microbiology 63, 734–742.

Südekum KH, Puls J, Brandt M and Vearasilp T 1992. Site and extent of cell-wall neutral monosaccharide digestion in dairy cows receiving diets with ear and husk meal maize silages from three different stages maturity. Animal Feed Science and Technology 38, 143–160.

Sweeney RA and Rexroad PR 1987. Comparison of leco fp-228 'nitrogen determinator' with AOAC copper catalyst Kjeldahl method for crude protein. Journal of AOAC International 70, 1028–1030.

Van Soest PJ 2006. Rice straw, the role of silica and treatments to improve quality. Animal Feed Science and Technology 130, 137–171.

Vérité R and Peyraud JL 1988. Nutrition azotée. In Alimentation des Bovins, Ovins et Caprins (ed. R Jarrige), pp. 75–93. INRA Editions, Paris, France.

Wang DL, Fan J, Xing F and Yang L 2008. Alfalfa as a supplement of dried cornstalk diets: associative effects on intake, digestibility, nitrogen metabolisation, rumen environment and hematological parameters in sheep. Livestock Science 113, 87–97.

Wang JK, Liu JX, Li JY, Wu YM and Ye JA 2007. Histological and rumen degradation changes of rice straw stem epidermis as influenced by chemical pretreatment. Animal Feed Science and Technology 136, 51–62.