Reprod Nutr Dev (1990) 30, 533-540

Original article

Microbial degradation in the rumen of wheat straw and anhydrous ammonia treated wheat straw observed by electron microscopy

E Grenet, P Barry

INRA, Station de recherches sur la Nutrition des Herbivores, Unité de l'Ingestion, Theix, 63122 Ceyrat, France

(Received 18 October 1989; accepted 25 May 1990)

Summary — With the exception of the phloem and the crown of the parenchyma, which borders the medullary lacuna, the walls of the tissues of both treated and untreated straw were lignified. The walls of the treated straw were not fluorescent in the ultraviolet probably because the treatment had modified the phenolic acids. They also had a stronger reaction to Schiff reagent particularly in the sclerenchyma indicating that their polysaccharides were more accessible. The tissues of the treated straw degraded faster in the rumen. The walls of the sclerenchyma of the treated straw were attacked by micro-organisms. Both treated and normal straw were abundantly colonized by rumen fungi, especially in the sclerenchyma. The increase in the digestibility of the treated straw was due to the greater access the micro-organisms had to the polysaccharides of the lignified walls.

wheat straw / anhydrous ammonia / rumen / microbial degradation / electron microscopy

Résumé — Observation au microscope électronique de la dégradation microbienne dans le rumen de la paille de blé traitée ou non à l'ammoniac anhydre. *Les parois de tous les tissus de la paille, qu'elle soit traitée ou non, sont lignifiées, à l'exception de celles du phloème et de la couronne de parenchyme qui borde la lacune médullaire. Les parois de la paille traitée ne sont pas fluorescentes dans l'ultra-violet, contrairement à celles de la paille normale probablement parce que le traitement a modifié les acides phénoliques. La réaction des parois de la paille traitée au réactif de Schiff est plus intense, au niveau du sclérenchyme en particulier, que celle de la paille normale, ce qui montre que les glucides de ces parois sont plus accessibles. La dégradation dans le rumen des tissus de la paille traitée est plus rapide que celle de la paille normale. Les parois du sclérenchyme de la paille traitée sont attaquées par les micro-organismes, contrairement à celles de la paille normale. Nous avons observé une colonisation très abondante de la paille normale et de la paille traitée par les champignons du rumen, particulièrement au niveau du sclérenchyme. En conclusion, l'augmentation de digestibilité de la paille traitée s'explique par une plus grande accessibilité des glucides des parois lignifiées par les micro-organismes.*

paille de blé / ammoniac anhydre / rumen / dégradation microbienne / microscopie électronique

INTRODUCTION

Although wheat straw is abundantly available in temperate regions it is seldom used as a feed. It is particularly rich in cell walls, which, according to Chesson and Ørskov (1984), account for 80% of straw dry matter (DM). Only about 40% of these walls are digestible (Jarrige, 1981), because they are highly lignified; non-lignified cellulose and hemicelluloses are readily degradable in the rumen but become resistant to microbial attack as the wall lignifies. Covalent bonds are established between the hemicelluloses and lignin (Chesson *et al,* 1983a) and the lignified cell wall becomes indigestible.

To improve the digestibility of straw, technological treatments have been developed (see Chenost and Dulphy, 1987) that allow rumen micro-organisms greater access to the polysaccharides of lignified walls. Sodium hydroxide treatment, for example, was used with success (Dulphy *et al,* 1982) but because of certain drawbacks (difficult to realize, high sodium content in feed) it has gradually been abandoned and replaced by anhydrous ammonia treatment (Sundstøl *et al,* 1978), which is easy to implement and has the advantage of considerably increasing the small amount of nitrogen in straw (Dulphy *et al,* 1984). The organic matter digestibility is increased by 10 to 12%. The mode of action of alkalis in general and of ammonia in particular is not yet understood. It is known that the ester linkages between the hemicelluloses and phenolic acids in the wall are alkali-labile (Hartley and Jones, 1978; Hartley, 1981). Cleavage of these linkages occurs (Chesson *et al,* 1983b) enabling the micro-organisms to attack the hemicelluloses.

We studied the mechanisms of action of anhydrous ammonia on straw cell walls and on their degradation in the rumen by light and electron microscopy.

MATERIALS AND METHODS

The straws and their treatment

Wheat straw (Hardy variety) was used, untreated or treated for 2 months in stacks with anhydrous ammonia (50 g/kg straw) (Sundstøl *et al,* 1978).

The digestibility of the straws was measured in a group of 6 sheep (Ramihone, 1987; Ramihone and Chenost, 1988; Chenost, in press). The *in vivo* digestibility of the organic matter of treated straw was 51.6% as against 34.8% in normal straw and crude fibre digestibility increased from 41.2 to 59.1% (Ramihone and Chenost, unpublished). These values indicate that the NH_3 treatment had a positive effect.

The rate of dry matter loss was measured *in sacco* by the method of Demarquilly and Chenost (1969) in 2 fistulated sheep fed lucerne hay *ad libitum* (Ramihone, 1987; Ramihone and Chenost, 1988; Chenost, in press). The extent of disappearance of straw was greater after treatment than before (Ramihone and Chenost, unpublished) and the difference increased with incubation time, reaching 9% after 72 h (fig 1).

The internode beneath the flag leaf was removed by hand and cylindrical samples 0.5 cm long cut halfway along it for microscopic examination.

Nylon bag method

The kinetic study of the degradation of the plant tissues was made with the nylon bag method of Demarquilly and Chenost (1969). Ten internode fragments from normal and treated straw were placed in nylon bags, which were then introduced for 8, 24, 48 and 72 h into the rumen of a fistulated sheep fitted with a permanent cannula and fed lucerne hay *ad libitum.* On removal from the rumen the bags were rinsed in water and residues of the plant fragments reserved and prepared for microscopic examination.

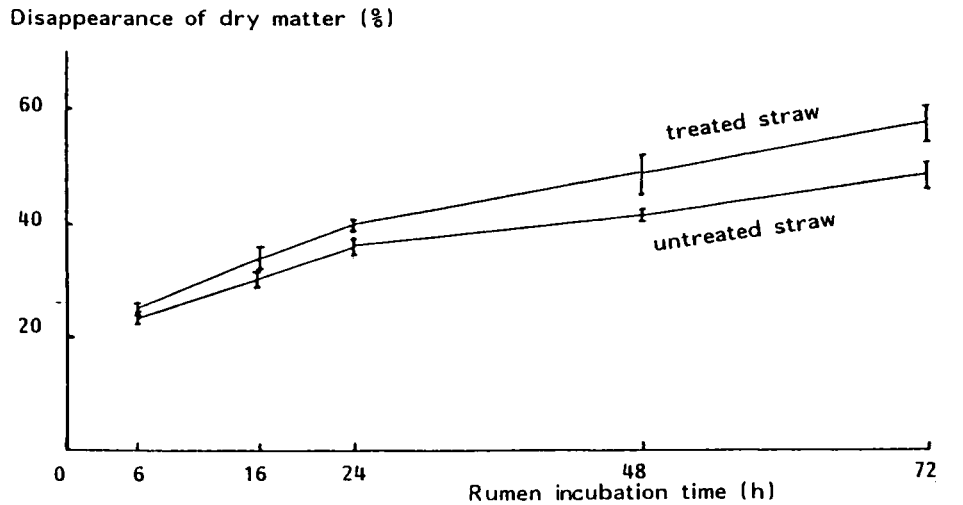


Fig 1. Kinetics of the *in sacco* dry matter disappearance of normal and treated straw in the rumen of a sheep fed lucerne hay (Chenost *et al*, unpublished results).

Preparation of internode samples for microscopic examination

Light microscopy

Free-hand sections were taken from 3 control samples to evidence lignin rich in syringyl, as indicated by reaction to the Maüle test (Langeron, 1949). Six control samples and 5 samples taken from the rumen were fixed for 3 h at ambient temperature with 1.5% glutaraldehyde in 0.2 mol/l cacodylate buffer at pH 7.4, then post-fixed in 1% osmic acid for 3 h and dehydrated in increasingly concentrated alcohol solutions (Grenet and Barry, 1988). Three of the control samples were then embedded in paraffin (Langeron, 1949) and sections were cut at 16 µm thickness with a microtome. The other samples were embedded in Epon 812 resin. Six micrometer semi thin sections were cut with an ultra-microtome (Reichert-Jung Ultracut).

The 16 µm sections were stained with acid phloroglucinol (Johansen, 1940) to evidence lignin rich in cinnamaldehyde units. The 6 µm semi-thin sections were treated with Schiff's reagent (Gabe, 1968) to evidence the wall polyosides. Phenolic compounds in the semi-thin sections were revealed by autofluorescence in the ultra-violet (Harris and Hartley, 1976).

Electron microscopy

Three control samples and 5 samples from the rumen were prepared for scanning electron microscopy (SEM) and for transmission electron microscopy (TEM) as previously described (Grenet and Barry, 1988). Ultra-thin sections cut at 80 nm thickness were contrasted with uranyl acetate and lead citrate.

RESULTS AND DISCUSSION

Observation of samples by light microscopy

All straw tissues were stained violet by Schiff's reagent. The walls of treated straw stained more intensely, especially in the sclerenchyma, both before and after passage in the rumen.

With the exception of the phloem and the crown of the parenchyma which borders the medullary lacuna, the walls of the tissues of both treated and untreated straw were lignified. This was indicated by positive reaction to acid phloroglucinol and Maüle reagent, the former staining more particularly the primary walls of the sclerenchyma and parenchyma and the latter the secondary walls.

Phenolic compounds were revealed in all the untreated straw walls by fluorescence in the ultra-violet. After incubation times in the rumen of up to 72 h the tissues which had resisted degradation were still fluorescent in the ultra-violet. In contrast, the walls of the treated straw were fluorescent neither before nor after incubation in the rumen.

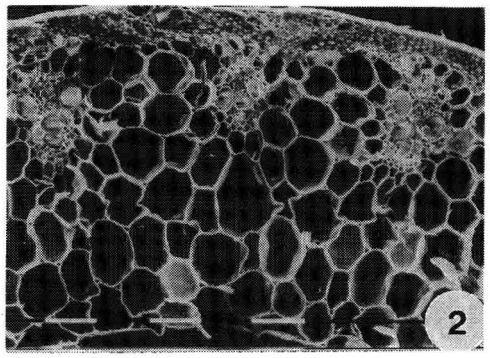
Straw is a substrate that has practically no cell content and almost all of its walls are lignified. Alkalis act on lignified walls by removing the phenolic acids (Hartley and Jones, 1977; Chesson, 1981; Besle *et al*, 1988). NH_3 modifies phenolics, so they possibly lose fluorescence in the ultra-violet. Hartley and Jones (1978) and Chesson (1981) have shown a relation between the loss of phenolic acids and the increase in digestibility that occurs when cereal straw is treated with alkalis. Likewise, Besle *et al* (1989) have shown that the digestibility of straw can be predicted from the concentration of aromatic compounds released by the treatment, as measured by optical density.

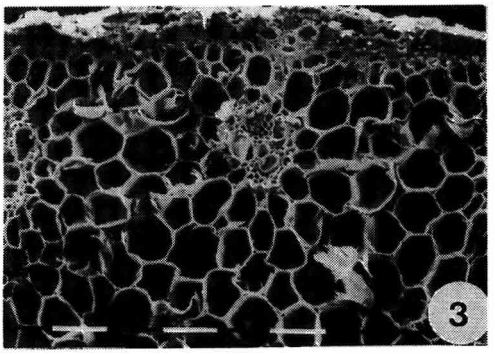
Electron microscope observation of the degradation of normal and treated straw in the rumen

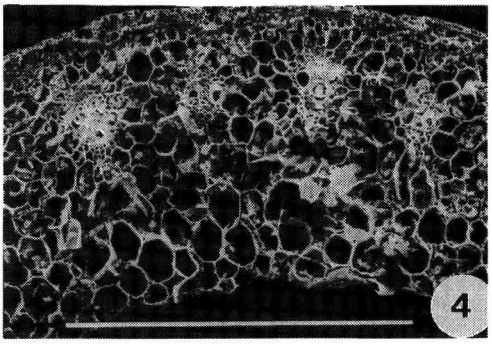
Observation of samples by SEM before incubation in the rumen (figs 2 and 3) showed the following arrangement of cell layers from the exterior of the stem inwards: the epidermis, thick-walled sclerenchyma, the parenchyma containing vascular bundles and, in the centre, the medullary lacuna.

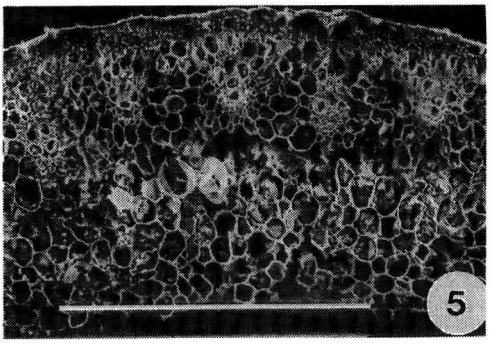
After 8 h in the rumen, the cell walls were clearly degraded (figs 4 and 5) in both treatments; the phloem had disappeared and half of the cell walls of the parenchyma were degraded. Bacteria were attached to the cell walls, and the sclerenchyma of both normal straw (fig 6) and treated straw (fig 7) were abundantly colo-

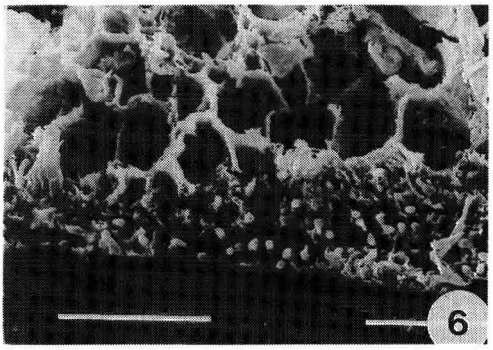
Fig 2. Untreated wheat straw before introduction into the rumen. From the exterior of the stem inwards there is the following arrangement: epidermis, sclerenchyma, vascular bundles and parenchyma surrounding the medullary lacuna. Bar = 1 mm.
Fig 3. Treated wheat straw before introduction into the rumen. There is no apparent difference between treated and untreated straw (fig 2). Bar = 1 mm.
Fig 4. Untreated wheat straw after 8 h in the rumen. The phloem and part of the parenchyma are degraded. Bar = 1 mm.
Fig 5. Treated straw after 8 h in the rumen. The phloem and part of the parenchyma, larger than in untreated straw, are degraded. Bar = 1 mm.
Fig 6. Untreated straw after 8 h in the rumen. Sclerenchyma is colonized by rumen fungi. Bar = 0.1 mm.
Fig 7. Treated wheat straw after 8 h in the rumen. Sclerenchyma is as abundantly colonized by fungi as in untreated straw. Bar = 0.1 mm.
Fig 8. Untreated straw after 24 h in the rumen. The degradation of the parenchyma is greater than at 8 h and the fungi are still abundant. Bar = 1 mm.
Fig 9. Treated wheat straw after 24 h in the rumen. The degradation of the parenchyma is greater than in untreated straw. Bar = 1 mm.

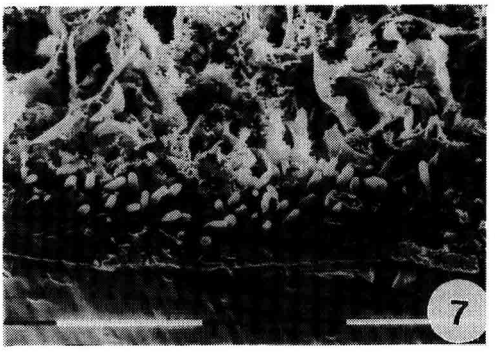

2

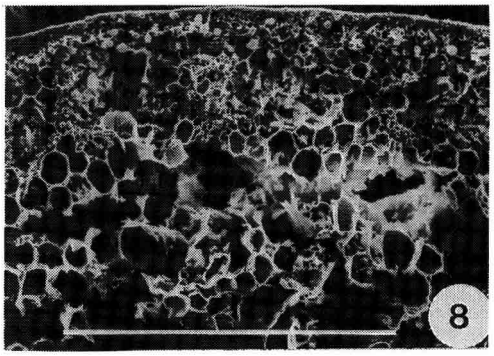

3

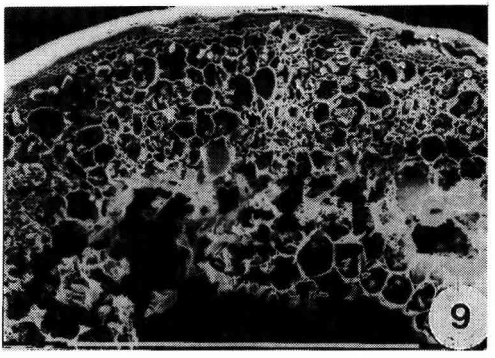

4


5


6


7


8


9

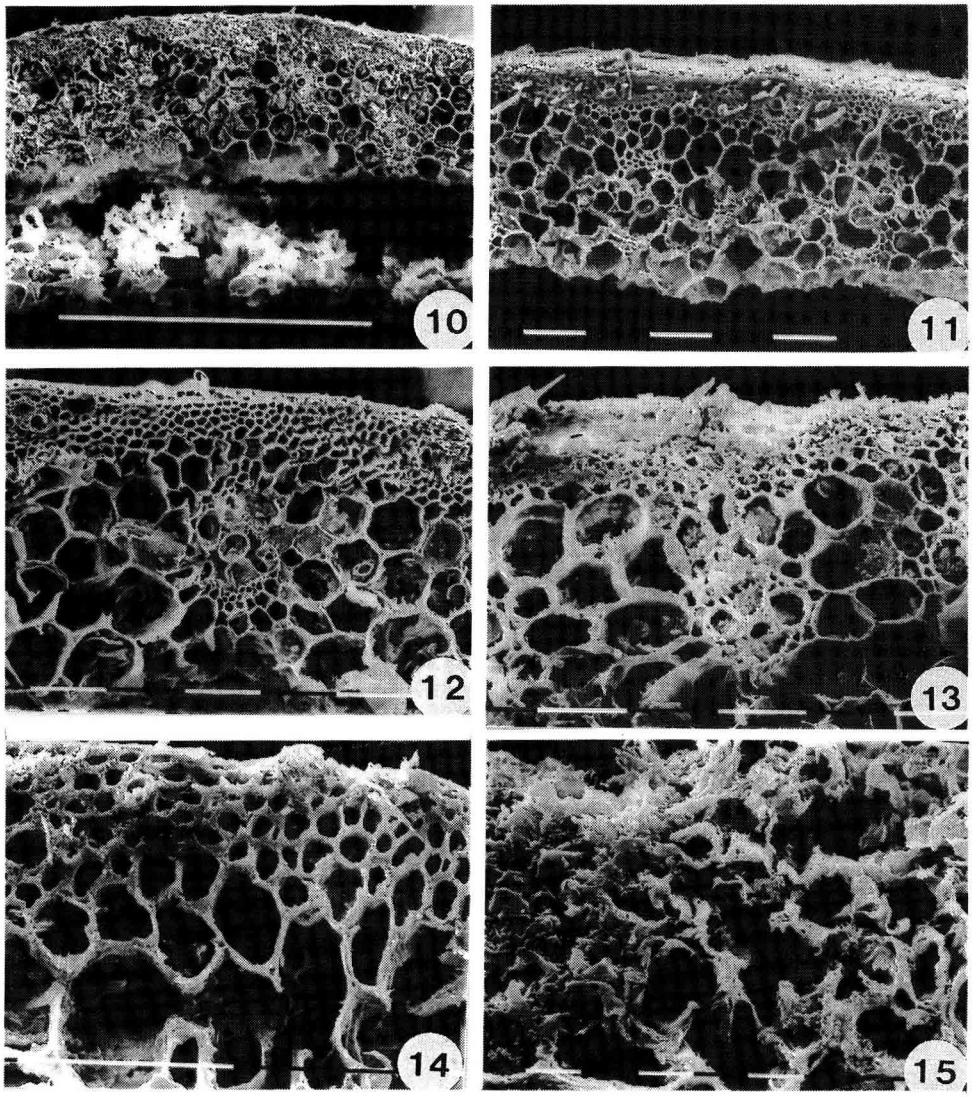


Fig 10. Untreated straw after 48 h in the rumen. A small portion of the internal parenchyma is not yet totally degraded. Bar = 1 mm.
Fig 11. Treated straw after 48 h in the rumen. The extent of the degradation of the parenchyma of treated straw is greater than for untreated straw. Bar = 0.1 mm.
Fig 12. Untreated straw after 72 h in the rumen. The medullary parenchyma has disappeared. Bar = 0.1 mm.
Fig 13. Treated straw after 72 h in the rumen. The sclerenchyma is partially degraded. Bar = 0.1 mm.
Fig 14. Untreated straw after 72 h in the rumen. The sclerenchyma walls are intact. Bar = 0.1 mm.
Fig 15. Treated straw after 72 h in the rumen. Sclerenchyma walls, abundantly colonized by bacteria, are partially degraded. Bar = 0.1 mm.

nized by fungi. At this stage it was al-ready possible to observe difference in the extent of degradation between treated and untreated straw.

After 24 h degradation was more advanced (figs 8 and 9). The walls of the internal parenchyma were beginning to degrade and this part of the stem had become separated from the rest.

After 48 h only the external crown of the stem remained; it was still abundantly colonized by anaerobic fungi (figs 10 and 11). The treated straw was more degraded since part of the medullary parenchyma of the normal straw still remained.

After 72 h (figs 12 and 13) there was a disorganization of the sclerenchyma cells of the treated straw (figs 14 and 15) not observed with normal straw.

Transmission electron microscopy also clearly showed degradation of the sclerenchyma secondary wall, which was colonized by bacteria.

This increase in the digestibility of treated straw can be explained by the greater access the micro-organisms have to the polysaccharides of the walls. For probably the same reason, staining with Schiff's reagent is stronger, provided that the size of the molecules of the microbial enzymes is the same as that of the reagents used (Tarkov and Feist, 1969). Treatment with alkali therefore results in the breakage of covalent bonds, which limit the accessibility of the polysaccharides. From our results this breakage occurs particularly in the walls of sclerenchyma cells, which in treated straw were colonized by bacteria. Harbers *et al* (1982) have also described a separation of the sclerenchyma cells after straw was treated with 10% sodium hydroxide, while Latham *et al* (1979) have shown that the lignified walls of sodium hydroxide treated straw are colonized by bacteria. Spencer and Akin (1980) observed a disorganization of the primary wall of a tropical grass, *Cynodon dactylon,* treated with potassium hydroxide. It would appear therefore that the increase in digestibility produced by treatment with ammonia, as by treatment with other alkalis, can be explained by a greater accessibility of the polysaccharides of the lignified walls.

To confirm this conclusion, other specific methods, such as immunological methods, should be used to assess the amount of polysaccharides and phenolic acids released by alkali treatment or, more interestingly, to determine the linkages between polysaccharides and polyphenolics.

REFERENCES

Besle JM, Ramihone B, Gochard O, Jouany JP, Tollier MT, Chenost M (1988) Disparition des acides phénoliques de la paille de blé en fermenteur semi-continu; influence de l'acide p coumarique sur la dégradation des glucides pariétaux. *Reprod Nutr Dév* 28, 155-156

Besle JM, Signoret C, Chenost M, Aufrère J, Jamot J (1989) Prediction of ammoniated and untreated straw organic matter digestibility by densitometry. Comparison with other predictors. *In: Evaluation of Straws in Ruminant Feeding* (Chenost M, Reiniger P, eds) Elsevier Applied Science, UK

Chenost M, Dulphy JP (1987) Amélioration de la valeur alimentaire (composition, digestibilité, ingestibilité) des mauvais foins et des pailles sur les différents types de traitement. *In: Les Fourrages secs: récolte, traitement, utilisation* (Demarquilly C, ed) INRA, Paris, 199-230

Chesson A (1981) Effects of sodium hydroxide on cereal straws in relation to enhanced degradation of structural polysaccharides by rumen microorganisms. *J Sci Food Agric* 32, 745-758

Chesson A, Ørskov ER (1984) Microbial degradation in the digestive tract. *In: Straw and other Fibrous By-products as Feed* (Sundstøl F, Owen E, eds) Elsevier, 305-339

Chesson A, Gordon AH, Lomax JA (1983a) Substituent groups linked by alkali-labile bonds to arabinose and xylose residues of legume,

grass and cereal straw cell walls and their fate during digestion by rumen microorganisms. *J Sci Food Agric* 34, 1330-1340

Chesson A, Gordon AH, Lomax JA (1983b) Cell wall organization and the biodegradation of cereal straws. *In: Biodeterioration 5* (Oxley TA, Barry S, eds) John Wiley and Sons Ltd

Demarquilly C, Chenost M (1969) Étude de la digestion des fourrages dans le rumen par la méthode des sachets de nylon; liaisons avec la valeur alimentaire. *Ann Zootech* 18, 419-436

Dulphy JP, Breton J, Bienaimé A, Louyot JM (1982) Étude de la valeur alimentaire des pailles de céréales traitées ou non à la soude. I. Influence du traitement à la soude. *Ann Zootech (Paris)* 31, 195-214

Dulphy JP, Boisseau JM, Leblanc F (1984) Influence du traitement à l'ammoniac sur la valeur alimentaire des pailles distribuées à des génisses. *Bull Techn* CRZV Theix, INRA, 62, 19-25

Gabe M (1968) *Techniques histologiques.* Masson, Paris

Grenet E, Barry P (1988) Colonization of thick-walled plant tissues by anaerobic fungi. *Anim Feed Sci Technol* 19, 25-31

Harbers LH, Kreiner GL, Davis GV, Rasmunssen MA, Corah LR (1982) Ruminal digestion of ammonium hydroxide-treated wheat straw observed by scanning electron microscopy. *J Anim Sci* 6, 1309-1319

Harris PJ, Hartley RD (1976) Detection of bound ferulic acid in cell walls of the graminae. *Nature* 259, 508-510

Hartley RD (1981) Chemical constituents and processing of lignocellulosic wastes in relation to nutritional quality for animals. *Agric Environ* 6, 91-113

Hartley RD, Jones EC (1977) Phenolic components and degradability of cell walls of grass and legume species. *Phytochemistry (Oxf)* 16, 1531-1534

Hartley RD, Jones EC (1978) Effect of aqueous ammonia and other alkalis on the *in vitro* digestibility of barley straw. *J Sci Food Agric* 29, 92-98

Jarrige R (1981) Les constituants glucidiques des fourrages: variations, digestibilité et dosage. *In: Prévision de la valeur nutritive des aliments des ruminants.* INRA Publ, Versailles

Johansen DA (1940) *Plant Microtechnique.* McGraw-Hill Book Company, New York

Langeron M (1949) *Précis de microscopie.* Masson, Paris

Latham MJ, Hobbs DG, Harris PJ (1979) Adhesion of rumen bacteria to alkali-treated plant stems. *Ann Rech Vét* 10, 244-245

Ramihone B (1987) Facteurs limitant la fermentation dans le rumen des pailles de céréales (à petits grains) traitées à l'ammoniac anhydre. Thèse de Doctorat, Université des Sciences et Techniques du Languedoc, Montpellier, p 122

Ramihone B, Chenost M (1988) Effet de la nature du complément protéique sur la digestion dans le rumen d'une paille de blé traitée ou non à l'ammoniac. *Reprod Nutr Dév* 28, 91-92

Ramihone B, Jouany JP, Chenost M (1988) Part de l'azote apporté par le traitement à l'ammoniac dans la digestion microbienne d'une paille de blé en fermenteur semi-continu (Rusitec). *Reprod Nutr Dév* 28, 151-152

Spencer RR, Akin D (1980) Rumen microbial degradation of potassium hydroxide-treated coastal bermudagrass leaf blades examined by electron microscopy. *J Anim Sci* 51, 1189-1196

Sundstøl F, Coxworth E, Mowat DN (1978) Improving the nutritive value of straws and other low quality roughages by treatment with ammonia. *World Anim Rev* 26, 13-21

Tarkov H, Feist WC (1969) A mechanism for improving the digestibility of lignocellulosic materials with dilute alkali and liquid ammonia. *In: Gould, Cellulases and their Applications. Adv Chem Ser* 95, 197-217

Thiéry JP (1967) Mise en évidence des polysaccharides sur coupes fines en microscopie électronique. *J Microsc (Paris)* 6, 987-1018