The Effects of Microwave Radiation on Rumen Degradation Characteristics of Barley Straw Cut at Two Different Stages of Maturity

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ABSTRAK

NATSIR, A. 2007. Pengaruh radiasi microwave terhadap karakteristik degradasi rumen jerami barley yang dipotong pada tingkat umur yang berbeda. *JITV* 12(2): 112-117.

Pendekatan yang umum dilakukan dalam meningkatkan nilai nutrisi hijauan berkualitas rendah dan limbah tanaman pangan adalah perlakuan, baik secara fisik, kimia atau biologi. Radiasi mikrowave dapat dianggap sebagai salah satu cara fisik yang kemungkinan dapat diaplikasikan dalam pengelolaan hijauan berkualitas rendah. Penelitian dilaksanakan untuk mempelajari pengaruh radiasi mikrowave terhadap karateristik degradasi rumen dari jerami barley yang diperoleh dari dua tingkat umur yang berbeda. Percobaan dilakukan secara faktorial berdasarkan pola rancangan kelompok lengkap teracak. Faktor pertama adalah waktu pemotongan yang berbeda yakni pemotongan pada saat awal terbentuknya biji (C1) dan pemotongan pada saat biji barley siap dipanen (C2). Faktor kedua adalah lamanya waktu radiasi mikrowave (WRMW) (T0 = kontrol-tanpa RMW, T1 = RMW selama 1menit, T2 = RMW selama 2 menit). Hasil penelitian memperlihatkan bahwa nilai nutrisi jerami barley yang diperoleh pada umur pemotongan C1 nyata lebih baik dari C2 dalam hal tingkat kecepatan degradasi dan total potensi degradasi dalam rumen. Sebaliknya, radiasi mikrowave tidak memberikan pengaruh nyata terhadap kecepatan degradasi dan total potensi degrdasi jerami di dalam rumen.

Kata Kunci: Radiasi Mikrowave, Jerami Barley, Degradasi Rumen

ABSTRACT

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A common approach for improving the nutritive value of low quality roughages and crop by-products is by pre-treatment or processing either physical, chemical, or biological treatments. Microwave radiation is one type of physical treatment that could be used to treat low quality roughages. Research was carried out to investigate the effects of microwave radiation on the rumen degradation characteristics of barley straw obtained from two different stages of maturity. The experiment was run factorially based on completely randomized block design. The first factor was stage of maturity, straw cut during the soft elongation time (C1) and during the harvest time (C2). The second factor was levels of microwave radiation times (MWRT) (T0 = control, without MWR; T1 = MWR for 1 minute, T2 = MWR for 2 minutes). The results indicated nutritive values of barley straw obtained from C1 sampling time were significantly better than that obtained from the C2 sampling time in terms of a higher rumen degradation rate and a much greater total potential rumen degradability. In contrast, MWR did not have significant effects on the rate of degradation and total potential degradability of straw in the rumen.

Key Words: Microwave Radiation, Barley Straw, Rumen Degradability

INTRODUCTION

A common approach for improving the nutritive value of low quality roughages and crop by-products is by pre-treatment or processing either physical, chemical, or biological treatments. Microwave radiation is one type of physical treatment that could be used to treat low quality roughages. Studies with wood have shown that when intensive radiation is applied this results in rapid generation of high internal steam pressure in the wood cells which in turn disrupts tissues and increases the permeability of the cells/tissues several thousands-fold (TORGOVNIKOV, 1993).

Furthermore, studies on wood have indicated that microwave irradiation enhanced enzymatic hydrolysis of lignocellulose material mainly due to partial degradation of cellulose and lignin (AZUMA *et al.*, 1984). Similarly MAGARA *et al.* (1988) reported that the pore widths of ground wood of *Fangus crenata* pretreated with microwave significantly increased associated with the partial degradation of cellulose and lignin, and this in turn was related to increased enzymatic hydrolysis of this lignocellulose material. This is supported by the finding of NATSIR *et al.* (2002) who reported that Microwave Radiation (MWR) of barley straw reduced lignin concentration and increased *in vitro* dry matter and organic matter digestibilities.

However, the reason is not clear whether the changes occurring after MWR mainly due to a change in the percentage of DM or water content or the differences in relative strength and composition of cell wall (NATSIR *at al.*, 2002). Therefore, it is interesting to look at how different stage of maturity of the straw might response to microwave radiation because as the plant matures, the cell walls become a greater proportion of the dry matter, as the fibrous constituents increase and become more lignified (JONES and WILSON, 1987; MINSON, 1990).

The aim of this study was to investigate rumen degradation characteristics of barley straw obtained from two different stages of maturity after microwave treatment.

MATERIALS AND METHODS

Straw

The barley straw used in this experiment was provided by the Department Natural Resources and Environment Experimental Farm, Gnerawarre, Southern Victoria. The straw samples were taken directly from the field at two different times: the first cutting was obtained at the soft elongation of the grain (soft dough stage, C1), and the second cutting was obtained at the stage of grain maturity (ready for harvest, C2). To get the straw dry matter content approximately the same between the two stages of growth before exposing to MWR, the samples obtained from the first cut were allowed to dry at room temperature. The straw samples then were separated manually into stem, leaf, and leaf sheath. Only the stem component of the straw was subjected to the microwave treatment.

Microwave radiation (MWR)

Domestic microwave (MW) (Sharp, model R-4A52) operated with 1.5 kW of electric power (maximum 750 watt power output) and at frequency of 2450 MHz was used. Before exposing to the MW, the samples were cut into 5 cm long segments. Approximately 50 g of the sample was placed in a MW container then exposed to MWR according to the treatment (time of exposure).

The longest time of MWR exposure was determined by first exposing the straw to the MWR for 30 seconds, and then increasing the time of radiation in 30 seconds steps gradually until a maximum time was reached where the straw sample started burning. For these particular straw samples, the longest microwave radiation time (MWRT) that could be applied was 2 minutes.

Experimental design

This experiment was arranged factorially according to completely randomised block design. The treatments consisted of combination of two factors, namely cutting time (C1 = sample taken during soft grain elongation; C2 = sample taken at the harvest time) and MWRT (T0 = control, without MWR; T1 = MWR for1 minutes, T2 = MWR for 2 minutes). Total combination of treatments was 6. Untreated- and treated-straw served as samples for *in sacco* study.

In sacco study

Rumen degradation characteristics of control and MW-treated barley straw were determined using the protocol described by ØRSKOV *et al.* (1980). Bags were made from a nitrogen-free, woven polyester cloth, have heat-sealed edges, pore size 35-65 μm with outer dimensions of 5 x 12 cm (Ankom technologies, USA). Prior to incubation, the nylon bag samples were prepared by coarsely milling it through a hammer mill to pass a 3-mm screen. The weight of the clean, dry, and labelled nylon bag plus a glass marble (to act as a weight) was recorded before transferring approximately 2.5 g of air-dry sample into each bag. A duplicate bag for each treatment was incubated in the rumen of each animal. The total number of bags required for each animal experiment was 180 (6 treatments x duplicate x 5 incubation time x 3 sheep). The number of bags incubated in the rumen at one time was kept the same (12 bags).

The bags containing straw samples (control and MW-treated samples) were incubated in the rumen of each sheep for 6, 12, 24, 48, and 72h. The first set of 12 bags was incubated in the rumen of each sheep for 72h. Following the removal of the first set, a second set was incubated for 48h and so on until all incubation times were completed. To minimize variation, the incubation in the rumen always commenced in the morning just before feeding. Immediately after each withdrawal, the bags containing the residues were rinsed under cold running tap water to remove excess ruminal contents and microorganisms on the surface of the bags and then kept in the freezer (-20°C) for later analysis.

After completing the incubation program, all collected bags were washed in washing machine without spinning for about 15 minutes and then dried at 55°C for 48 h to determine dry weight of the residue in each bag (AOAC, 1990). To determine the zero time loses, duplicate bags containing the same samples were soaked in warm tap water (39°C) for 1 h followed by washing and drying as before.

Animal and feeding

This experiment was approved by Animal Experimentation Ethics Sub-Committee, the University of Melbourne. Three mature merino sheep (wethers) with existing rumen cannula (internal diameter 40 mm) were used in this study. The average body weight, BW ± SD, was 75 ± 3.0 kg. The sheep were penned and fed individually in the Animal House, School of Agriculture and Food Systems, I.L.F.R., the University of Melbourne, Parkville, Victoria, throughout the study. The animals were cared for according to the guidelines on animal care established as standard operating procedure by NH&MRC/CSIRO.

The wethers were fed *ad libitum* at 09:00 in the morning and a new feed was added (to top up if needed) at 17:00 in the afternoon. The diet, a combination (50% : 50%) of Oaten chaff and lucerne chaff (Table 1), was given to the animal as the sole diet throughout the study at maintenance level. The diets used in this experiment were purchased from a commercial company (Essendon Produce, Essendon, Victoria). No supplementary vitamins and minerals were given but animals had a free access to water.

Table 1. Chemical composition of diet (g/kg DM) fed to sheep during the *in sacco* study

Measurement	Lucerne chaff	Oaten chaff
DM (g/kg)	860	875
Nutrient (g/kg DM)		
OM	892	922
CP	244	56
NDF	474	748
ADF	299	416
*ME (MJ/kg DM)	8.5	7.2

*Adopted from AFRC (1993)

Rumen fluid sample

Rumen conditions of the sheep were monitored by taking rumen fluid samples from each animal after completing the *in sacco* experiment. A plastic tube with an attached syringe covered by nylon cloth was inserted through the cannula into the mid ventral region of the rumen. Rumen fluid samples were withdrawn at 0h (before morning feeding) and at 3, 6, 9, 12 h and 24h. The fluid was withdrawn using a-20 ml disposable syringe. On each sampling time, the first 10 ml of the fluid was discarded. Approximately 40 ml of the rumen fluid was collected from each sheep, 20 ml of this fluid was dispensed into a tube and immediately tested for pH (measured within 1 minute) using a portable pH meter (HI 8424, Hanna Instruments Srl, Italy), and the rest for rumen fluid samples taken at 0 and 6 h was acidified with 5-6 drops of H_2SO_4 concentrate before freezing it for later analysis for rumen NH_3 and VFA.

Laboratory analysis

Prior to chemical analysis all samples (diets and *in sacco* samples) were ground to pass a 1-mm screen. Sample DM content was determined by drying at 100°C in the oven for 24 h. The percentage of ash was determined by combustion of samples for 6 h at 550°C. Organic matter (OM) was calculated as 100-%ash (DM basis). Total N content was determined by the Kjeldahl procedure (AOAC, 1990) and percentage of crude protein (CP) was calculated as total N*6.25. Fibre composition (NDF, ADF, and ADL) was analysed according to the procedure of GOERING and VAN SOEST (1970), while hemicellulose was calculated as NDF–ADF and cellulose as ADF-ADL.

Total N content of feeds & rumen fluid samples were determined by the Kjeldahl procedure (AOAC, 1990) with automatic titration (Radiometer, Copenhagen, Denmark) while rumen VFA concentration was analysed by a gas chromatograph (GC; Hewlett-Packard, Model 5890, Series II).

Calculation of rumen degradation characteristics

Analysis of ruminal degradation pattern for DM was performed by fitting the degradation values to the equations of ØRSKOV and MCDONALD (1979):

$P = a + b\,(1 - e^{-ct})$, where

- p = rumen degradation at time t
- a = intercept, which is highly correlated with the water soluble fraction (WSF)
- b = + he portion of feed DM (other than WSF) which is degraded in time t
- c = the degradation rate of the insoluble (b) fraction (%/h)
- t = incubation time

In addition, $a + b$ (asymptote) shows the value of rumen potential degradability.

The calculation of the rumen degradation characteristics was performed using the program "NEWAY EXCELL" (CHEN, 1997).

Statistical Analysis

Data for the *in sacco* experiment was analysed factorially-according to completely randomized block design using minitab zoo. The model:

$$Y_{ijk} = u + \beta_i + T_j + C_k + T_jC_k + \epsilon_{ijk},$$

where:

Y_{ijk} = observation value,
u = mean,
β_i = effects of animals (i = 1,2,3),
T_j = effects of MWRT (j = 1, 2, 3),
C_k = cutting time (k= 1, 2),
T_jC_k = interaction between factor T and C,
ϵ_{ijk} = experimental error

Rumen fermentation conditions

The mean values for rumen fermentation parameters of the animals used for the *in sacco* experiment are given in Table 2. (But no statistical analysis performed for the rumen data since all animals received the same experimental diet).

RESULTS

Rumen degradation characteristics of straw

Data for dry matter disappearance of barley straw samples are given in Table 3.

The basic data for DM disappearance were not statistically analysed, but the data were used to calculate the degradation constants as presented in Table 4.

In general, the characteristics of rumen degradation parameters were significantly (P<0.01) affected by the cutting time. However, within each state of maturity, MWR did not affect the degradation parameters. Significant interactions was observed only for the constant *a*.

For all parameters, the values of *a*, *b*, *a+b*, and *c* for C1 were significantly higher (P<0.01) than those for C2: 21.8% vs. 15.13%, 42.21% vs. 32.84%, 64.0% vs. 47.98%, 0.0460 vs. 0.0295, respectively. For constant *a*, the value was significantly higher (P<0.05) for T1 and T2 compared to that of T0 within C1 but within C2 there was no difference among the MWRT

DISCUSSION

It is well known that the maximum rate of ruminal degradation of forage dry matter and particularly fibrous constituents can be achieved when the rumen conditions are not a limiting factor for activity of microorganisms. As illustrated in Table 2, the mean of rumen pH was 6.38. This value was higher than the minimum rumen pH of 6.2 reportedly required for optimum fibre digestion (DIXON and STOCKDALE, 1999), much higher than the cellulolytic threshold point of pH 6.0 (MOULD and ØRSKOV, 1983), but slightly lower than a value of 6.5 recommended by STEWART (1977). The average of rumen NH_3-N and VFAs concentrations for this experiment was 13.16 mg N/100 ml rumen fluid and 7.23 mmol/100 ml, respectively.

Table 2. Mean (n=3) of ruminal fluid pH, ruminal NH_3-N and total ruminal VFAs concentration

Measurement	Sheep 1	Sheep 2	Sheep 3	Mean ± SD	Measurement
pH*	6.41	6.45	6.27	6.38 ± 0.09	pH*
NH_3-N (mg/l)**	102.6	159.5	132.8	131.6 ± 28.5	NH_3-N (mg/l)**
VFA (mmol/l)**	64.4	75.9	76.7	72.3 ± 6.9	VFA (mmol/l)**

*The value for each sheep is the average of six sampling times
**The value for each sheep is the average of 0h and 6h sampling times

Table 3. Mean (n=3) of cumulative DM disappearance (mg/g) of barley straw samples from the nylon bag at different incubation time

Incubation time (h)	Treatment					
	C1T0	C1T1	C1T2	C2T0	C2T1	C2T2
6	310	368	367	240	214	203
12	346	408	388	264	242	227
24	481	508	510	346	318	300
48	533	567	572	387	388	373
72	580	627	627	449	431	421

Table 4. DM degradation constants of barley straw at 2 different stages of maturity in sheep fed oaten chaff:lucerne chaff (1:1) according to equation, $P = a + b\,(1 - e^{-ct})$

Measurement	Treatment							Difference (P value)			
	C1T0	C1T1	C1T2	C2T0	C2T1	C2T2	sem*	Sheep	C	T	C*T
a	14.8^{a}	25.6^{b}	25.0^{b}	17.7^{ab}	13.9^{a}	13.8^{a}	1.93	0.035	0.001	0.176	0.003
b	43.5^{b}	43.2^{b}	39.9^{ab}	30.5^{a}	33.3^{ab}	34.8^{ab}	2.26	0.059	0.000	0.852	0.245
a + *b*	58.4^{bc}	68.8^{c}	64.9^{c}	$48.2b^{a}$	47.2^{a}	48.5^{ab}	2.40	0.028	0.000	0.162	0.093
c	0.055^{b}	0.042^{ab}	0.041^{ab}	0.030^{a}	0.032^{a}	0.027^{a}	0.0052	0.004	0.002	0.309	0.376

* Standard error of means
^{a,b} Means sharing different superscripts at the same row differ ($P<0.05$)
C1 and C2: samples cut at soft grain elongation and harvest time respectively
T0, T1, T2: MWR at 0, 1, 2 minutes respectively

SATTER and SLYTER (1974) reported that the maximum microbial growth rate should be achieved when the concentration of rumen NH_3-N was between 5 and 8 mg/100 ml. In another study, the minimum rumen NH_3-N required for optimising rumen microbial fermentation was in the range of 10 to 20 mg N/100ml (PERDOK *et al.*, 1988). From those studies, it appears that rumen conditions in this experiment were satisfactory to support a high level of microbial activity.

Data on degradation characteristics indicated that MWR did not significantly affect the rumen degradation rate of barley straw. However, stage of maturity of plant (C1 vs. C2) did have very significant effects on the rumen degradation characteristics of barley straw. Degradation rates and potential degradability of barley straw of C1 were significantly higher than those of C2. This is consistent with the finding of SULLIVAN (1973) who reported the effects of maturity on rumen degradation rate of forages. Similarly, the values for the degradable fraction (*b*) and total potential degradability (*a*+*b*) for C1 were significantly greater than those of C2. As reported by CLEALE and BULL (1986) and PANDITHARATNE *et al.* (1988) as a grass matures, the values for rumen degradation parameters are markedly decreased. This phenomenon is highly correlated with the structural deposition and lignifications of the cell wall of the roughage. Lignin concentration as analysed by the NDF/ADF/ADL of C2 samples (data not shown) system was almost 2-fold that of the C1 samples.

Many studies have been demonstrated a strong negative correlation of dry matter and fibre digestion with forage lignin concentration (JUNG and VOGEL, 1986; CHESSON and FOSBERG, 1988; FARIANI *at al.*, 1994). The degradation rate was significantly different between C1 and C2 while MWR did not have any effect on rate of digestion. The lower value of *c* for C2 suggests that the significant increase of lignin content due to advancing maturity of the plant was associated with reduction of rumen DM degradation. Therefore even though MWR significantly decreased the lignin content of barley straw of C2 samples, the reduction of lignin was not sufficient to be comparable with that of barley straw from C1 samples. Much of the cellulose and hemicellulose in forage is protected from the attack of rumen microorganisms by a layer of indigestible lignin that can only be disrupted by ball milling or chemical treatment (MINSON, 1990).

In general, cellulose and hemicellulose can be divided into potentially digestible and indigestible fractions separated by a presumptive layer of lignin, with the indigestible fraction increasing proportionally as advancing maturity of forage. A review by JONES and WILSON (1987) has indicated the importance of lignin in reducing digestibility of fibre. They stated that the variation in total content of structural constituents or their components is of lesser significance in the nutrition of herbivore than the interrelationship between constituents. When lignin is removed, the polysaccharides of the cell wall of herbage are totally digestible. Their association with lignin, however, deters attack by microbial enzymes to a varying extent depending mainly upon the degree and site of lignifications.

CONCLUSION

Nutritive values of barley straw obtained from C1 sampling time were significantly better than that obtained from the C2 sampling time in terms of a higher rumen degradation rate and a much greater total potential rumen degradability. However, microwave radiation did not give any benefits in improving nutritive values of barley straw in terms of rumen degradation characteristics, suggesting that application of this technique for improving the nutritive value of low quality roughage needs a second thought.

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REFERENCES

AFRC. 1993. Energy and protein requirements of ruminants. CAB international, Wallingford, UK.

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Washington, DC., USA.

AZUMA, J., F. TANAKA and T. KOSHIJIMA. 1984. Microwave irradiation of lignocellulosic materials: I. Enzymatic susceptibility of microwave-irradiated woody plants. *J. Japan Wood Res. Soc.* 30: 501-509.

CHEN, X.B. 1997. Neway Excell, a utility for processing data of feed degradability and *in vitro* gas production. Version 5. XBC Laboratory, Rowett Research Institute, Aberdeen, UK.

CHESSON, A. and C.W. FORSBERG. 1988. Polysaccharide degradation by rumen microorganisms. pp. 251-284. *In*: The rumen microbial system. P.N. Hobson (ed.). Elsevier Applied sci., New York, USA.

CLEALE, R.M. and L.S. BULL. 1986. Effect of forage maturity on ration digestibility and production of dairy cows. *J. Dairy Sci.*, 69: 1587-1594.

DIXON, R.M. and C.R. STOCKDALE. 1999. Associative effects between forages and grains: consequences for feed utilisation. *Aust. J. Agric. Res.* 50: 757-773.

FARIANI, A., L. WARLY, T. MATSUI, T. FUJIHARA and T. HARUMOTO. 1994. Rumen degradability of Italian Ryegrass (*Lolium multiflorum*, L.) harvested at three different growth stages in sheep. *Asian-Aust. J. Anim. Sci.* 7: 41-48.

GOERING, H.K. and P.J. VAN SOEST. 1970. Forage Fibre Analysis (Apparatus, reagents, procedures, and some application). Agric. handbook 379, ARS., USDA., Washington, DC. USA.

JONES, D.I.H. and A.D. WILSON.1987. Nutritive quality of forage. pp. 65-89. *In*: The nutrition of herbivores. J.B Hacker and J.H. Trenouth (Eds.). Academic Press.

JUNG, H.G. and K.P. VOGEL. 1986. Influence of lignin on digestibility of forage cell wall material. *J. Dairy Sci.* 62: 1703-1712.

MAGARA, K., M. TSUBOUCHY and T. KOSHIJIMA. 1988. Change of pore-size distribution of lignocellulose by microwave irradiation. *J. Japan Wood Res. Soc.* 34: 858-862.

MINITAB. 2000. Minitab Statistical Software for Windows Rel. 13.1. Minitab Inc.

MINSON, D.J. 1990. Forage in ruminant nutrition. Academic Press.

MOULD, F.L. and E.R. ØRSKOV. 1983. Manipulation of rumen fluid pH and its influence on cellulolysis *in sacco*, dry matter degradation and the rumen microflora of sheep offered either hay or concentrate. *Anim. Feed Sci. Technol.* 10: 1-14.

NATSIR, A., A.R. EGAN and B.J. LEURY. 2002. Effects of microwave radiation on physico-chemical properties and *in vitro* digestibility of barley straw. *Anim. Prod. Aust.* 24: 153-156.

ØRSKOV, E.R., F.D. DEB HOVELL and F. MOULD. 1980. The use of nylon bag technique for evaluation of feedstuffs. *Trop. Anim. Prod.*, 5: 195-213.

ØRSKOV, E.R. and I. MCDONALD. 1979. The estimation of protein degradability in the rumen for incubation measurement weighed according to rate of passage. *J. Agric. Sci. (Camb.)* 92: 499-503.

PANDITHARATNE, S., V.G. ALLEN, J.P. FONTENOT and M.C.N. JAYASURIYA. 1988. Effect of stage of gowth and chopping length on digestibility and palatability of Guinea 'A' grass silage. *J. Anim. Sci.*, 66:1005-1009.

PERDOK, H.B., R.A. LENG, S.H. BIRD, G. HABIB and M. VAN HOUTERT. 1988. Improving livestock production from straw based diets. pp. 81-91. *In*: Increasing small ruminant productivity in semi arid area E.F. Thomson and F.S. Thomson (Eds.). ICARDA, Syria.

SATTER, L.D. and L.L. SLYTER. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.* 32: 194-208.

STEWART, C.S. 1977. Factors affecting the cellulolytic activity of rumen contents. *Appl. Environ. Microbiol.* 33: 497-502.

SULLIVAN, J.T. 1973. Drying and storing herbage as hay. p. 1-31. *In*: Chemistry and biochemistry of herbage. G.W. Butler and R.W. Bailey (Eds.). Academic Press. London and New York.

TORGOVNIKOV, G.I. 1993. Dielectric properties of wood and wood based materials. Springer-verlag. Berlin.