DYNAMICS OF *IN VITRO* FERMENTATION OF STRUCTURAL CARBOHYDRATES IN TROPICAL GRASSES[1] /

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Resumen

Se estudió la dinámica de la fermentación in vitro *de celulosa y hemicelulosa potencialmente digestibles en ocho gramíneas tropicales, a cuatro diferentes edades, estimadas como el número de días después del último corte. La variación observada en la tasa de fermentación de ambos carbohidratos fue pequeña y no significativa, tanto entre especies como entre edades. La tasa de fermentación varió de 4 a 10% h^{-1}. La demora para iniciar la fermentación estimada indirectamente, también fue uniforme en todas las especies y edades. Ni el porcentaje de lignina ni la edad influenciaron los parámetros de la fermentación. La edad, y en menor grado la lignina, estuvo correlacionada con la baja digestibilidad de la hemicelulosa y la celulosa.*

Introduction

A relatively large body of information is available regarding the proximal composition and digestibility of tropical grasses (2, 3). Nevertheless, current views on the nutritive value of forages for ruminants demand accurate information on the contents of specific chemical compounds, as well as estimates of the factors that influence the rate of fermentation of these entities in the rumen (11, 17). These are not readily available for tropical grasses.

It is widely recognized that the nutritive value of forages is related to the composition and digestibility of the cell wall contents. Cellulose and hemicellulose are quantitatively the main components of cell walls, and their digestibility is variable, depending upon the stage or maturity, species and lignin content, as well as on residence time in the rumen (10). To represent the dynamics of fermentation of these two carbohydrates, a two-pool model was proposed by Waldo *et al* (18), and subsequently widely used on studies of temperate forages (14, 15), and in computer simulations of rumen function (11). In that model, cellulose and hemicellulose are represented as composed of two fractions: an indigestible pool which disappears from the rumen by passage to the lower tract, and a "potentially digestible" fraction subject to passage and fermentation (18). It has been postulated that the partition of structural carbohydrates into these two pools is determined by the content of lignin, and perhaps silica (10), while the rate of fermentation of the potentially digestible pool is independent of lignin (10). The effects of age of the forage on the above parameters are not well documented.

The objective of this study was to evaluate the rate of fermentation of potentially digestible cellulose and hemicellulose in tropical grasses, in relation to age.

Materials and methods

Samples of eight grasses cut at four different stages of maturity (21, 42, 63 and 84 days respectively) were obtained from a previous experiment (13). The eight grasses were coded as follows: M = *Melinis minutiflora;* J = *Hyparrhenia rufa;* T = *Panicum maximum* var. *trichoglume* (green panic); G = *Panicum maximum* var. *gongyloides* ("coloniação); R = *Ghloris gayana;* SN = *Setaria sphacelata* cv. Nandi; SK = *Seta-*

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ria sphacelata cv. Kazungula, and N = *Pennisetum purpureum*. Chemical analyses and agronomic conditions of the samples have been described (13); lignin was determined as the organic matter residue insoluble in 72 percent H_2SO_4. To study rate of fermentation, samples were incubated for 6, 12, 24 and 48 h with the reagents of the first stage of the *in vitro* digestibility technique (16); nitrogen was not added to the incubation medium. After the filtration step, the residues were analyzed for hemicellulose and cellulose, as described previously (13). Trial runs showed that there were no significant differences in fermentation between 48 – and 72 h incubations. Due to the large number of samples and analyses, only composite samples of three field replicate were analyzed.

The amount of structural carbohydrates fermented in 48 h, expressed as percentage of that initially present in the sample, was considered "potentially digestible" (18). The amounts fermented at 6, 12 and 24 h were expressed as percentage of the potentially digestible carbohydrate, and the data was fitted to Waldo *et al* model (18) in the form of equation 1,

$$Y = b_0 \exp(-b_1 T) \qquad (1)$$

were Y is the percentage of the potentially digestible hemicellulose or cellulose, remaining at T hours, b_0 = (100 – % digestibility) and can be used to estimate the initial time lag of fermentation (10), while b_1 represents the rate of fermentation, in h^{-1}. The parameters of the regression equations thus calculated, were compared by standard analysis of covariance (5). In some opportunities, the effect of age on the value of the parameters was evaluated by linear regression.

Results and discussion

The parameters of the regression equations relating the disappearance of the potentially digestible hemicellulose or cellulose of time of fermentation, for all species and ages are included in Table 1. As an example of the small variation encountered, Table 1 shows the parameters for the disappearance of the potentially digestible cellulose of each species, in each of the two extreme ages studied. Overall, the range in rate of fermentation of hemicellulose was 4.35 to 10.53% h^{-1}, and that of cellulose was 4.89 to 8.11% h^{-1}, depending on age and species. In none of the four stages of maturity were the differences between species significant ($P > 0.05$). In every case, a single regression equation was adequate to represent the dynamics of fermentation of the potentially digestible hemicellulose and cellulose (Table 2). Comparable results for tropical grasses do not appear to have been published. Pezo and Vohnout (12) studied the *in vitro* fermentation of dry matter in six grasses, each at three ages, by application of a sigmoidal model relating extent of dry matter fermentation to time of incubation. Not having separated the dry matter into two-pools, they reported as expected, significant differences between speces and ages within species, concluding that stage of maturity is more important than species. Smith *et al* (14, 15) observed a wide range in the rate of fermentation of the poten-

Table 1. Values of the parameters in Waldo *et al* model for the disappearance of potentially digestible cellulose in relation to species and ages.

Species	21 days			84 days		
	b_0	b_1	r^2	b_0	b_1	r^2
M	103.75	0.0498 ± 0.0355	0.99	116.24	0.0681 ± 0.0492	0.96
J	115.85	0.0678 ± 0.0495	0.94	120.78	0.0714 ± 0.0526	0.93
I	112.38	0.0607 ± 0.0440	0.95	114.80	0.0594 ± 0.0430	0.96
G	112.05	0.0546 ± 0.0397	0.94	116.76	0.0622 ± 0.0453	0.95
R	120.67	0.0724 ± 0.0534	0.92	123.15	0.0707 ± 0.0521	0.92
SN	114.90	0.0608 ± 0.0445	0.93	121.53	0.0694 ± 0.0510	0.92
SK	115.67	0.0604 ± 0.0440	0.94	108.21	0.0518 ± 0.0370	0.98
N	133.69	0.0812 ± 0.0693	0.94	113.36	0.0567 ± 0.0410	0.95

Table 2. Values of the parameters in Waldo *et al* model, for the disappearance of potentially digestible hemicellulose and cellulose.

Parameter	Age, days			
	21	42	63	84
			Hemicellulose	
b_0	117 50 ± 2.452	115.74 ± 2.137	120 30 ± 2.577	117.68 ± 3 363
b_1	0.0672 ± 0 0048	0.0622 ± 0 0042	0.0711 ± 0 0050	0 0668 ± 0 0051
r^2	0.88 (P < 0 01)	0.89 (P < 0 01)	0 89 (P < 0.01)	0.88 (P < 0 01)
			Cellulose	
b_0	114 67 ± 2 043	117 543 ± 1 927	117.919 ± 1 605	116.73 ± 1.734
b_1	0 0635 ± 0.0029	0.0664 ± 0 0044	0 0656 ± 0 0040	0.0637 ± 0 0036
r^2	0 89 (P < 0 01)	0 88 (P < 0 01)	0 93 (P < 0.01)	0 95 (P < 0.01)

tially digestible neutral detergent fiber of several temperature grasses, ranging from 4 to 23%.h^{-1}, depending on stage of maturity and species. On the other hand, Mertens (10) reported a non significant correlation between rate and lignin content. A similar observation was made by Lechtenberg *et al* (8) comparing the rate of disappearance of the potentially digestible cellulose of two isogenic lines of corn differing in lignin content. The effect of maturity on rate fermentation was studied by Darcy and Belyea (4) who found no difference in the digestion rate of potentially digestible cellulose in early vs. late cut orchardgrass.

To evaluate the effect of stage of maturity on both time lag and rate of fermentation, the values of the parameters reported in Tables 1 and 2 were regressed on days of age. When this technique was applied to ages within species, only seven of the 32 possible correlations were significant ($P < 0.05$). None of the correlations between the parameters in Table 2 and ages across species were significant ($P > 0.05$). The same lack of correlation was noted when lignin content, rather than age, was related to either time lag or rate of disappearance of the digestible pools of both structural carbohydrates. Nonsignificant correlations between rate of fermentation and lignin have been noted by several authors (4, 8, 10) but there are exceptions (14, 15). The former authors, as well as McLeod and Minson (9) have suggested that the main effect of lignin is on the extent of indigestibility, and not on the rate of fermentation of the potentially digestible fiber. This hypothesis was tested by correlating indigestibility to age and lignin (Table 3). Since the latter two variables were confounded and highly correlated between themselves the data should be interpreted with caution. Nevertheless, it does suggest that lignin may not be the only factor determining indigestibility, since the correlations with age were generally larger than those involving lignin. Other factors may be involved, such as chemical entities not presently determined or morphological peculiarities not detectable by chemical analysis (1, 6).

The value of the parameter b_0 (Table 2) can be used to estimate the initial time lag of fermentation (9). In the present study, these estimates ranged from 2 h 09 min. to 2 h 36 min, and are comparable to those reported by other authors (7, 10).

Lastly, it should be noted that the model of rumen disappearance of structural carbohydrates, although fitting the present experimental data very well, could mask differences between stages of maturity, associated with changing proportions of plant tissues. Different tissues are colonized at different speeds (7), and since colonization is probably the major cause of the time lag of fermentation, the latter could be expected to increase with maturity. A similar mechanism may operate with regard to rate of fermentation, since increasing age may also increase the degree of cristallinity of cellulose (10), which has been shown to affect the rate of enzymatic hydrolysis (6).

The experimental results presented suggest that the pool of potentially digestible structural carbohydrates of tropical grasses is extremely uniform from

Table 3. Coefficients of correlation of hemicellulose and cellulose indigestibilities (%), with age and lignin content.

Species	Hemicellulose indigestibility	Cellulose indigestibility
		Correlation with age
M	0.9603 *	0.9997 **
J	0.9699 *	0.9874 *
I	0.9898 **	0.9930 **
G	0.9755 *	0.9980 **
R	0.9635 *	0.9759 *
SN	0.9623 *	0.9868 *
SK	0.9980 **	0.9924 **
N	0.9666 *	0.9584 *
		Correlation with lignin
M	0.9181	0.8620
J	0.4995	0.4746
I	0.8394	0.8403
G	0.9380	0.9384
R	0.9180	0.9158
SN	0.8706	0.9085
SK	0.9032	0.9358
N	0.8206	0.9720*

* ($P < 0.05$)

** ($P < 0.01$)

the point of view of the kinetics of anaerobic fermentation, and that the latter is unaffected either by species, age or lignin content On the other hand, indigestibility of hemicellulose and cellulose are closely related to age.

Summary

The influence of age of the forage, expressed as number of days elapsed since previous cutting, on the rates of fermentation of the potentially digestible hemicellulose and cellulose of eight tropical grasses was studied *in vitro*. Rates of fermentation for both carbohydrates did not differ significantly between species or ages within species. The range in rate of fermentation was 4 to 10% h^{-1}. The time lag of fermentation estimated indirectly was also uniform across species and ages.

Age and lignin content did not influence significantly either parameter of fermentation. Age, and to a lesser extent lignin, was correlated to hemicellulose and cellulose indigestibilities.

Resumo

Foi estudada *in vitro*, a dinâmica da fermentação da celulose e hemicelulose potencialmente digestiveis em oito capins tropicais, em quatro diferentes idades cada. A variação observada na taxa de fermentação de ambos carboidratos foi pequena e não significativa entre espécies e idades. A taxa de fermentação variou entre 4 e 10% h^{-1}. A demora no inicio da fermentação, estimada indiretamente, também não foi afetada por espécies ou idades. A porcentagem de lignina e a idade não influenciaram significativamente os parâmetros da fermentação. Porém, a idade, e em menor grau a lignina, estiveram correlacionadas com a indigestibilidade da hemicelulose e da celulose.

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