Arq. Bras. Med. Vet. Zootec., **v.57, n.2, p.223-228, 2005**

Digestibility and ruminal digestion kinetics of corn silage

[*Digestibilidade e cinética da digestão no rúmen de silagem de milho*]

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ABSTRACT

The *in situ* dry matter (DM) disappearance of corn silages in two maturity stages (milk grain and half milk line) of known *in vivo* and *in vitro* digestibility was determined, with the main purpose of comparing digestibility values with the ruminal disappearance at 24 and 48h of incubation. A secondary goal was the description of their ruminal digestion kinetics, from which the effective degradability was calculated at an assumed passage rate of 4%/h. Data of *in vivo*, *in vitro* and *in situ* degradability at 24 and 48-h were analyzed with a linear model that included as fixed effects the maturity and the methodology of evaluation, and the kinetic data were described by the exponential model of McDonald. There was a significant effect (P<0.05) of methodology in the estimation of digestibility, but not of maturity or interaction maturity × methodology. The *in vivo* digestibility (52.9%) was not different from the 24-h *in situ* degradability (55.6%) with numerical values in the range of the effective degradability. The *in vitro* digestibility (61.6%) was not different from the 48-h *in situ* degradability (61.9%), being both estimates higher than the *in vivo* digestibility. The 24-h *in situ* degradability was a closer estimator of the *in vivo* digestibility and the 48-h *in situ* degradability and the *in vitro* digestibility overestimated the *in vivo* parameter by 15-20%.

Keywords: corn silage, *in situ, in vitro* and *in vivo* digestibility, ruminal kinetics

RESUMO

A degradabilidade in situ *da matéria seca (MS) de silagens de milho em dois estados de maturidade (grão leitoso e meia linha de leite), de conhecida digestibilidade* in vivo *e* in vitro*, foi determinada com o propósito principal de comparar valores de digestibilidade com a degradabilidade no rúmen após 24 e 48h de incubação. Também foi analisada a cinética da digestão no rúmen, pelo modelo exponencial de McDonald, e foi calculada a degradabilidade efetiva, assumindo uma taxa de passagem de 4%/h. Dados de digestibilidade* in vivo, in vitro *e de degradabilidade* in situ *a 24 e 48h foram analisados com um modelo linear que incluiu o efeito do estado de maturação e metodologia de avaliação. Houve efeito significativo (P<0,05) da metodologia na estimação da digestibilidade, mas não foi encontrado efeito da maturação ou da interação maturação × metodologia. A digestibilidade* in vivo *(52,9%) não foi diferente da degradabilidade* in situ *a 24h (55,6%), e apresentou valores numéricos na amplitude dos valores da degradabilidade efetiva. A digestibilidade* in vitro *(61,6%) não foi diferente da degradabilidade* in situ a 48h *(61,9%), e ambas as alternativas foram maiores do que a digestilidade* in vivo. *A degradabilidade* in situ *a 24h de incubação é um bom preditor da digestibilidade* in vivo *da silagem de milho. Este parâmetro*

Recebido para publicação em 19 de agosto de 2003
Recebido para publicação, após modificações, em 21 de setembro de 2004
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foi superestimado em 15-20% pela digestibilidade in vitro *e pela degradabilidade* in situ *a 48h de incubação.*

Palavras-chave: silagem de milho, digestibilidade in situ, in vitro *e* in vivo*, cinética ruminal*

INTRODUCTION

Whole corn plant silage is a complex feedstuff consisting of a mixture of grain and finely chopped forage, being both plant components of different nutritional value for the animal. While grains are highly digested (Andrae et al., 2001) the green part of the plant (stover) seems to be of limited digestibility. Leaf and stem tissues are hard to digest because of the complex structure of their cell walls (Buxton and Redfearn, 1997). Then, since silage particles remain less than 24h in the rumen (Arieli et al., 1998; Satter et al., 1999), a great proportion of the stover particles will be expected to escape digestion. Consequently, the more resistant particles will end up in feces with the consequent depression of the stover and the whole corn plant silage *in vivo* digestibility (Morrison et al., 1998). For this reason diets based on corn silage were identified as having the most severe over-estimation of energy content by laboratory procedures (Siciliano-Jones and St. Pierre, 1997).

It is well known that the *in vitro* technique (Tilley and Terry, 1963) is accepted as the most appropriate and utilized lab methodology to estimate the digestibility of feedstuffs for ruminants (Fahey Jr. and Hussein, 1999). So, it is not surprising that the most available information on corn plant, corn stover and corn silage quality has been obtained with this methodology. However, it has to be considered that in this procedure samples are incubated in the test tube for a period of time of 48h, which is longer than the retention time that feed silage particles are exposed *in vivo* to microbial attack (<24h). Siciliano-Jones and St. Pierre (1997) pointed out that this technique has shown a consistent over-estimation of the *in vivo* digestibility of corn silages, being such over-estimation of approximately 15% (Nomdedeu and Di Marco, 2001; Di Marco et al., 2002). These findings suggest that the *in vitro* digestibility could have severe limitations to estimate the *in vivo* digestibility in those situations in which the ingested material is suspected or known to be retained in the rumen for a time period shorter than the incubation one (48h). To avoid such over-estimation, a silage working team has recently proposed to estimate corn silage digestibility through the *in situ* ruminal disappearance at 24h of incubation, procedure that has been termed *in situ* digestibility (Results…, 2001).

In situ data not only are useful for a more precise evaluation of silage energy concentration (Siciliano-Jones and St. Pierre, 1997) but also to quantify rates and pools being degraded in the rumen (Adesogan et al., 2000). In this sense, results obtained with this technique revealed that the soluble fraction of corn silage is, at least, as an important source of degradable substrates as the insoluble degradable one (Arieli et al., 1998). This kind of information, which can not be obtained by the *in vitro* procedure, may help to progress in the understanding of factors affecting silage digestion.

The objective of this study was to compare the *in vivo* digestibility of two corn silages at different maturity stages with the *in vitro* digestibility and the *in situ* degradability at 24 and 48h of ruminal incubation, and to describe their ruminal digestion kinetics.

MATERIALS AND METHODS

An experiment was carried out in Balcarce, Buenos Aires province, Argentina (37° 45´ S, 58° 18´ W, altitude 130 m), to complement a previous study, in which the effect of crop maturity on *in vivo* and *in vitro* DM digestibility was determined (Nomdedeu and Di Marco, 2001). Samples of two corn silages from a crop[1] harvested in milk grain (R3) and half milk line (R5), according to the phenological scale of Ritchie et al. (1996), were incubated in two Holstein cows (440 and 450kg body weight) with ruminal fistula. Animals were fed to maintenance body weight with a medium quality lucerne hay twice a day, at 9:00 (33%) and 16:00h (67%) in

[1] Suco, Novartis Seed Company

individual outdoor corrals for a period of 10 days of adaptation previous to the incubation period. Two ground (2mm) samples (5g DM) per animal were incubated in the rumen for 0, 4, 9, 15, 24, 48, 72 and 96h (Mehrez and Ørskov, 1977). Samples were placed in dacron bags (10×20cm, 50µm pore size) and previous to incubation they were hydrated for 5 minutes (37ºC). Four additional bags per animal at 24 and 48h were used (n=12). The 0-h corresponded to a ruminal incubation of 5 minutes to estimate the soluble fraction. After extraction, bags were rinsed thoroughly with cold tap water until the rinse water was clear. Then, they were dried until constant weight and weighed. Rumen liquor samples were taken by hand at 0, 2.5, 5 and 8h after feeding during two consecutive days and ruminal pH and ammonia nitrogen (NH_3-N) concentration were determined.

The analysis of ruminal DM degradation kinetics was carried out following two successive steps. In the first one, the lag-time was estimated using the model described by McDonald (1981). Then, data of DM degradation beyond the lag-time were further adjusted to the model:
$P=a+b\ (1-e^{-ct})$, where
P= fraction degraded in the time t, a= soluble fraction, b= degradable fraction, c= fractional degradation rate and t= incubation time.

Finally, the effective degradability (ED) was estimated assuming a passage rate (kp) of 4%/h, as follows:
ED= a'+(b'c)/(c+kp), where
a'= observed degradability at time 0, b'= a+b-a'

Digestibility and *in situ* degradability data were analyzed with a linear model including the fixed effects of maturity stage and methodology of evaluation. Dunnett test was used for the comparison between the *in vivo* digestibility and the other methodologies. The parameters of the McDonald (1981) model were estimated by the Marquart method and were compared by Tukey test considering each animal as a block. The analysis of data was carried out using the SAS (User's… 1996) package.

RESULTS AND DISCUSSION

As shown in Table 1, the *in vivo* DM digestibility (52.9%) was not different (P>0.05) from the ruminal DM degradability at 24h (55.6%) in agreement with Siciliano-Jones and St. Pierre (1997). However, it was lower (P<0.05) than the ruminal DM degradability at 48h of incubation (61.9%) and than the *in vitro* DM digestibility (61.6%). In other words, these procedures over-estimated the *in vivo* digestibility by approximately 15%. It is important to note that both silages differed in DM (26 *vs*. 32%), starch (13 *vs*. 28%) and neutral detergent fiber contents (55 *vs*. 41%), in accordance with their respective stages of maturity. However, no effects of maturity and interaction maturity × methodology on digestibility were observed (data not shown). Di Marco et al. (2002) reported that whole corn plant silage digestibility was not affected by maturity because the consequent depression in silage fiber digestibility was counteracted by an increase in starch content.

Table 1. Digestibility and ruminal degradability of DM of corn silage from a crop in two maturity stages

Methodology	n	Stage R3	Stage R5	Mean	EEM
In vivo digestibility (%)	9	52.5±0.87	53.5±0.64	52.9a	0.89
In vitro digestibility (%)	10	60.1±0.33	63.1±0.34	61.6b	0.84
24-h degradability (%)	12	54.4±1.49	56.5±1.19	55.6a	0.79
48-h degradability (%)	12	60.9±1.42	62.8±1.38	61.9b	0.74

Effect of maturity and interaction maturity x methodology was not significant (p>0.05).
n = number of data for each stage of maturity.
Values followed by different letters indicate significant differences by test of Dunnett (P<0.05).
EEM = standard error of the mean.

The over-estimation of the *in vivo* digestibility by the *in vitro* technique or by the 48-h *in situ* incubation suggests that a 48-h incubation period, used in both cases, might have exceed the time that the silage was retained in the rumen for *in vivo* digestion. Data of Arieli et al. (1998), Satter et al. (1999) and Kuehn et al. (1999) suggested that in high producing ruminants the ruminal retention time of corn silage might not be longer than 24h, which helps to explain the

close similarity between values of *in vivo* DM digestibility and *in situ* degradability at 24 h. Andrae et al. (2001) also reported data of 24-h *in situ* DM degradation (52%) in range with the *in vivo* digestibility (55%) of corn silage. On the other hand, values of 48-h *in situ* degradability or 48-h *in vitro* digestibility show that degradation of corn silage samples were similar in the rumen than in the test tube, in spite that conditions of fermentation in both cases might have been quite different (Adesogan et al., 2000).

It was found that maturity did not affect (P>0.05) the McDonald (1981) model parameters. The soluble fraction (a) was in average 32.3%, the degradable fraction (b) 42.2% and the fractional degradation rate (c) 4.45%/h, with a lag time (L) period of 7.3h (Table 2). The numerical valued of the estimated average effective DM degradability (ED = 52.9%) was in range with the *in vivo* digestibility, suggesting that the passage rate (kp) assumed in its estimation (4%/h) might have been close to that *in vivo*. In fact, Kuehn et al. (1999) reported rate of passages between 4.0 to 5.5%/h in high producing dairy cattle fed diets based on corn silage. In other words, coincidence among values of *in vivo* digestibility, 24-h-degradability and ED at a kp= 4%/h is indirectly indicating that particles of silage might not be retained in the rumen for digestion more than 24h. On the other hand, it is important to note that approximately 60% of the effective degradability is explained by the contribution of the soluble fraction (Table 2), which is in agreement with data reported by Arieli et al. (1998). Data indicated that the insoluble but degradable fraction was less important that the soluble one as a source of degradable substrates.

Table 2. Parameters of ruminal digestion kinetic and effective degradability (ED, kp= 4%/h) of corn silages from a crop in two maturity stages

Parameters	Stage R3	Stage R5	EEM
Soluble fraction (a, %)	32.1	32.5	0.61
Degradable fraction (b, %)	43.4	40.9	0.67
Fractional degradation rate (c, %/h)	3.69	5.20	0.17
Lag time (L, h)	7.7	6.9	1.12
R^2	0.92	0.91	
ED (%)	51.5	54.3	0.88

Differences between maturity stage were not significant, test Tukey (P>0.05).
EEM = standard error of the mean.

The *in vivo* DM digestibility of silages was in average 52.9% (Table 1), then if the gross energy is 4.4Mcal/kg DM and the losses for methane and urine are 18% of the digestible energy (Energy…, 1993), the concentration of metabolizable energy (ME) should be of 1.9Mcal/kg DM. The same estimation from the 24-h *in situ* degradability is 2.0Mcal ME/kg DM. and from the *in vitro* digestibility 2.2Mcal ME/kg DM, which clearly shows that *in vitro* data over-estimates the silage energy value, as pointed out by Siciliano-Jones and St. Pierre (1997).

Ruminal degradability was measured in a favorable environment for cellulolytic activity with an average ruminal pH of 6.73±0.21 and an average $N\text{-}NH_3$ concentration of 17.6±4.73mg/100ml (Satter and Slytter, 1974; Grant and Mertens, 1992). In spite of it, the DM degraded in 24h was in average only 55.6% (Table 1). This low degradability value (55.6%) is the result of the degradation of soluble carbohydrates, starch and part of the fiber fraction. Since soluble carbohydrates are completely degraded in the rumen and the starch is of high ruminal digestion (Owens et al., 1986; Johnson et al., 1999; Cammell et al., 2000), it is evident that the fibrous portion of the stover fractions (stalks, leaves, husks and cobs) might have been poorly degraded in 24. In fact, as previously pointed out, 60% of the DE was accounted by the contribution of the soluble fraction. Although that in the area in which this experiment was carried out, this fraction will be expected to be depressed. In fact, Uhart and Andrade (1991) reported remobilization of assimilates from stalk to ears during kernel filling due to insufficient sunlight radiation. This should reduce the stalk soluble fraction, which

might depress stalk degradability and, thus, the quality of the stover. The eventual effects of hybrids, agronomic practices (like irrigation, plant density or sowing date) and climate, upon stover degradability remain to be investigated, to understand the proper combination of factors that maximize grain yield and stover quality.

CONCLUSIONS

Corn silage *in vivo* DM digestibility was estimated satisfactorily by the *in situ* DM degradability at 24h of ruminal incubation and by the effective degradability at a kp of 4%/h. The soluble fraction explained the main proportion of corn silage degraded *in situ* in 24-h. The *in vivo* DM digestibility was over-estimated by the *in vitro* digestibility and by an *in situ* incubation of 48h by 15-20%, which indicates that both procedures over-predict silage energy concentration. However, digestibility estimated by any methodology was not affected (P>0.05) by the stage of maturity.

ACKNOWLEDGEMENTS

This work is part of the project 15/A106 "Evaluation of quality of plant and silage of maize", which is supported by the National University of Mar del Plata (Argentina).

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