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Simulation and validation of the ruminal digestion of carbohydrates in cattle from kinetic parameters obtained by *in vitro* gas production technique

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ABSTRACT - This study aimed to validate the estimates of the ruminal degradation of total carbohydrates (TC), ruminal and total digestion of fibrous carbohydrates (FC) and microbial nitrogen flow in the abomasum evaluated by *in vitro* gas production technique (IVGP). Six ruminally and abomasally cannulated steers arranged in a double 3 × 3 latin square were used to measure described parameters with indigestible neutral detergent fiber (INDF) utilization as marker. Total and fibrous carbohydrates degraded in the rumen were estimated through digestion rates obtained for fibrous (FC) and non fibrous carbohydrates (NFC) using *in vitro* gas production technique, corrected for its respective ruminal and post-ruminal passage rates. The estimation of the total digestion of FC was done by the sum of ruminal and post-ruminal digestion of these compounds. The microbial nitrogen flow in the abomasum was estimated by the calculating the microbial efficiency of bacteria that ferment FC and NFC, utilizing the microbial growth rate obtained by the ruminal digestion rate for carbohydrate fractions in IVGP. The utilization of the *in* vitro gas production technique allows obtaining accurate estimates of the ruminal digestion of total carbohydrates, total and ruminal digestion of fiber carbohydrates and microbial protein flow in the abomasum.

Key Words: degradation, fiber, flow, microbial nitrogen, passage rate

Introduction

The knowledge of the feeds' nutritive value is primary on the process of diet formulation, as well as in the selection and improvement of plants designated to ruminants feeding. The forage nutritive value determination need of studies which evaluate the intake, digestibility and nutrients metabolism in animals. Thus, ruminant feed evaluations imply on conduction of many digestion experiments with cannulated animals on one or more sections of the gastrointestinal tract, in order to quantify the digestion, as well as to understand where it happens. However, these studies are expensive, laborious and with a long period of work, which justify the development of lab methods that are easy, accurate and with low costs to estimate the nutritive values (Pell & Schofield, 1993).

Although there are a number of methods which estimate digestibility and ruminal degradation of feeds, including biological methods and equations, there is a constant difficulty in predicting the NDF or fibrous carbohydrates digestion, which is influenced by chemical, physical and anatomic characteristics of the plant cell wall (Jung & Deetz, 1993; Wilson, 1994), besides being dramatically affected by variations in the digesta passage rate of the gastrointestinal tract (Mertens, 1994).

Among the biological methods, the *in situ* incubation has been routinely used for this propose (Nocek, 1988); however, peculiar size and bags' porosity variations, as well as the necessity of surgically preparing animals, increase the expectation for other technique developments. In this respect, the *in vitro* gas production technique deserves attention, since it is not subject to the influences above cited and additionally is inexpensive, nondestructive, precise, fast and enables the estimation of the digestion rate of the non fibrous carbohydrates (Pell & Schofield, 1993).

The current systems used to estimate nutrient requirement and diet formulations for ruminants require kinetic parameters of nutrients degradation (protein and carbohydrates). However, for the practical use of the estimates of nutrients' digestion rate obtained by biological methods, the accuracy in predicting the event measured in the animal must be verified of different dietary conditions, through a process called validation (Mertens, 1976; Pell & Schofield, 1993).

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Therefore, the present study aimed to verify the accuracy of predictions for rumen digestion of fibrous and total carbohydrates, total digestion of the carbohydrates and the flow of microbial nitrogen in the abomasum by the use of digestion rate obtained by the *in vitro* gas production technique.

Material and Methods

The study involved three stages: the first consisted of the ruminal and total nutrient digestion determination obtained in bovine fitted on rumen and abomasum, which were fed diets based on silage corn, elephant-grass silage and Tifton 85 grass hay (Cabral et al., 2004); the second stage was done to obtain estimates of the digestion rates of carbohydrate fractions by in vitro gas production technique, and the last one involved the simulation of ruminal and total digestion predicted values of the total and fibrous carbohydrates from kinetics parameters obtained in stage two, along with the validation of these estimates by comparing them with those measured in animals (Cabral et al., 2006; 2008).

The study with cattle was carried out at the Laboratório Animal of the Departamento de Zootecnia of the Universidade Federal de Viçosa, Minas Gerais, for evaluation of diets based on silage corn (*Zea mays*, L.), elephant-grass silage (*Pennisettum purpureum*, Schum) cv. Cameroon or Tifton 85 grass hay (*Cynodon spp.*), suppemented with 10% of soybean meal. The Tifton 85 grass hay was additionally supplied with 0.5% of urea:ammonia sulfate mixture (9:1) in order to maintain the same crude protein percentage in diets (Table 1).

Six ruminal and abomasum cannulated crossbred cattle with average initial body weight of 351 kg were distributed in a double 3 × 3 Latin square design, whose periods lasted 16 days each: 10 d for adaptation to diets, and the final six for feces and abomasum sampling. The apparent intestinal digestibility of nutrients was measured by the difference between the total and rumen digestibility.

Animals were weighed at the beginning and end of each experimental period, housed in covered individual pens with concrete floor, and fed once a day (7 h 30 min). Daily orts were quantified to fit and evaluate the intake, allowing approximately 5% of orts in dry matter basis.

The sample collections of feces and abomasal digesta were taken every 26 hours starting at 8 h on the 11th day and finishing at 18 h of the last day of each experimental period. Feces, abomasal and orts samples were dried in forced ventilation oven at 55 ^{o}C for 72 h. At the end of the process, a composite sample was prepared for each period and treatment for further chemical analysis.

Samples were ground in a knife mill with 1-mm sieves for subsequent analysis of dry matter (DM), nitrogen (N), ether extract (EE), ash (Silva & Queiroz, 2002), NDF and NDF corrected for ash content and protein (NDFap) (Van Soest et al., 1991), total carbohydrates (TC = 100 - (% CP +% EE +% ash)) (Sniffen et al., 1992) and non-fibrous carbohydrates (NFC = 100 - (% CP +% EE +% NDF +% ash)) (Sniffen et al., 1992). B_2 carbohydrate fraction was obtained by subtracting the indigestible NDF from the NDFap.

Fecal excretion and flow of abomasal dry matter were obtained from INDF use as marker after 144 h of *in vitro* incubation (Cochran et al., 1986), and utilized for the estimation of ruminal and total nutrients digestion, respectively.

The abomasal flow and fecal dry matter excretion (g/animal/day) were estimated by the ratio between the amount of consumed marker (iNDF) and its concentration on abomasal digesta and feces, in the same order. The ruminal and total apparent digestion were obtained by the difference between dry matter intake and amounts flown to the abomasum and present in the fecal excretion, respectively. The post ruminal digestion of dry matter was estimated by deducting the ruminal digestion from total apparent digestion.

The rates of digestion of fibrous and non fibrous carbohydrates in each ingredient of diets included the roughage and soybean meal (Table 2), which were obtained according to Cabral et al. (2004).

Table 1 - Chemical composition of the experimental diets (% DM)

Item	Diet		
	Corn silage	Tifton 85 grass hay	Elephant grass silage
Crude protein	11.33	10.66	10.63
Ash	5.79	6.73	10.65
Ether extract	2.39	1.38	1.52
Total carbohydrates	80.47	82.63	71.20
Neutral detergent fiber	52.08	80.02	69.03
FDNap	48.92	73.72	63.07
Non fibrous carbohydrates	31.56	8.90	14.13
iNDF	17.43	35.40	29.43

NDFap = neutral detergent fiber corrected for ash and protein; iNDF = indigestible neutral detergent fiber.

Table 2 - Average values for the total carbohydrates fractions and their respective digestion rates estimated for the feeds used in experimental diets

Fraction	Corn silage	Tifton 85 grass hay	Elephant-grass silage	Soybean meal
		% of total carbohydrates		
NFC	37.05	7.02	14.74	75.75
B_2	39.91	51.20	51.70	16.88
iNDF	23.04	41.77	33.56	7.37
		Digestion rates (h^{-1})		
NFC	0.1905	0.1294	0.1410	0.1783
B_2	0.3000	0.0254	0.0263	0.0300

NFC = non-fiber carbohydrate; iNDF = indigestible neutral detergent fiber.

Thus, for each feed, the *in vitro* rumen degradation rates of fiber and non fiber carbohydrates were obtained. Integrated in the total diet provided to the animals, and before adjustments for respective passages rates of each fraction estimated for roughage and concentrates according Cannas & Van Soest (2000), they allowed the obtainment of the ruminal degradation.

The predictions of ruminal digestion of carbohydrates of total (RDTC) and fibrous carbohydrates (RDFC) were obtained by the following equation: RDTC (kg/day) = ingested CNF * kd/(kd + kp) + ingested FC * kd/(kd + kp), where: RDTC = ruminal degradability of total carbohydrates, NFC = non-fiber carbohydrates; Kd = ruminal degradation rate estimated for each fraction through the *in vitro* gas production technique and, kp = rumen passage rate of each fraction .

The total digestion of fibrous carbohydrates was estimated by the sum of ruminal digestion (RDFC) and intestinal digestion, from the following equation: TDFC (kg/day) = FCE + KDI */(KDI + kpi), where FCE = fibrous carbohydrates which escape from digestion, KDI = rate of intestinal digestion of fiber carbohydrates, considered as 90% of the rumen digestion rate, and kpi = rate of intestinal passage of fibrous carbohydrates, whose value used was 0.125 h^{-1} and corresponds to the average observed by Detmann et al. (2001). Although Ulyatt et al. (1974), cited by Mertens & Ely (1979), have suggested that the activity of microbial cells in the large intestine is equal or greater than that observed in the rumen, partly attributed to the increased susceptibility to digestion of hemicellulose due to acidity of the abomasal, Bailey & MaCraee (1970) and Hungate (1966) suggested that the enzymatic activity in this compartment is smaller than the rumen. Thus, according to Mertens & Ely (1979), the digestion rate of fibrous carbohydrates in the large intestine was assumed to be 90% of the observed in rumen, since the fiber that escapes the rumen would be more resistant to digestion.

The microbial nitrogen flow in the abomasum was estimated from the microbial efficiency calculated by the equation PIRT (1965): $1/Y = M/\mu + 1/Ymax$, where Y = estimated microbial efficiency (g cell. g digested carbohydrate^{-1}), M = maintenance requirement; μ = microbial growth rate considered proportional to the degradation of carbohydrates rates, and Ymax. = Theoretical maximum yield. The values of M and Ymax utilized in this study were those suggested by Russell et al. (1992), where M ranges from 0.05 to 0.15 g carbohydrate/g cell per hour for microorganisms fermenting structural and nonstructural carbohydrates, respectively, and Ymax is 0.4 g cell/g carbohydrate. Once obtained for each population, microbial efficiency was multiplied by its previously estimate ruminal degradation, obtaining thereby the dry matter microbial flow in the abomasum, which, when multiplied by the average percentage of nitrogen (18%) of isolates obtained by Cabral et al. (2008), allowed the calculation of the nitrogen microbial flow in the abomasum.

The validation of the predictions concerning the total carbohydrates degradability, total and ruminal digestion of carbohydrates (kg/day), nitrogen microbial flow in the abomasum (kg/day) by the *in vitro* gas production technique were carried out regardless of treatment, by adjusting the simple linear regression (full model) of predicted values relative to that observed. Estimates of regression parameters were tested under the hypotheses: $H_0^{(a)}$: $\beta_0 = 0$ and $H_0^{(b)}$: $\beta_1 = 1$. When the null hypothesis was not rejected by the test, the occurrence of similarity between the predicted and observed values was considered. Conversely, when there was rejection of the null hypothesis, a new regression equation was drawn, removing the parameter for the intercept (reduced model) and estimating the global estimates addiction as: $B = (\beta - 1)*100$; where B = addiction global estimates (%) and β = estimate of the slope of the fitted equation without consideration of the intercept parameter (reduced model). For all statistical procedures used, $\alpha = 0.05$, using the statistical package SAS (2001).

Results and Discussion

The means to predict the values of total carbohydrates' ruminal degradation, total and ruminal digestion of fibrous carbohydrates and microbial nitrogen flow in the abomasum were similar to those obtained with the animals (Table 3). The estimated passage rates are within the limits found in the national literature (Detmann et al., 2001).

For ruminal degradation of fibrous carbohydrates, there were no differences (P>0.05) between predicted and observed values for both intercept β_0 (H_0 is accepted $^{(a)}$) and the slope (H_0 is accepted $^{(b)}$: $\beta_1 = 1$) (Table 4), which indicates that predicted values were accurate with the observed values. In Figure 1-a, we can observe the high frequency of points along the equation line Y = X, which indicates the accuracy of estimates (Figure 1).

In contrast, Vieira et al. (2000) observed underestimation for fibrous carbohydrates' digestion in cattle kept under grazing conditions, which can be attributed to flaws at obtaining estimates of degradation kinetics parameters from *in vitro* gravimetric methods for fibrous carbohydrates, as well as the errors from estimate of passage rates. Thus,

Table 3 - Observed and estimated values for the ruminal and total digestion of the total and fibrous carbohydrates (kg/animal/day), microbial nitrogen flow in the abomasum (g/day) and passage digesta rate (h^{-1}) for experimental diets

Item	Diets		
	Corn silage	Tifton 85 grass hay	Elephant-grass silage
Observed rumen digestion of fibrous carbohydrates	1.45	1.83	1.31
Estimated rumen digestion of fibrous carbohydrates	1.45	1.90	1.32
Observed rumen digestion of total carbohydrates	3.07	1.96	1.77
Estimated rumen digestion of total carbohydrates	3.10	2.30	1.91
Observed microbial nitrogen flow in the abomasum	67.25	48.94	53.14
Estimated microbial nitrogen flow in the abomasum	70.57	43.61	40.73
Observed total digestion of fibrous carbohydrates	1.69	2.11	1.36
Estimated total digestion of total carbohydrates	1.74	2.26	1.55
Rate of roughage (h^{-1}) passage through digesta	0.0322	0.0303	0.0279
Rate of concentrate (h^{-1}) passage through digesta	0.0414	0.0384	0.0346

Table 4 - Estimative of the regression parameters between predicted and observed values for the analyzed variables

Variable	Complete model		Reduced model
	b_0	b_1	b_1
Rumen digestion of the total carbohydrates	0.37*	0.91	-
Rumen digestion of the fibrous carbohydrates	0.23	0.87	-
Total apparent digestion of the fibrous carbohydrates	0.37*	0.86	-
Flow of the microbial N in abomasum	–14.17	1.16	-

* Significant difference (P<0.05) for $H_0^{(a)}: \beta_0 = 0$ and $H_0^{(b)}: \beta_1 = 1$, complete ($Y = \beta_0 + \beta_1X$) and reduced model ($Y = \beta_1X$).

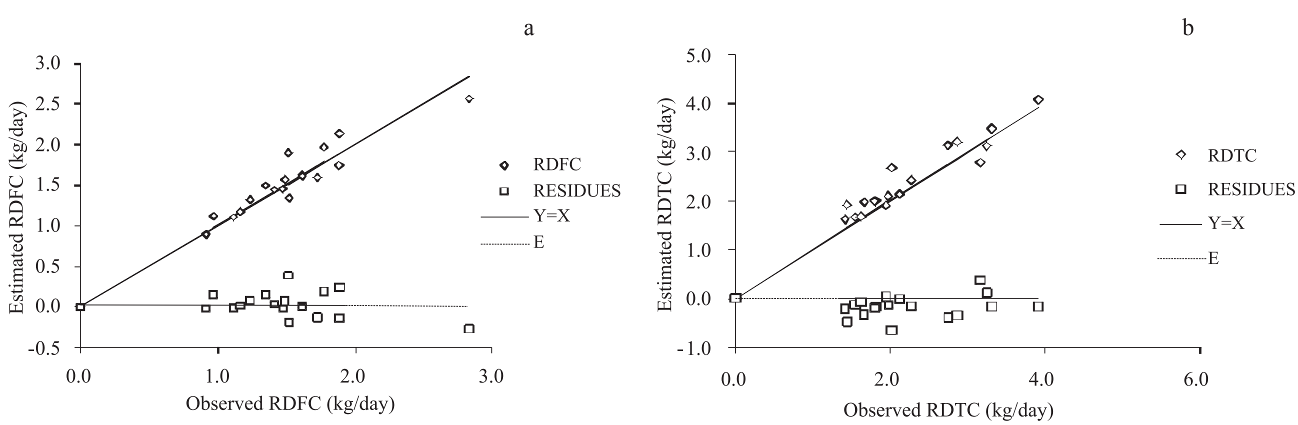

Figure 1 - Relation between predicted and observed values and your respective ordinary residue for ruminal degradation of (a) fibrous (RDFC) and (b) total carbohydrates (RDTC).

given that these authors obtained values for digesta passage rate by chromium mordant fiber use, the passage rate of marked particles can be higher than that observed for fiber of the non-marked food, since the extraction with neutral detergent can alter the physical cell wall structure, and the bind with chromium may increase the particles density.

According to Mertens (1994), variations in the passage rate of rumen digesta have a high effect on the prediction of digestion of fibrous carbohydrates, which have slower digestion rate, and therefore are affected by little changes in the ruminal passage rate and their estimates.

For the ruminal degradation of total carbohydrates, the intercept was different from the parametric zero value, which leads to rejection of the hypothesis $H_0^{(a)}: \beta_0 = 0$; there is a constant bias of 0.37 kg / day, while the angular coefficient does not differ from one, accepting the hypothesis $H_0^{(b)}: \beta_1 = 1$. It means that there was no difference between the predicted and observed values for that variables.

A close relationship between predicted and observed values for total carbohydrates degraded in the rumen was observed (Figure 1-b), since most of points are near to the equation line Y = X. It indicates the existence of a linear relationship between predicted and observed values. The distance of the predicted values from the line Y = X can indicate the occurrence of under or overestimated values for the ruminal digestion of carbohydrates, which weren't evident for these variable.

Estimated fibrous carbohydrates digested in the total digestive tract were also accurate from digestion rates obtained using the *in vitro* gas production technique, when compared with values measured in animals. Although there is a constant tendency of 0.37 kg/day for this variable, because the intercept was statistically different from parametric value zero, the regression slope did not differ from the parametric value 1 (Table 4). Considering that estimates of fibrous carbohydrates digested in the rumen were accurate, it could be said that their estimates of NFC digested in the intestine were too. Since the digestion of these compounds in the total digestive tract is the sum of ruminal and intestine digestion, it indicates that the assumptions (Mertens & Ely, 1979) for its calculation have biological significance.

Assuming that the availability of digestible carbohydrates in the rumen is the main limiting factor for ruminal microbial growth and hence to the microbial protein flow to the abomasum, estimated values for these compounds were used to predict the microbial nitrogen (Nmic) flow in the abomasum. The use of carbohydrate digested in the rumen resulted in accurate estimates of microbial N in the abomasum (Tables 3 and 4); there were no differences ($P<0.05$) between the intercept and the slope of parametric values 0 and 1, respectively, in contrast to the observed by Vieira et al. (2000). The intercept value of -14.17 g of microbial nitrogen is close to the -12 g found by Russell et al. (1992).

Although the passage rates were not measured directly in animals in this experiment (estimated values were used), the results presented enable, at first, the use of *in vitro* gas production technique to estimate rumen kinetic parameters of carbohydrates degradation in foods. Such estimates accurately predicted the digestion of total fibrous carbohydrates. Moreover, these digestion rates allowed estimating the contribution of microbial protein in the intestines and, thus, supplemental protein escape may result in better responses to dietary adequacy.

In this study three different diets with different values for ruminal carbohydrates degradation were investigated, ranging from 1.77 to 3.07 kg.animal^{-1}.day^{-1} (Table 3), for diets based on elephant grass silage and corn silage, respectively. Curiously, the use of the kinetic parameters from *in vitro* gas production technique allowed obtaining accurate values for the analyzed variables, showing the sensibility of the system at predicting variations in the energy (carbohydrates) availability in the rumen.

Detmann et al. (2005) evaluated the accuracy of the estimates using *in vitro* gas production technique and observed overestimation for total carbohydrates' degradability. However, considering that the abovementioned authors used grazing steers and the forage intake was estimated and not measured, mistakes associated to prediction of forage intake could be affecting the estimate of total carbohydrate digested in the rumen.

Additionally, these authors obtained the estimates of digestion rates for carbohydrate fractions from gas production curves for 120 hours, which can lead to a mistaken interpretation of results. Because part of measured gases in long incubation periods may reflect autolysis and recycling of microorganisms cells compounds and nor food constituents, such as the carbohydrates, according by Cone & Van Gelder (1999), the use of data obtained from long periods of incubation could lead to and underestimates of carbohydrates digestion rates, which do not reflect the real values for foods and diets.

Although experiments with other dietary conditions are necessary, the validation of these estimates may greatly contribute to diet formulation, animal performance prediction and feeds evaluation, reducing thus, the cost and labor involved in animal experimentation.

Considering that carbohydrates are the major constituents of feeds used in ruminant feeding, it could be stated that these are the main diets' compounds for accurately predicting the ruminal and total digestion, especially for fibrous carbohydrates. These last compounds correspond to the largest proportion of energy in tropical forages. The last compounds correspond to largest proportion of energy in tropical forages. However, because they present incomplete availability in gastrointestinal tract of herbivores are responsible for the lowest use of the energy in diets based in these roughages. Due to the intrinsic nature of the cell wall of these forages, related to their chemical, physical and anatomical characteristics, the relationship of chemical composition and availability of this fraction is variable, which hampers the better understanding of the factors that limit their digestion.

Considering also that carbohydrates represent the main primary energy source for microbial growth in the rumen (Russell et al., 1992), which is the primary source of amino acids for ruminants, it could be understood that digestion rates obtained by *in vitro* gas production technique enables accurate estimation of the microbial protein flow in the abomasum.

The NRC (2001) proposed the estimation of total digestible nutrients (TDN) of feed from its chemical composition and suggests that NDF digestibility can be estimated by *in vitro* incubation during 48 hours. However, variations in the digestion rate of this fraction, on the indigestible fiber proportion among feeds and on the digesta passage rates influenced by different dietary conditions and animal physiological status limit the use of these estimates.

The use of kinetic parameters could be more accurate to estimate total NDF digestion because it takes into account the inherent characteristics of fiber (digestion rate) and passage rate, which is the main factor that affects the fiber availability in the gastrointestinal tract. This fact would allow better estimation of available energy of diets, since fibrous compounds are the main source of dietary energy for ruminants under tropical conditions, in addition to being responsible for energy availability variation.

Conclusions

The use of estimates of digestion rates by the *in vitro* gas production technique allows an accurate obtainment of total carbohydrates and fiber digestion in the rumen, as well as the microbial nitrogen flow to abomasums.

References

BAILEY, R.W.; MaCRAEE, C.J. The hidrolysis by rumen and caecal microbiol enzymes of hemicellulose in plant and digesta particles. **Journal of Agricultural Science**, v.75, p.321, 1970.

CABRAL, L.S.; VALADARES FILHO, S.C.; DETMANN, E. et al. Taxas de digestão das frações protéicas e de carboidratos para as silagens de milho e de capim-elefante, o feno de capim-tifton-85 e o farelo de soja. **Revista Brasielira de Zootecnia**, v.33, n.6, p.1573-1580, 2004.

CABRAL, L.S.; VALADARES FILHO, S.C.; DETMANN, E. et al. Consumo e digestibilidade dos nutrientes em bovinos alimentados com dietas à base de volumosos tropicais. **Revista Brasileira de Zootecnia**, v.35, n.6, p.2406-2412, 2006.

CABRAL, L.S.; VALADARES FILHO, S.C.; DETMANN, E. et al. Eficiência microbiana e parâmetros ruminais em bovinos alimentados com dietas à base de volumosos tropicais. **Revista Brasileira de Zootecnia**, v.37, n.5, p.919-925, 2008.

CANNAS, A.; VAN SOEST, J.P. Simple allometric models to predict rumen feed passage rate in domestic ruminants. In: McNAMARA, J.P.; FRANCE, J.; BEEVER, D.E. (Eds.) **Modeling nutrient utilization in farm animals**. London: CAB Inernational, 2000. p.49-62.

COCHRAN, R.C.; ADAMS, D.C.; WALLACE, J.D. et al. Predicting digestibility of different diets with internal markers: Evaluation of four potential markers. **Journal of Animal Science**, v.63, n.5, p.1476-1483, 1986.

CONE, J.W.; VAN GELDER, A.H. Influence of protein fermentation on gas production profiles. **Animal Feed Science Technology**, v.76, n.3-4, p.251-264, 1999.

DETMANN, E.; CECON, P.R.; PAULINO, M.F. et al. Estimação de parâmetros da cinética de trânsito de partículas em bovinos sob pastejo por diferentes seqüências amostrais. **Revista Brasileira de Zootecnia**, v.30, p.222-230, 2001.

DETMANN, E.; PAULINO, M.F.; CABRAL, L.S. et al. Simulação e validação de parâmetros da cinética digestiva em novilhos mestiços suplementados a pasto, por intermédio do sistema *in vitro* de produção de gases. **Revista Brasileira de Zootecnia**, v.34, n.6, p.2112-2122, 2005.

HUNGATE, R.E. **The rumen and its microbes**. New York: Academic Press, 1966. 533p.

JUNG, H.G.; DEETZ, D.A. Cell wall lignification and degradability. In: JUNG, H.G.; BUXTON, D.R.; HATIFIELD, R.D. et al. (Eds.) **Forage cell wall structure and digestibility**. Madison: America Society of Agronomy, Crop Science Society of America, Soil Science Society of America, 1993. p.315-46.

MERTENS, D.R. Principles of modeling and simulation in teaching and research. **Journal of Dairy Science**, v.60, n.7, p.1176-1186, 1976.

MERTENS, D.R.; ELY, L.O. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. **Journal of Animal Science**, v.49, n.4, p.1085-1095, 1979.

NATIONAL RESEARCH COUNCIL – NRC. **Nutrients requirements of dairy cattle**. 7.ed. Washington, D.C.: National Academy Press, 2001. 381p.

NOCEK, J. In situ and other methods to estimate ruminal protein and energy digestibility: a review. **Journal of Dairy Science**, v.71, p.2051-2069, 1988.

PELL, A.N.; SCHOFIELD, P. Computerized monitoring of gas production to measure forage digestion *in vitro*. **Journal of Dairy Science**, v.76, n.9, p.1063-1073, 1993.

PIRT, S.J. The maintenance energy of bacteria in growing cultures. **Proceedings of Royal Society (series B)**, v.163, p.224-231, 1965.

RUSSELL, J.B.; O'CONNOR, D.J.; FOX, D.G. et al. A net carbohydrate and protein system for evaluating cattle diets: ruminal fermentation. **Journal of Animal Science**, v.70, n.12, p.3551-3581, 1992.

SILVA, D.J.; QUEIROZ, A.C. **Análise de alimentos**: métodos químicos e biológicos. 3.ed. Viçosa: UFV, 2002. 235p.

SNIFFEN, C.J.; O'CONNOR, D.J.; VAN SOEST, P.J. et al. A net carbohydrate and protein system for evaluating cattle diets: carbohydrate and protein availability. **Journal of Animal Science**, v.70, n.12, p.3562-3577, 1992.

STATISTICAL ANALYSIS SYSTEM - SAS. **The statistical analyze systems for windows**: version 8. Cary: 1999-2001. (CD-ROM).

VIEIRA, R.A.M.; PEREIRA, J.C.; MALAFAIA, P.A.M. et al. Simulação da dinâmica de nutrientes no trato gastrintestinal: aplicação e validação de um modelo matemático para bovinos a pasto. **Revista Brasileira de Zootecnia**, v.29, n.3, p.898-909, 2000.

WILSON, J.R. Cell wall characteristics in relation to forage digestion by ruminants: review. **Journal of Agricultural Science**, v.122, n.2, p.173-182, 1994.