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Parameterization of ruminal fibre degradation in low-quality tropical forage using *Michaelis–Menten* kinetics

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ABSTRACT

This work aimed to parameterize the ruminal degradation of neutral detergent fibre (NDF) from low-quality tropical forage using *Michaelis–Menten* kinetics. The intake, rumen outflow (*L*), fractional degradation rate (kd), discrete lag (LAG) and effective degradability (ED) of NDF, and the microbial flow of nitrogenous compounds into the small intestine (Nmic) were assessed in two 5 × 5 Latin square experiments by using five Holstein × Zebu heifers cannulated in the rumen. The experiments were carried out sequentially and the treatments were formed by increasing the level of supplementation with nitrogenous compounds. A low-quality signal grass (*Brachiaria decumbens*) hay was used as roughage. The nitrogen supplement was a mixture of urea, ammonium sulfate and albumin, at the ratios of 4.5:0.5:1.0, respectively. The crude protein contents in the diets ranged from 51.9 to 136.3 g/kg of dry matter. The rumen ammonia nitrogen (RAN) concentration was used as an independent variable. The NDF intake, *L* and Nmic showed a quadratic pattern ($P<0.05$) as a function of RAN concentration, and the critical points (maximum responses) were observed with 15.17, 16.28, and 14.52 mg of RAN/dL of rumen fluid, respectively. On the other hand, ED and LAG presented a *linear-response-plateau* ($P<0.05$) according to the RAN concentration, with break points close to 8 mg/dL for ED (maximum estimate) and LAG (minimum estimate). The RAN concentrations to optimize NDF degradation and intake were defined as 8 and 15 mg/dL, respectively. This difference between estimates appears to be due to a better adequacy of the metabolizable protein:metabolizable energy ratio in the animal metabolism, which increases the animal intake even after the rumen NDF degradation has been optimized. This observation was supported by Nmic pattern. An adapted *Michaelis–Menten* model was applied to the data, where RAN was the independent variable and kd the dependent variable. The relationship between these variables was found to be significant by using the *Hanes–Woolf* plot ($P<0.01$). Based on this model, the rate of NDF degradation as a function of RAN concentration indicates that fibre degradation in the rumen could be considered a second order process. In this context, the RAN concentration of 8 mg/dL was assumed as the limit where zero order (below limit) and first order (above limit) reactions become predominant for NDF degradation in the rumen.

Abbreviations: ADFom(n), Acid detergent fibre corrected for ash and nitrogenous compounds;ADIP, Acid detergent insoluble protein;BCVFA, Branched-chain volatile fatty acids;CP, Crude protein;DM, Dry matter;ED, Effective degradability of neutral detergent fibre;EE, Ether extract;kd, Fractional degradation rate of NDF; km, The Michaelis–Menten constant;*L*, Time-dependent rate parameter associated with rumen flow of fibrous particles;LAG, Discrete lag for fibre degradation; Lignin (sa), Lignin determined by solubilization of cellulose with sulphuric acid;aNDFom(n), Neutral detergent fibre assayed with a heat stable amylase and corrected for ash and nitrogenous compounds;NDF, Neutral detergent fibre;pdNDF, Potentially degradable fraction of neutral detergent fibre;NFC, Non-fibrous carbohydrates;Nmic, Intestinal flow of microbial nitrogenous compounds;OM, Organic matter;RAN, Rumen ammonia nitrogen.

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1. Introduction

The carbohydrate components of tropical grasses insoluble fibre (cellulose and hemicellulose) are the main energy resources for cattle production in tropical regions. This can be attributed to an adequate production of digestible energy at lower costs compared to other energetic feedstuffs (Detmann et al., 2008).

However, the climate characteristics of such regions, mainly rainfall and temperature, divide the year in two different seasons: rainy and dry. Although during the rainy season tropical forages under grazing have high quality, in the dry season its nutritive value is severely reduced, with increased lignin content in the cell wall and a decreased total content of nitrogenous compounds. These modifications can compromise the availability of energy from forage (Paulino et al., 2001, 2006) by reducing the neutral detergent soluble compounds and decreasing neutral detergent fibre (NDF) digestibility (Van Soest, 1994).

In this context, the strategic supplementation with limiting nutrients become the best option for cattle management, specially when this supplementation is based on feeding nitrogenous compounds, which stimulates the fibrolytic activity in the rumen and increases the utilization of low-quality fibrous carbohydrates (Costa et al., 2008; Detmann et al., 2008; Paulino et al., 2006).

The main challenge for nutritionists in tropical conditions is to understand the dynamics of NDF degradation for low-quality forages as a function of nitrogenous compounds supplementation, under the point of view of productive and economic optimization of interactions between basal nutrient resource (forages) and supplements. Under non-tropical conditions, some modeling approach has been developed to take into account the effect of the nitrogen deficiency on fibre utilization in the rumen for prediction of animal performance (Tedeschi et al., 2000), but similar information is not currently available in the tropics.

In the ruminant nutrition literature, it is frequently assumed that NDF utilization in the rumen consists of a first order dynamic process. This argument is based on the assumption that rumen degradation parameters would be defined only by substrate characteristics (Detmann et al., 2005), without limitations in enzyme availability for NDF degradation.

However, recent studies under tropical conditions indicated that enzymatic limitation could explain, at least in part, the reduction of NDF utilization in the rumen of animals fed low-quality forage, as observed for forages under grazing during the dry season (Costa et al., 2008; Lazzarini, 2007; Sampaio, 2007; Zorzi, 2008). This pattern suggests that ruminal fibre degradation is a process that follows a second order kinetics, or a *Michaelis–Menten* process, being defined simultaneously by enzyme availability and substrate characteristics (Detmann et al., 2005, 2008).

Some experimental evidences indicated that the low nitrogen content of low-quality forages could limit the availability of microbial fibrolytic enzymes in the rumen. Thus, the main effect of the supplementation with nitrogenous compounds would be the higher supply of nitrogenous precursors for the synthesis of microbial enzymes (Costa et al., 2008; Detmann et al., 2008; Souza, 2007).

Several parameters have been suggested to evaluate the availability of dietary nitrogenous compounds in the rumen. However, the concentration of rumen ammonia nitrogen (RAN) has been used as a qualitative reference to understand the adequacy of the rumen environment according to the microbial activity on fibrous carbohydrates (Hoover, 1986). This strategy is possibly associated with the fact that RAN is the preferred nitrogen source for the growth of fibrolytic microorganisms (Russell, 2002).

Most models used to describe fibre degradation in the rumen are based on simple first order assumptions (Mertens, 2005). However, this assumption interferes with the correct interpretation of rumen degradation dynamics when the diet is based on low-quality tropical forages. In these cases, a second order model might be a better tool for interpreting NDF degradation.

The *Michaelis–Menten* model allows the evaluation of enzyme kinetics when the ratio of enzyme:substrate is variable ((Nelson and Cox, 1999); Voet and Voet, 2006), such as the situation described above. However, it has not been verified, at least in the tropics, any research applying this model to interpret NDF degradation in the rumen.

The objective of this work was to evaluate the NDF degradation dynamics in the rumen of cattle fed low-quality tropical forage as a function of rumen ammonia nitrogen concentration using the *Michaelis–Menten* kinetics.

2. Material and methods

2.1. Location and animals

Two experiments were sequentially carried out in the Animal Laboratory at the Department of Animal Science of Federal University of Viçosa (UFV), Viçosa, Brazil, from December of 2005 to May of 2006. Five crossbred heifers (Holstein × Zebu) averaging 180 ± 21 and 209 ± 13 kg of body weight (BW), were used for the first and second experiment, respectively.

The animals were surgically fitted with ruminal cannulae, approximately 60 days prior to the beginning of Experiment 1. All surgical and animal care procedures were approved by the University Animal Care Committee. Ruminal fistula and their surrounding areas were cleaned routinely during the experiments. The animals were treated for endo and ecto parasites at the beginning of the experiments and kept into individual stalls (which were cleaned daily) of approximately 10 m^2. Water and mineral mixture were available to the animals at all times.

2.2. Experimental diets and feeding

The forage fed to the animals consisted of low-quality signal grass (*Brachiaria decumbens* Stapf.) hay. The hay was produced from a dry season cutting (August 2005) of the forage available in a signal grass pasture located in the central region of Brazil (latitude 18°41′S, longitude 49°34′W, average altitude 620.2 m). The climate is classified as Aw type, hot and humid, with coldest monthly temperatures above 18 °C, annual average rainfall between 1400 and 1600 mm, rainy season from November to March and dry season from April to October.