Kinetics of accumulative gas production from horse colon digesta

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Introduction

There are many methods to measure diet quality and the gas production method its one with facilities and restrictions (*Pell* and *Schofield* 1993; *Theodorou* et al. 1993). The technique of accumulative gas production measures forage digestibility and parameters kinetics fermentation of rumen fluid. It's a simple technique, reliable, low cost to laboratory studies of forage quality (*Thiago* and *Barrocas* 1998). The in vitro technique of cumulative gas production was also used in non-ruminant herbivores, like horses, rabbits, and swine. Most of these studies were focused on microbial fermentation of feeds. However, the caecum position at the end of digestive tract reduces the accuracy of this approach, since extensive digestive process occur on feeds before reaching to the hind gut (*Fondevila* et al. 2002). Thus, it seems more accurate to use hind gut contents as fermentation substrate, so this work aimed to evaluate accumulative gas production in vitro of contents of ventral colon in horses fed different diets with roughage and concentrate.

Material and Methods

The experiment was carried out at Equine Health Laboratory of Universidade Federal Rural do Rio de Janeiro with four horses with cannula fitted at right ventral colon. Surgical procedures were adapted to technique described by *Lopes* (2002). Four diets were supplied: Diet I - Tifton-85 hay (Cynodon dactylon); Diet II - Tifton-85 hay (50%) and alfalfa hay (Medicago sativa) (50%); Diet III - Tifton-85 hay (30%), alfalfa hay (30%) and concentrate I (40%); Diet IV - Tifton-85 hay (60%) and concentrate II (40%). The experimental design was 4x4 Latin Square, with diets supplied at six hours intervals during 21 days. At 21st day, colon contents were colleted, about 700gr, at 2:30 hours after morning meal. Samples were stored at -20°C and at the end of trial were dried in forced ventilation oven (55°C), during 72 hours and grounded until 1 mm. Chemical analysis procedures of diets and colon contents were described by *Silva* and *Queiroz* (2002). Inoculum source to in vitro fermentation procedure was obtained by rumen-fistulated bovine fed elephant grass (Pennisetum purpureum) ad libitum and 1.0 kg of concentrate. The inoculum was collected before morning meal, filtered and conditioned at 39°C to microbial preservation and, immediately, taken to the Lab. Replicates aliquots (100 mg DM) of colon samples were added in to flasks of 50mL containing McDougall's buffer solution (8 mL) and ruminal inoculum (2mL), closed with rubber stopper and aluminium crimp seal. Bottles were incubated at 39°C during 96 hours. Readings were made at 0, 0.5, 1, 2, 3, 4, 6, 9, 12, 15, 21, 27, 33, 39, 48, 60, 72 and 96 hours after incubation with a needle coupled to an electronic transdutor linked directly to a multimeter. Readings obtained in mV were later transformed to gas volume (mL) through procedure described by *Pell* and *Schofield* (1993), using a conversion factor and 1 volt corresponds to 8.68 mL. Kinetics of accumulative gas production was fitted to the model:

$$V = V0 + VF (1 - \exp[-kd (t - l)]); t > l; t \leq l$$

where V represents the accumulative gas production (mL), V0 is initial volume (mL), VF is final volume (mL), Kd is specific degradation rate of substratum, t is incubation time (h) and l corresponds to the lag time (h). Values were submitted by variance analysis and means compared by Tukey test at 5% probability.

Results

Chemical composition of roughage and concentrate supplied to horses can be observed in Table 1.

Table 1 Chemical composition of roughage and concentrates.

	Feeds			
Nutrient	Alfafa hay	Tifton-85 hay	Concentrate I	Concentrate II
Dry Matter (%)	84.73	83.88	86.91	87.83
Crude Protein (%)	15.94	9.11	13.91	18.16
NDF (%)	46.19	72.31	11.9	10.98
ADF (%)	32.36	34.03	3.61	4.66
Gross Energy[1]	3796.1	3813.1	4090.6	3875.8

[1]- Kcal/kg

Table 2 Parameters of the accumulative gas production of colon contents.

Diet:	V_0 (mL)	V_F (mL)	K_d	L (h)
I	0.38[a]	5.68[b]	0.000147[a]	27.4[a]
II	0.31[a]	9.35[ab]	0.019000[a]	20.4[a]
III	0.49[a]	13.21[a]	0.019400[a]	7.7[a]
IV	0.87[a]	13.68[a]	0.010200[a]	19.4[a]
CV (%)	83.3	26.8	79.5	38.2

Means with different letters in line differ by SNK test ($P<0.05$).

Parameters of accumulative gas production of colon contents can be observed in Table 2. In relation to final volume of gas produced, differences were observed ($P<0.05$) with values varying from 5.68mL/100mgDM with samples from horses that fed Tifton-85 hay to 13.68/100mgDM with samples from horses that fed Tifton-85 hay and concentrate II, indicating that diet IV probably provides a better enviroment for fermentation in relation to diet I. Values of degradation rate of colon digesta didn't present differences ($P>0.05$).

Colon contents of horses fed tifton-85 hay presented smaller cumulative gas production suggesting lowest substratum to microbial fermentation at the colon and can be supposed that gas produced in vitro resulted from microbial turnover. Colon contents of horses fed diets composed by tifton-85 hay and alfalfa hay also presented smaller gas production but there weren't microbial turnover evidences. Largest cumulative gas production were observed from samples of colon content of horses fed diets composed by tifton-85 hay and concentrate suggesting the largest contribution of fermentative substrate from diets at colon in horses, acording to figure 1.

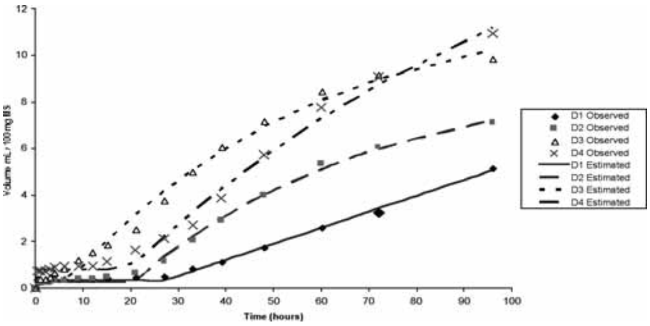

Fig 1 Accumulative gas production of colon contents.

The lag phase is the period in which microorganisms develop digestion capacity in relation to substratum and those parameters were similar (P>0.05), the values suggesting that there were an adaptation of ruminal microorganisms from inoculum to samples of colon content.

Discussion

Colon content of horses fed diet composed by Tifton-85 hay presented a low VF and a very slow Kd, probably due a lack of substrate in the colon resulted from a completely digestion of soluble fractions until the caecum. By another way, gas production resulted from fermentation of microorganisms presented in inoculum. Colon content of horses fed diet composed by Tifton-85 hay and alfalfa hay presented smallest initial gas production, 0.31 mL /100mg DM, and also smallest final gas production, 9.35 mL /100 mg DM. *De Fombelle* et al. (1999) evaluate microbial activity in hind gut in relation to hay proportion and concentrate in horse diets and observed higher changes of microorganisms in colon than in caecum. Those authors observed that exclusive hay diet did not promote significant changes in microbial activity.

Fondevila et al. (2002) proposed that gas production rate is associated with fast microbial growth, microorganisms interactions and feed digestion at hind gut. There were substrate differences by different diets and those authors suggest that caecum content composition affected gas production. The smallest lag phase was observed with diet composed by tifton-85 hay, alfalfa hay and concentrate, 7.7 hours, suggesting adequate nutrients to ruminal microorganisms and larger degradation rate, 0.0194. Diet composed by tifton-85 hay and concentrate presented largest initial and final gas volume, 0.87 mL/100mg DM and 13.68 mL/100 mg DM, respectively, but lag phase has been delayed, 19.4 hours and substrate degradation rate was the smallest, 0.102.

Conclusions

The colon digesta of horses fed diet composed by Tifton-85 hay presented smaller cumulative gas production perhaps due smaller dry matter content of colon and low substrate was available to colon bacteria fermentation. The largest gas production observed in horses fed Tifton-85 hay, alfalfa hay and concentrate.

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