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RUMEN MICROBIAL GROWTH ESTIMATION USING IN VITRO RADIOPHOSPHOROUS INCORPORATION TECHNIQUE

Ives Cláudio da Silva Bueno, Mariana de Carvalho Machado, Sergio Lucio Salomon Cabral Filho, Sarita Priscila Gobbo, Dorinha Miriam Silber Schmidt Vitti and Adibe Luiz Abdalla

Centro de Energia Nuclear na Agricultura – CENA/USP
Av. Centenário, 303 – C.P. 96
13400-970 – São Dimas, Piracicaba, SP, Brazil

ABSTRACT

Rumen microorganisms are able to transform low biological value nitrogen of feedstuff into high quality protein. To determine how much microbial protein that process forms, radiomarkers can be used. Radiophosphorous has been used to mark microbial protein, as element P is present in all rumen microorganisms (as phospholipids) and the P:N ratio of rumen biomass is quite constant. The aim of this work was to estimate microbial synthesis from feedstuff commonly used in ruminant nutrition in Brazil. Tested feeds were fresh alfalfa, raw sugarcane bagasse, rice hulls, rice meal, soybean meal, wheat meal, Tifton hay, leucaena, dehydrated citrus pulp, wet brewers' grains and cottonseed meal. ^{32}P-labelled phosphate solution was used as marker for microbial protein. Results showed the diversity of feeds by distinct quantities of nitrogen incorporated into microbial mass. Low nutrient availability feeds (sugarcane bagasse and rice hulls) promoted the lowest values of incorporated nitrogen. Nitrogen incorporation showed positive relationship ($r=0.56$; $P=0.06$) with the rate of degradation and negative relationship ($r=-0.59$; $P<0.05$) with fiber content of feeds. The results highlight that easier fermentable feeds (higher rates of degradation) and/or with lower fiber contents promote a more efficient microbial growth and better performance for the host animal.

Keywords: microbial synthesis, ruminant, animal nutrition, animal science

I. INTRODUCTION

Protein requirements for ruminant production or maintenance are result of microbial protein synthesis from protein degradation in the rumen, recycling endogen nitrogen (saliva), rumen undegraded protein and endogen protein [1].

Ruminants have microorganisms that are able to use low biological value nitrogen of feedstuff and to change it into high quality protein. The process is known as rumen microbial synthesis. Better quality feed promotes more efficient microbial growth. Thus, to evaluate nutritive value of ruminant feeds, the microbial synthesis ability is a good parameter. To determine how much microbial protein that process synthesized, radiomarkers can be used. Radiophosphorous (^{32}P) has been used as mark microbial protein marker, as P is present in all rumen microorganisms (as phospholipids) and its relationship with nitrogen in rumen biomass is quite constant [2]. Then, if one of them can be measured, the other one could be predict.

The aim of this work was to estimate microbial synthesis from feedstuff commonly used in ruminant nutrition in Brazil.

II. MATERIAL AND METHODS

Feeds. Eleven feeds commonly used in ruminant nutrition were tested: fresh alfalfa (*Medicago sativa*), raw sugarcane bagasse, rice hulls, rice meal, soybean meal, wheat meal, Tifton (*Cynodon sp.*) hay, leucaena (*Leucaena leucocephala*), dehydrated citrus pulp, wet brewers' grains and cottonseed meal.
All feeds were collected in triplicates, at different places (n=33). They were dried at 60ºC, grounded (1 and 2mm) and stored in plastic flasks. Chemical composition of each one was done to characterize feeds.

Animals and Rumen Liquor. As donors for rumen liquor at least three of a group of six Santa Ines sheep were used. They were kept in a known diet to keep ideal conditions for rumen environment. All animals had rumen cannulas. The same animals were used for in situ degradability assay.

Before collection of rumen liquor, animals were kept in fastening to minimize the contribution of remains of diet on microbial growth. The rumen liquor was collected from each animal and mixed. The collection was done using a probe adapted to a syringe, immediately before handling for inoculation. The rumen liquor was filtered through 2 layers of gauze and kept at 39ºC until inoculation.

Microbial Synthesis Technique. The estimative of microbial growth was obtained from the in vitro radiophosphorous incorporation modified technique [2, 3 and 4]. Each substrate (1g) was placed in plastic tubes and 4ml of buffered solution (3g of sodium bicarbonate and 25g of glucose per liter) and 16ml of filtered rumen liquor were added. As marker, 25µl of ^{32}P-labelled phosphate solution were used, corresponding to 3700Bq (0.1µCi) per tube. Three tubes were prepared for each feed and for a blank (no substrate). One of the tubes had its microbial activity stopped by addition of 1ml of sulphuric acid (18N). That was for measuring of sample and rumen liquor natural activity. All tubes were incubated at 39ºC for 8h, under CO_2.

After that period, incubations was stopped in the two remaining tubes.

The material was centrifuged at ~40000×*g*. The supernatant was separated and 1ml was used for ^{32}P detection in a liquid scintillator (Packard mod. 1600 TR). The remaining supernatant was used to determine total phosphorous content [5]. The pellet was washed four times with saline solution and centrifuged at each washing. The final pellet was diluted with water, dried at 105ºC for 24h, ashed for 4h and digested with hot sulphuric acid for 1:30h. ^{32}P activity of the pellet was determined.

Calculations. To estimate the microbial synthesis from ^{32}P incorporation, the following calculations are used. Extra-cell ^{32}P specific activity (SA_e) is obtained as:

$$SA_e = \frac{A_e}{P_e} \quad \text{.....(1)}$$

where, A_e is the extra-cell (supernatant) ^{32}P activity and P_e is the total extra-cell phosphorous content.

From the Eq. (1), incorporated phosphorous (P_i) can be determined as following:

$$P_i = \frac{A_i}{SA_e} \quad \text{.....(2)}$$

where, A_i is the intra-cell (pellet) ^{32}P activity.

The N:P ratio in the microbial mass is quite constant and it is assumed as 8.37±0.75 [2], then, the total microbial nitrogen (*MN*) syntesized during the process is predicted as:

$$MN = 8.37 \times P_i \quad \text{.....(3)}$$

In Situ Rumen Degradability Assay. This technique was carried out according to Ørskov and McDonald [6]. Approximately 3g of substrate (dried and grounded to 2mm) were weighted in nylon bags (35µm porosity) and incubated in the rumen of two animals for 3, 8, 16, 24, 48, 72 and 96h. Washing loss was also measured, according to McDonald [7]. Results were fitted by simple exponential model:

$$p = a + b \times \left(1 - e^{-c \times t}\right) \quad \text{.....(4)}$$

where, p is the rumen degradability at time t; a and b are mathematical parameters; c is the rate of feed disappearance; and $(a+b)$ represents the potential degradability.

Washing loss, or immediately soluble fraction, is represented by A. The insoluble fermentable fraction (B) can be represented by:

$$B = (a + b) - A \quad \text{.....(5)}$$

Statistical Analysis. Variables were analyzed in a completely randomized design. Observations were characterized by means and standard deviations and/or standard errors and compared between different feeds by Tukey test. Relationships were tested and compared by Pearson correlation coefficient.

III. RESULTS AND DISCUSSION

The chemical composition of tested feeds is presented on Table 1. Results show that feeds had distinct composition and this could be usd to validate the technique using feeds with different microbial growth availabilities. All groups of feeds [8] are presented: fibrous feeds as fresh alfalfa, raw sugarcane bagasse, rice hulls and Tifton hay, energetic feeds as rice meal, wheat meal and dehydrated citrus pulp, and proteic feeds as soybean meal, wet brewers' grains and cottonseed meal.

In situ assay also demonstrated that feeds had distinct characteristics of fermentability. Fig 1 illustrates the rate of disappearance of each substrate (c).

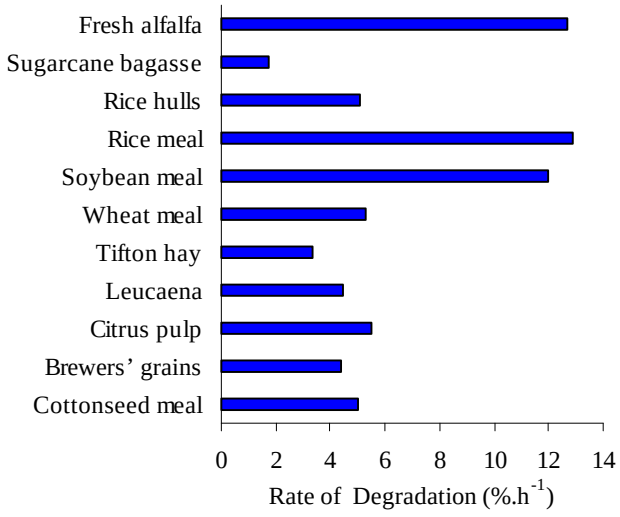

Figure 1. Rate of Degradation of Tested Feeds.

TABLE 1. Chemical Composition of Tested Feeds*

Feeds	DM	OM	CP	NDF	ADF	ADL	TDN
Fresh alfalfa	43.3±0.8	92.0±0.5	21.6±1.0	34.8±2.1	27.7±1.7	5.7±0.1	70.6±9.4
Sugarcane bagasse	60.4±7,2	93.7±3.1	1.9±0.2	87.2±6.1	67.5±6.0	13.9±3.7	47.1±18.7
Rice hulls	90.1±0.1	84.8±5.7	2.4±0.3	76.1±6.0	68.4±2.6	17.1±1.5	39.2±35.1
Rice meal	90.0±2.2	90.1±1.7	13.3±1.1	21.3±4.2	14.7±4.4	4.8±1.1	83.5±19.9
Soybean meal	88.2±0.3	92.9±0.3	43.4±2.0	13.2±3.6	11.6±1.0	0.8±0.1	82.1±17.3
Wheat meal	88.9±1.9	94.8±0.5	15.0±0.3	38.4±4.5	12.6±1.7	3.7±0.5	75.7±7.4
Tifton hay	88.9±0.8	94.4±1.9	9.1±4.0	71.7±1.8	39.6±5.3	5.2±1.8	57.1±28.1
Leucaena	36.8±9.2	95.5±0.1	14.0±0.8	61.6±9.3	48.0±9.9	2.8±1.2	64.5±25.1
Citrus pulp	90.4±2.1	94.7±0.9	6.1±0.3	23.6±3.7	24.8±1.5	2.4±0.6	74.4±8.2
Brewers' grains	21.6±4.4	95.4±0.4	23.8±4.0	58.3±2.6	25.2±1.0	5.7±0.9	77.9±21.8
Cottonseed meal	89.2±0.4	94.3±0.8	34.3±4.7	38.6±9.9	28.4±6.8	12.6±2.2	66.7±82.7

* results are expressed in % DM; DM = dry mater; OM = organic mater; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ADL = acid detergent lignin; and TDN = total digestible nutrients.

The degradability parameters of tested feeds were similar to those found in literature [9, 10 and 11]

The ^{32}P incorporation technique results showed lower values for incorporated nitrogen (Table 2) for low easily fermentable nutrient content feeds (sugarcane bagasse and rice hulls).

TABLE 2. Microbial Nitrogen Synthesis (in mg MN/g DM) Estimated by In Vitro ^{32}P Incorporation Technique

Feed	Microbial Nitrogen
Fresh alfalfa	5.6 ^{a}
Sugarcane bagasse	1.1 ^{c}
Rice hulls	2.5 ^{bc}
Rice meal	3.6 ^{abc}
Soybean meal	4.2 ^{ab}
Wheat meal	3.2 ^{abc}
Tifton hay	3.2 ^{abc}
Leucaena	4.8 ^{ab}
Citrus pulp	5.0 ^{ab}
Brewers' grains	3.3 ^{abc}
Cottonseed meal	4.4 ^{ab}

$^{a, b, c}$ means with different superscripts are significantly different (Tukey test, P<0.05)

The relationships between N incorporation and rate of degradation (*c*) from the Eq. (4) (Fig. 2a), show that there is a trend (r=0.56; P=0.06) to increase the N incorporation with higher rates of degradation, i.e., feeds that promotes easy fermentation (degradation) also promotes higher incorporation of nitrogen into microbial cells.

On the other hand, when microbial growth is compared to fiber contents (Fig. 2b), there is a negative relationship (r=-0.59; P<0.05). Those results showed that fibrous feeds are more difficult to be fermented and then they promote a lower microbial N incorporation.

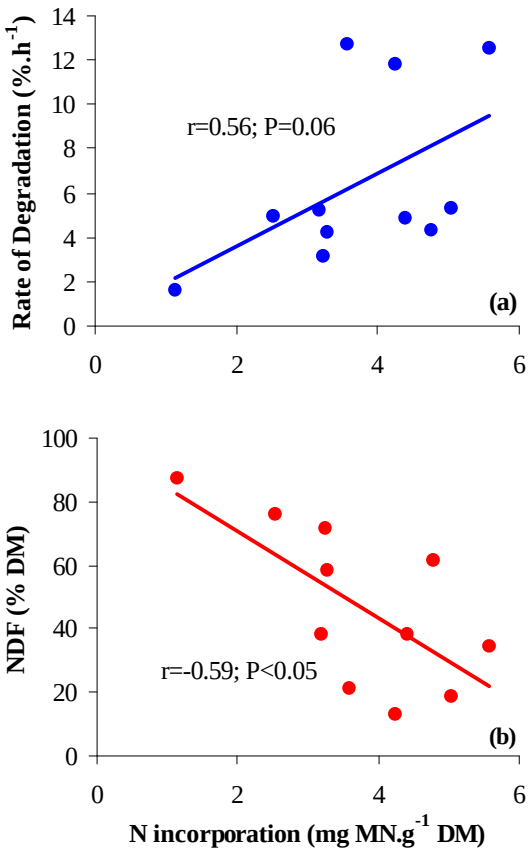

Figure 2. Relationships Between N Incorporation and either a. Rate of Degradation (*c*) or b. Neutral Detergent Fiber (*NDF*)

Those facts highlight that easier fermentable feeds (higher rates of degradation) and/or lower fiber content feeds allow a more efficient microbial growth, promoting a better performance for the host animal.

IV. CONCLUSION

Microbial synthesis estimated from ^{32}P incorporation can be used as a parameter to compare different feeds for ruminants.

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