Comparison of Indirect and Direct Determination of Microbial Growth in The Rumen Simulation Technique (RUSITEC)

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ABSTRACT

A study to compare the indirect and direct measurements of microbial protein synthesis in Rusitec using stable isotope nitrogen (^{15}N-urea with 95% enrichment) as a tracer have been conducted. Six types of ration containing 16% CP, 24 – 36% CF, 49 – 62% NDF and 27 – 41% ADF with gradual content of non protein nitrogen (NPN) have been put into nylon bag and fed daily to Rusitec fermentor for 15 days observation. Indirect microbial protein synthesis (MPS) estimated MPS from N-turnover of the ammonia pool, while direct method estimated MPS from microbial isolated. The direct measurement of microbial-N in bacterial isolates resulted in higher microbial protein synthesis compared to the indirect method based. The results obtained are compared and discussed in relation to theoretical stoichiometric data of rumen fermentation.

Key words: direct measurement, indirect measurement, microbial protein synthesis, Rusitec

INTRODUCTION

Low protein content of tropical grasses (8 – 12%) forces the farmer to use high protein concentrate feed. This condition limits ruminant production in the tropical area. To avoid utilisation of expensive high protein feed, sometimes, non protein nitrogen (NPN) is added into the ruminant ration. In this condition, microbial protein (MP) has become the major crude protein contributor and provides high quality protein for the ruminants. To have a figure of protein supply for ruminant, it is therefore important to quantify microbial protein synthesis in the rumen.

Several methods have been used to assess microbial protein synthesis. For instance, (1) measurement of microbial protein on protein free diets and assuming that all protein flowing to the duodenum is of microbial origin, (2) distinguishing feed and microbial protein on the basis of amino acid profiles, (3) use of endogenous or exogenous markers such as ^{35}S, ^{15}N, ^{3}H-Leucine, ^{32}P, diaminopimelic acid (DAPA), RNA, and purine and pyrimidine bases to label microbial protein material, and (4) near infrared reflectance spectroscopy methods (Obispo and Dehority, 1999; Dewhurst *et al.*, 2000).

So far there is no sophisticated method available. Dewhurst *et al.* (2000) reviewed that each of the methods has its own problems including safety (radioisotopes), cost (^{15}N and amino acid profiles), difficulties in analysis (RNA and DNA) and contamination of feeds, thereby lacking specificity to the microbial fraction (DAPA, nucleic acid and their bases).

To overcome the cost problem in using ^{15}N exogenous marker, Abel *et al.* (1990) tried to measure microbial protein synthesis in rumen simulation fermentor (Rusitec) by using small quantities of ^{15}N and applying the continuous tracer infusion method. The method is based on the rate of incorporation of nitrogen into microbes through ammonia pools (indirect method). It is assumed that ammonia was the only nitrogen source for microbial protein synthesis.

Van Nevel *et al.* (1975) had expressed their objection to the ^{15}N based methods which do not take into consideration the direct incorporation of amino acid or peptide nitrogen into microbial cells instead of ammonia. The direct incorporation of amino acid or peptide nitrogen could amount to 20% of the total nitrogen incorporated. With the indirect ^{15}N-based methods the microbial synthesis could, therefore, be under-estimated.

The objective of this study is to compare the indirect (ammonia pool) and direct (microbial isolate) measurements of microbial protein synthesis using tracer stable isotope nitrogen ^{15}N in rumen simulation fermentor (Rusitec).