Livestock Science 167 (2014) 144–153

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Comparison of purine bases and ^{15}N for quantifying microbial nitrogen yield using three marker systems and different sampling sites in zebu cross breed bulls

P.P. Rotta [a,b,*], S.C. Valadares Filho [a], L.F. Costa e Silva [a,b], E. Detmann [a], F.A.C. Villadiego [c], T.E. Engle [b], R.F.D. Valadares [c], B.C. Silva [a], M.V. C. Pacheco [a], D. Zanetti [a]

[a] Department of Animal Sciences, Federal University of Viçosa, Viçosa 36571-000, Brazil
[b] Department of Animal Sciences, Colorado State University, Fort Collins, CO 80523, USA
[c] Department of Veterinary, Federal University of Viçosa, Viçosa 36571-000, Brazil

ARTICLE INFO

Article history:
Received 17 February 2014
Received in revised form
8 June 2014
Accepted 10 June 2014

Keywords:
Abomasum
Omasum
Purine bases
Reticulum
Zebu
^{15}N

ABSTRACT

The present experiment was designed to evaluate the use of microbial markers (MM), sampling sites (SS), and marker systems (MS) to estimate microbial nitrogen (MN) synthesis in bulls and to develop equations to correct MN estimates when only one of the aforementioned techniques was utilized. The MM systems evaluated were (1) purine bases (PB) and (2) ^{15}N labeling. The SS evaluated were: (1) reticulum, (2) omasum, and (3) abomasum, and the single, double and triple MS were evaluated. Eight crossbred (Holstein × Zebu) bulls (353 ± 36.9 kg of BW; 24 ± 1 mol) with ruminal and abomasal cannulas were utilized in this experiment. The following experimental diets were used: (1) 60% corn silage + 40% concentrate, (2) 40% corn silage + 60% concentrate, (3) 60% fresh sugarcane + 40% concentrate, and (4) 40% fresh sugarcane + 60% concentrate. Four experimental periods lasting 16 d each were completed with 10 d for adaptation to the experimental diet and 6 d for sampling. Bulls were randomly distributed into two 4 × 4 Latin squares balanced for residual effects. Data were analyzed in a Latin square design using PROC MIXED. Interactions were observed ($P < 0.05$) in MN, microbial crude protein/total digestible nutrients (MCP/TDN), microbial nitrogen/rumen fermented organic matter (MN/RFOM), microbial nitrogen/rumen truly fermented organic matter (MN/RTFOM), and microbial dry matter/rumen fermented total carbohydrates (MDM/RFTCHO) between SS and MM. For PB, the greatest ($P < 0.01$) values of MN were observed for the digesta sampled in the reticulum and abomasum. In contrast, for ^{15}N, the greatest ($P < 0.01$) values were observed for digesta sampled in the omasum and abomasum. Microbial nitrogen yield was only different ($P < 0.05$) when using reticulum and ^{15}N from those estimated using abomasum and ^{15}N. Thus, the equation developed to correct MN value was: MN (g/d) = 27.93 ± 2.46 + 0.99 ± 0.09 × reticulum ^{15}N. The triple MS exhibited the greatest ($P < 0.01$) value of MN compared to the single and double MS. No interactions ($P > 0.05$) were observed between MS and MM or SS; thus, the equation established to correct MN value used only the MS. In conclusion, we have demonstrated that there is no difference using ^{15}N to estimate MN yield if omasum or abomasum are used. Therefore, the omasum can be used as an accurate SS to predict MN. The triple MS presented higher

* Corresponding author. Tel.: +1 970 818 6713.
E-mail address: polyana.rotta@ufv.br (P.P. Rotta).

http://dx.doi.org/10.1016/j.livsci.2014.06.010

values than the single and double MS. Thus, if single or double MS is used the value must be corrected by the equation obtained using the triple MS.

1. Introduction

Microbial nitrogen yield (MN) is of great importance to protein metabolism in ruminants (Bach et al., 2004; Broderick et al., 2010). The quantification of its flow to the small intestine is therefore important in calculating the amount of digestible MN available to the animal. Various microbial markers (MM) can be used to estimate MN flow (Carro and Miller, 2002; Ipharraguerre et al., 2007). Among the techniques used, ^{15}N, purine bases (PB), and urinary excretion of purine derivatives are the most common (Blummel and Lebzien, 2001; Belenguer et al., 2002; Ma et al., 2014).

However, according to Reynal et al. (2005) and Ipharraguerre et al. (2007), when omasal sampling of digesta and bacteria are performed to calculate MN flow, the use of ^{15}N is recommended. In contrast, these authors did not observe differences between ^{15}N and PB when the samples were taken from the duodenum. Using ^{15}N, Krizsan et al. (2010) suggested that digesta sampled from the reticulum could be used, as opposed to digesta sampled from the omasum. However, there is no study with other MM using the digesta sampled in the reticulum. In addition, studies whose objective was to evaluate the flow of MN used the triple marker system (MS) (Reynal et al., 2005; Krizsan et al., 2010) without reference to single and double MS.

Most of the studies that evaluate MN yield have used digesta sampled in the abomasum or duodenum (Gonzalez-Ronquillo et al., 2004; Ipharraguerre et al., 2007). However, fitting animals with abomasum and/or duodenal cannulas is costly, and the cannulas can be difficult to maintain (Harmon and Richards, 1997). If the digesta sampling could be performed in the omasum or reticulum through a ruminal cannula, it would be easier to obtain the samples and manage the animals. Additionally, due to the high cost and labor-intensive nature of performing triple MS experiments, it would be beneficial to be able to use a single or double MS and make adjustments with equations derived from triple markers.

Our hypothesis is that reticulum and omasum could be used as SS to estimate MN yield and that the double MS could be used instead of the triple MS. We also believe that different diets could influence the MN yield depending on the SS.

The objectives of this study were to estimate MN yield and its efficiency calculated using PB and ^{15}N, with data obtained from three digesta SS (reticulum, omasum, and abomasum) using three MS (single, double, and triple) in beef cattle fed diets characteristic of a tropical climate. The objectives were also to develop an adjustment for using only the abomasum as the SS, ^{15}N as the MM, and the triple MS.

2. Materials and methods

2.1. Animals, experimental design, and diets

This study was approved by the Institutional Animal Care and Use Committee at the Federal University of Viçosa.

The experiment was conducted at the Experimental Feedlot of Animal Science Department in Viçosa, Brazil. Laboratory analyses were conducted at the Ruminant Nutrition Laboratory at Animal Science Department at Federal University of Viçosa, Brazil.

Eight crossbred (Holstein × Zebu) bulls (353 ± 36.9 kg of BW; 24 ± 1 mo) with ruminal and abomasal cannulas were randomly distributed into two 4 × 4 Latin squares balanced for residual effects. The bulls were offered feed as TMR twice daily at 7:00 and at 15:00 h, in amounts that allowed ad libitum access to feed throughout the day. Bulls were housed in tie stalls with free access to water throughout the experiment. Four experimental diets, three digesta SS, three MS and two MM were assessed for the estimation of ruminal outflow of MN and efficiency calculated as: microbial crude protein (CP)/total digestible nutrients (MCP/TDN), microbial nitrogen/rumen fermented organic matter (MN/RFOM), microbial nitrogen/rumen truly fermented organic matter (MN/RTFOM), and microbial dry matter/rumen fermented total carbohydrates (MDM/RFTCHO). The following experimental diets were used: (dry matter (DM) basis): (1) 60% corn silage (CS)+40% concentrate (CO), (2) 40% CS+60% CO, (3) 60% fresh sugar cane (SC)+40% CO, and (4) 40% SC+60% CO.

The DM of the CS and SC diets were determined daily to adjust the amount of urea (U) and ammonium sulfate [(AS); 9:1, U:AS] supplied to the bulls, and the U:AS mixture was used to adjust the CP content of the diets to 120 g/kg DM (19.2 g of nitrogen (N)/kg DM). The DM content was analyzed daily in duplicate, using a conventional microwave oven, according to recommendations from the National Forage Testing Association (1993). Feeds and orts were weighed daily, sampled, and frozen for later analysis.

The chemical compositions of the feeds used in the experimental diets are shown in Table 1. The concentrate used in all diets consisted of 90.4% ground corn, 7.90% soybean meal, 0.85% mineral mixture, and 0.85% NaCl (on

Table 1
Ingredients and chemical composition of feed used in experimental diets.

Feed	DM	OM	CP	EE	NDF	NFC	iNDF
	g/kg	DM					
Corn silage	301	947	66	32	516	333	140
Sugar cane	282	974	28	16	459	471	222
Corn	902	989	95	36	144	715	1.7
Soybean meal	875	952	528	15	124	288	0.6
Mineral mix[a]	975	160	–	–	–	–	–
Urea+ammonium sulfate	951	985	2,63	–	–	–	–

DM=dry matter, OM=organic matter, CP=crude protein, EE=ether extract, NDF=neutral detergent fiber, NFC=non fiber carbohydrates, iNDF=indigestible neutral detergent fiber.

[a] 266 g/kg calcium; 147 g/kg phosphorus; 7 g/kg magnesium; 3 g/kg potassium; 7 g/kg sulfur; 2 g/kg sodium; 118 mg/kg chromium; 1191 mg/kg copper; 5070 mg/kg iron; 1728 mg/kg manganese; 4198 mg/kg zinc; and 136 mg/kg cobalt.