A Rapid Technique for Determination of Total Disappearance of Dietary Nitrogen in the Digestive Tract Using Washed Fecal Sample after Freezing and Thawing

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ABSTRACT : Three Holstein steers, fitted with ruminal and duodenal cannulas, were used in a replicated 3×3 Latin square design to determine the digestibility of dietary nitrogen in total digestive tract by three methods, 1) mobile nylon bag (MNB); 2) total fecal collection (TFC); and 3) washed fecal sample after freezing and thawing through a sieve with a pore size of 45 μm (WFS). A basal diet of oaten hay-barley was supplemented with one of the following protein sources; soybean meal, fish meal or blood meal. Steers were fed at a level of 2% of body weight. The experimental diets were contained approximately 1.85% nitrogen. There were no differences ($p>0.05$) among the diets on DM, NDF and nitrogen disappearances, and the diet results were pooled to assess the methods. Total tract disappearances of dry matter and neutral detergent fiber were 61.6, 71.1 and 78.9 and 25.3, 63.2 and 64.6 for MNB, TFC and WFS methods, respectively. The lower digestibility of DM and NDF in the MNB method could be a result of low ruminal incubation time. The TFC method had the lower ($p<0.05$) determination of nitrogen disappearance in the total digestive tract than the MNB and WFS methods. On the other hand, nitrogen disappearance in the total digestive tract determined by the WFS technique was comparable to that in MNB technique, as there was no significant difference ($p>0.05$) between the methods. It is shown that the disappearance of dietary nitrogen in the total digestive tract could be estimated in the intact animals by using washed fecal sample prior to freezing and thawing. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 3 : 313-316*)

Key Words : Nitrogen Disappearance, Total Digestive Tract, Frozen Fecal Sample

INTRODUCTION

Dietary proteins not digested in the rumen or in the small intestine, will pass through the large intestine with only minor changes and be recovered in feces. Conventional *in vivo* digestion coefficients are quite reliable for plant cell-wall compounds because microbial or endogenous substances within the digestive tract make practically no contribution to feces. For nitrogen, these coefficients are likely to be rough underestimates of true digestion in the total alimentary tract because of a large proportion of non-feed nitrogen in the feces. Fecal nitrogen of microbial origin was found to be a large portion of fecal nitrogen excretion (Mason, 1969; Kamel et al., 1996). Therefore, detaching microbial nitrogen associated with fecal excretion is useful to determine the true digestion of dietary nitrogen in the total digestive tract. Kamel et al. (1995b) found that freezing and thawing technique could eliminate the attached microorganisms from ruminally incubated hay and digesta samples due to bursting of the microbial membrane as a result of ice crystals generate in the cell during storage at -30℃. Thereafter, the bacterial cell contents easily wash-out in the running water. Theoretically, the same procedure could be used to dislodge microbial nitrogen contributing to total fecal nitrogen.

Mobile nylon bag (MNB) technique was developed for the determination of protein disappearance in the rumen and total digestive tract (Kirkpatrick and Kennelly, 1984), and it has been used successfully by several researchers (Boer et al., 1987; Erasmus et al., 1990) for estimation of ruminal and intestinal availability of rumen undegraded protein.

Therefore, the current study aimed to determine the nitrogen disappearance in total digestive tract for diets supplemented with different protein sources using fecal samples after freezing and thawing, and also to compare these results with those obtained from MNB technique.

MATERIALS AND METHODS

Animals and diets

Three Holstein steers (mean BW±SD=466±15.3 kg) were fitted with ruminal and duodenal cannulae. Surgery and animal care followed the guidelines set out by the Animal Production Care and Use Committee as approved by the Japanese Prime Minister's Cabinet Secretary.

Each animal was fed one of three different diets over three different periods. Diets and periods were randomly assigned for each animal in a 3×3 Latin square design. A basal diet of oaten hay-barley was

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supplemented with one of the following protein supplements; soybean meal (D-SBM), fish meal (D-FM) or blood meal (D-BM). The experimental diets and their chemical composition are shown in table 1. Steers were offered diets at the level of 2% of initial BW on an air dry basis in two equal portions at 8:00 and 16:00 h, and had free access to fresh water and trace mineralized salt licks.

Table 1. Ingredient and chemical composition of the experimental diets

	D-SBM	D-FM	D-BM
Ingredient (kg/d of DM)			
Oats hay	6.48	6.81	6.79
Barley grain	0.98	0.98	0.98
Soybean meal	0.74	-	-
Fish meal	-	0.41	-
Blood meal	-	-	0.34
Chemical composition (% of DM)			
Nitrogen	1.89	1.82	1.84
NDF	46.6	48.7	48.9
ADF	24.6	24.9	25.2

Samples collection and analyses

Each experimental period was 21 d, with a 16 d for conditioning period and 5 d for carrying out the mobile nylon bag (MNB) technique and fecal collection.

Bags were made from polyester cloth (Swiss Nylon Monofilament, Switzerland) with a defined pore size of 45 μm. The bag size was 3.5×5 cm (De Boer et al., 1987). Each bag contained approximately 1 g (ground to 2 mm) of an air-dried sample similar in composition to that offered to the animals. Sixteen bags were held in a large net with a pore size of 2× 2 mm, connected by a nylon cord to the cap of the rumen cannulas. On day 17 for each treatment, the large bags were incubated in the rumen for 16 h. After withdrawal from the rumen, the bags were incubated in an acid-pepsin solution (1 g of pepsin/L of 0.01 HCl) at 39℃ for 3 h, in order to simulate abomasal digestion (Kirkpatrick and Kennelly, 1984). After digestion in the pepsin-HCl solution, the bags were kept on ice at 4℃ to stop the pepsin activity. Bags were then randomly inserted into the small intestine through the duodenal cannula at a rate of one bag per 45 min. Upon recovery from the feces the mobile bags were washed and stored frozen at -30℃. The bags were washed in washing machine for 15 min. followed by 5 min. in running water, then they were dried at 60℃. The bags were pooled to give one value per animal.

From d 17 to the end of each period daily fecal output was collected, weighed and recorded. Sub-samples approximately 10% of fecal excreted were grabbed every 4-h interval, composted by treatment and stored frozen at -30℃. Later, fecal samples were thawed and thoroughly mixed, then two portions (each approximately 150 g) were used as follow: 1) dried in a forced oven at 60℃ for 48 h and stored for further analyses as a total fecal collection (TFC) sample, and 2) transferred to a sieve with a pore size of 45 μm where it was washed in running water with a discharge rate of 20 L/min for 15 min., to represent washed fecal sample after freezing and thawing (WFS). Samples of TFC and WFS were ground through 2 mm screen prior to determination of DM, nitrogen and NDF.

Dry-matter and Kjeldahl-N were determined according to AOAC 1984. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by using the method of Goering and Van Soest (1970).

Dry matter, nitrogen and NDF disappearances in the total digestive tract were calculated as follows:

1) in the MNB method, [(initial g of DM, nitrogen or NDF-residual g of DM nitrogen or NDF after ruminal and intestinal incubation)/initial g of DM, nitrogen or NDF]×100;
2) in the TFC method, 100-{[(DM, nitrogen or NDF g/g fecal DM daily fecal output g DM)/ daily intake of DM, nitrogen or NDF g]×100};
3) in the WFS method, 100-{[(DM, nitrogen or NDF g/g fecal DM after freezing and washing ×daily fecal output DM g after freezing and washing)/daily intake of DM, nitrogen or NDF g]×100}.

Statistical analysis

Results were analyzed by ANOVA for a 3×3 Latin square design using the JMP procedure of SAS Institute, Inc. (1994).

RESULTS

Disappearances of DM, N, and NDF in the total digestive tract were not different among the diets when either the TFC, WFS or MNB method was used. Therefore, the mean values for the three diets were pooled to compare these methods for the determination of DM, nitrogen and NDF disappearance (table 2).

Disappearance of dry matter in the total digestive tract (DMDT) varied significantly ($p<0.05$) among the methods. The WFS method indicated a higher disappearance than did the TFC and MNB methods. Also, DMDT determined by MNB method was lower ($p<0.05$) than in the TFC method.

Disappearance of NDF in the total digestive tract was lower ($p<0.05$) when it was determined by the

Table 2. Daily intake and total tract disappearance percentage of dry matter, neutral detergent fiber and nitrogen determined by mobile nylon bag (MNB), total fecal collection (TFC) and washed fecal sample after freezing and thawing (WFS)

	D-SBM ± SE	D-FM ± SE	D-BM ± SE	Mean[1] ± SE
Dry matter				
Daily intake kg/d	8.2	8.2	8.1	8.16 ± 0.05
Disappearance, %				
MNB	62.0 ± 0.98[c2]	61.2 ± 1.56[c]	61.4 ± 1.43[c]	61.5 ± 0.68[c]
TFC	72.3 ± 1.18[b]	71.1 ± 1.56[b]	71.8 ± 0.14[b]	71.8 ± 0.51[b]
WFS	80.3 ± 1.41[a]	80.1 ± 1.13[a]	81.2 ± 1.02[a]	80.5 ± 0.63[a]
Neutral detergent fiber				
Daily intake g/d	3821.2	3993.4	3965.8	3926.8 ± 26.9
Disappearance, %				
MNB	24.0 ± 1.34[b]	24.8 ± 3.12[b]	27.0 ± 2.05[c]	25.3 ± 1.24[b]
TFC	64.2 ± 0.98[a]	61.1 ± 1.91[a]	64.4 ± 2.14[a]	63.2 ± 1.36[a]
WFS	66.1 ± 3.32[a]	63.5 ± 2.72[a]	64.3 ± 0.28[a]	64.6 ± 0.93[a]
Nitrogen				
Daily intake g d-1	156.0	152.3	151.2	153.1 ± 2.13
Disappearance, %				
MNB	94.4 ± 0.61[a]	93.5 ± 0.50[a]	92.0 ± 0.73[a]	93.3 ± 0.61[a]
TFC	67.0 ± 3.49[b]	67.3 ± 1.66[b]	68.2 ± 1.16[b]	67.5 ± 1.18[b]
WFS	95.5 ± 1.34[a]	94.4 ± 0.34[a]	94.6 ± 1.36[a]	94.8 ± 1.03[a]

[1] n=9 for 3 diets.
[2] Means within the same column lacking a common superscript differ ($p<0.05$).

MNB method than with the other two methods, however, no significant difference was found in NDF disappearance determined by the TFC and WFS methods (table 2).

Disappearance of dietary nitrogen in the total digestive tract (NDT) determined by TFC method was lower ($p<0.05$) than with the MNB and WFS methods, whereas no significant difference was observed between the MNB and WFS methods. The NDT for MNB, TFC and WFS were 93.3, 67.5 and 94.8%, respectively (table 2).

DISCUSSION

Mobile nylon bags and the sieve which was used in WFS method were made from the same material with a pore size of 45 μm. However, the DMDT in the MNB method was 19.0% lower than that determined by WFS method. This difference between DMDT in MNB and WFS is a result of low disappearance of NDF in the total digestive tract in the MNB method. This difference represents 18.9% of DM, [i.e., [(NDF g intake × difference between NDF disappearance percentage in MNB and WFS)/ DM intake g] × 100]. The lower disappearance of NDF in MNB than TFC and WFS is most likely a function of the ruminal incubation time, which was 16 h for the mobile bags. The mean retention time in the rumen for oaten hay (82% of the whole diets) found to be 33 h (Sekine et al., 1995).

Disappearance of NDF in the total digestive tract was not different between the WFS and TFC methods, however, the DMDT was higher with the WFS by 8.7 % than with the TFC method (table 2). These results might be due to washed-out of the soluble fraction through the sieve in WFS method. Eliminating the soluble fraction, by washing the fecal sample after freezing and thawing, increased the NDT from 67.5% in TFC to 94.8% in the WFS, this shows that the soluble fraction (less than 45 μm) contains a large amount of nitrogen. In the present study washed-out fraction contained about 6.0% nitrogen. Van Soest (1982) stated that the undigested dietary fraction in the feces is largely plant cell wall and the metabolic fraction makes up 10% of the fecal DM excreted by cows. The nitrogen content of the organic metabolic matter of ruminant feces averages about 7%. Nitrogen content of the washed-out fraction found in the present study is in agreement with is earlier report, inferring that the washed-out fraction consisted mainly of metabolic fecal nitrogen (i.e., abraded epithelial cell, unabsorbed enzymes, mucus, microbial cell wall and intact bacteria). The microbial nitrogen synthesized in the large intestine cannot serve as a source of protein for the ruminant and this protein is not digested and absorbed (Ørskov, 1992). Therefore, the washed-out fraction may have contained ruminal bacteria attached to feed particles; which escaped intestinal digestion; and bacteria colonized on the feed particles during retention in the caecum and large intestine.

Nitrogen content of oaten hay-NDF has been determined to be 0.58% (Kamel et al., 1995a). The true digestibility of cell wall nitrogen found to be 86% (Van Soest, 1982) and 79% (Masom, 1969) with an estimated average of 82%. The low content of nitrogen in oaten hay-NDF with its high digestibility could explain the similarity of NDT in both MNB and WFS methods, eventhough the disappearance of NDF in total digestive tract was 39.3% higher in the WFS method than in the MNB method.

Results obtained from the present study indicated that, the disappearance of DM and NDF from the mobile nylon bags was underestimated because of the short incubation time in the rumen. The NDT however, was not affected by the incubation time. Nitrogen disappearance in the total digestive tract determined by the WFS technique was comparable to that of the MNB technique. The WFS method could therefore be used for the rapidly determination of nitrogen disappearance in the total digestive tract for intact animals.

The value of nitrogen in the diets is derived from supplying ruminally degradable nitrogen for the synthesis of microbial protein and providing intestinally available nitrogen for growth or milk production. Cannulated animals are used to separate bacterial and protozoal protein and undegraded dietary protein as well as for measurements of total protein flow (i.e., *in vivo* technique). Although the *in vivo* method remains as a reference method, it is expensive, labor-intensive, time consuming and subject to error with the use of digesta flow rate markers, microbial markers and inherent animal variation (Janicki and Stallings, 1988; Stern et al., 1997). Attempts have been done by many researchers for more simpler and reliable techniques using intact animals (Topps and Elliott, 1965; Fujihara et al., 1987; Chen et al., 1990) to determine urinary purine derivatives in order to estimate microbial nitrogen supply to the small intestine. Also, *in vitro* technique to determine ruminal degradability of dietary nitrogen. Subsequently, fecal method described here could be used with these other methods for rapid evaluation of dietary nitrogen.

REFERENCES

AOAC. 1984. Association of Official Analytical Chemists. Official methods of analysis. Washington, DC.

Chen, X. B., F. D. DeB. Hovell, E. R. Ørskov and D. S. Brown. 1990. Excretion of purine derivatives by ruminants: Effect of exogenous nucleic acid supply on purine derivative excretion by sheep. Br. J. Nutr. 63:131-142.

De Boer, G., J. J. Murphy and J. J. Kennelly. 1987. Mobile nylon bag for intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977-982.

Erasmus, L. J., P. M. Botha, P. Lebzien and H. H. Meissner. 1990. Composition and intestinal digestibility of rumen fermented feeds and duodenal digesta in cows using bag techniques. J. Dairy Sci. 73:3494-3501.

Fujihara, T., E. R. Ørskov, P. J. Reeds and D. J. Kyle. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. J. Agric. Sci. Camb. 109:7-12.

Goering, H. K. and P. J. Van Soest. 1970. Forage fiber analysis (Apparatus, reagents and some applications) Agric. Handbook no. 379. Agric. Res. Serv. USDA, Washington, DC.

Janicki, F. J. and C. C. Stallings. 1988. Degradation of crude protein in forages determined by *in vitro* and *in situ* procedures. J. Dairy Sci. 71:2440-2448.

Kamel, H. E. M., J. Sekine, T. Suga and Z. Morita. 1995a. Degradation of dietary nutrient and purine in the rumen of sheep given oaten hay, timothy and alfalfa hays. Anim. Sci. Technol. (Jpn). 66:927-935.

Kamel, H. E. M., J. Sekine, T. Suga and Z. Morita. 1995b. The effect of frozen-rethawing technique on detaching firmly associated bacteria from in situ hay residues. Can. J. Anim. Sci. 75:481-483.

Kamel, H. E. M. and J. Sekine. 1996. Determination of nitrogen disappearance in the total digestive tract and apparent digestibility of microbial nitrogen in the post-ruminal tract using mobile nylon technique. Proceeding of the 8th AAAP Animal Science Congress. Tokyo, Japan. Vol. 2. 136-137.

Kirkpatrick, B. K. and J. J. Kennelly. 1984. Prediction of digestibility in cattle using a modified nylon bag technique. Can. J. Anim. Sci. 64:1104.

Mason, V. C. 1969. Some observations on the distribution and origin of nitrogen in sheep faeces. J. Agric. Sci., Camb. 73:99-111.

Ørskov, E. R. 1992. Host animal protein requirement and protein utilization. In: Protein Nutrition in Ruminants (2nd Ed.). Academic Press. London. pp. 94-152.

SAS. 1994. SAS User's Guide: Statistics. Version 2 edition. SAS Institute, Inc., Cary, NC, USA.

Sekine, J., M. Shinoda, H. E. M. Kamel and R. Oura. 1995. Effect of kinds of hay on population of rumen anaerobic fungi. Indian Journal of Animal Sciences. 65(12):1352-1355.

Stern, M. D., A. Bach and S. Calsamiglia. 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci. 75:2256-2276.

Topps, J. H. and R. C. Elliott. 1965. Relationship between concentrations of ruminal nucleic acids and excretion of purine derivative by sheep. Nature. London 205:498-499.

Van Soest, P. J. 1982. Fecal composition, mathematics of digestion balances and markers. In: Nutritional ecology of the ruminant. O&B Books, Inc., USA. pp. 39-57.