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Effect of Mobile Bag and Sample Sizes on Intestinal Digestibility of Forage in Sheep

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ABSTRACT : This study aimed to clarify the effect of mobile bag size and ratio of sample size to bag surface area on intestinal digestibility of forage in sheep. Four Suffolk ewes fitted with ruminal and proximal duodenal cannulae were fed second-cut Italian ryegrass (*Lolium multiflorum* Lam.) hay twice daily, and the same forage was used to measure intestinal digestibility. The forage samples were incubated in the rumen for 16 h and then in pepsin-HCl solution for 3 h before intestinal incubation. The incubated forage samples were placed in a nylon mobile bag. The bag sizes used were either 20 mm×20 mm (small bag size; SBS) or 30 mm×30 mm (large bag size; LBS) and the ratio of the sample size to the surface area of the bag was either 5.5 mg/cm^2 (low ratio; LR) or 11.0 mg/cm^2 (high ratio; HR) resulting in four different treatment conditions: SBS-LR, SBS-HR, LBS-LR and LBS-HR. Eight bags per animal were inserted through the duodenal cannulae at 15-min intervals and were subsequently collected from the feces of the animal. The mean intestinal bag transition time did not differ significantly between animals, but ranged from 23.2 to 27.0 h. The intestinal digestibility of dry matter (IDDM) ranged from 0.162±0.019 g/g in the SBS-HR treatment group to 0.195±0.018 g/g in the SBS-LR treatment. The intestinal digestibility of crude protein (IDCP) ranged from 0.610±0.031 g/g in the LBS-LR treatment to 0.693±0.018 g/g in the SBS-LR treatment. There was no difference in the IDDM and IDCP between different treatments. It was therefore concluded that the size of the mobile bag and the ratio of the sample size to the bag surface area did not influence the intestinal digestibility of forage. Future studies should use bags with high ratios of sample size to surface area in order to obtain sufficient residue for further analysis. (**Key Words :** Forage, Intestinal Digestibility, Mobile Bag, Sheep)

INTRODUCTION

The mobile bag technique is used to estimate the intestinal digestibility of feed in ruminants. This technique has been tested in several studies (Voigt et al., 1985; De Boer et al., 1987; Varvikko and Vanhatalo, 1990; Jarosz et al., 1994) and appears to be a promising method (Hvelplund et al., 1992). However, Beckers et al. (1996) stated that the mobile bag technique for ruminants comprised certain complications, particularly regarding the duration of the incubation time in the rumen, the pore size of the nylon bag, the site of bag recovery, and the necessity of subjecting the feed to acid digestion in the abomasum before determining intestinal digestibility. In addition to these complications, the size of the mobile bag and the ratio of the sample size to bag surface area (SS:SA) must be considered in estimating intestinal digestibility, since this ratio influences the transition time of the bag and, in turn, the intestinal digestibility. Moreover, the SS:SA ratio theoretically influences enzyme binding and microbial attachment to the feed samples in both the small and large intestine, thereby affecting the intestinal digestibility. Indeed, these factors are known to decrease *in situ* ruminal digestibility (Nocek, 1988; Vanzant et al., 1998).

The sizes of mobile bags used in different studies range from 9.6 cm^2 (2.4 cm×4 cm) to 50 cm^2 (5 cm×10 cm) for cattle (Voigt et al., 1985; Varvikko and Vanhatalo, 1990; Haugen et al., 2006) and from 7.1 cm^2 (3 cm across) to 11.3 cm^2 (2.5 cm×4.5 cm) for sheep (Beckers et al., 1996; González et al., 2001). Similarly, the SS:SA ratio ranges from 9.7 to 41.7 mg/cm^2 in concentrates and from 9.7 to 22.9 mg/cm^2 in forages (Voigt et al., 1985; Jarosz et al., 1994; Vanhatalo and Ketoja, 1995). However, systematic digestibility effects of mobile bag characteristics have not been explicitly tested. Understanding the influence of bag size and the SS:SA ratio on intestinal digestibility will be useful in developing a sophisticated and standardized

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mobile bag technique. The development of the mobile bag technique is particularly valuable in sheep (Beckers et al., 1996), since the relatively small and narrow digestive tract of this species may be more susceptible to modifications of bag characteristics than the spacious digestive tract of cattle. Therefore, the goal of this study was to determine the impact of bag characteristics (bag size and SS:SA) on intestinal dry matter (DM) and crude protein (CP) digestibility of forage in sheep.

MATERIALS AND METHODS

Animals and feed

Four Suffolk ewes (mean body weight±SD, 47.6±6.0 kg) fitted with ruminal and proximal duodenal cannulae were used in this study. The duodenal cannula was T-shaped with an internal opening diameter of 13 mm (Sanshin Industrial Co., LTD). None of the ewes were pregnant or lactating, and the animals were kept in individual pens during the experiment. The ewes were fed second-cut and early bloom Italian rye-grass (*Lolium multiflorum* Lam.) hay twice daily in equal proportions at 09:00 and 16:00. This diet met the maintenance requirements recommended by the Japanese Feeding Standard for Sheep (AFFRC, 1996). The Italian rye-grass hay contained 83.1% DM, 6.3% CP (DM basis), and 74.8% neutral detergent fiber (NDF) (DM basis). The ewes had free access to water and a mineral mixture. The experimental procedures and surgical operations for animals were approved by the Committee of Animal Care and Management, Gifu University.

Sample preparation

Italian rye-grass hay, similar to the feeding material, was used in the test forage sample. The forage sample was incubated in the rumen according to the standard *in situ* method (Nocek, 1988; Vanzant et al., 1998). The forage samples were dried at 60°C for 48 h and ground through a 1-mm screen using a Wiley mill. Samples of 2.5 g ground material were placed in nylon bags (5 cm×10 cm; pore size, 53 μm; Bar Diamond Inc.), and 10 bags were prepared for each animal. The opening of each bag was tightly closed with a rubber band. The bags were soaked in water at 39°C for 20 min prior to ruminal incubation. Pre-soaked bags were placed into a laundry net with an anchor and incubated in the ventral rumen for 16 h. Immediately after removal from the rumen, the bags were immersed in cold water to stop microbial activity. Subsequently, seven bags/animal were incubated at 37°C for 3 h with pepsin-HCl solution (1 g/L in 0.1 N HCl). These seven bags and the remaining three untreated bags were then rinsed five times for 1 min/rinse using a standard domestic washing machine. All bags were dried at 60°C for 48 h and then weighed. The three bags not subjected to pepsin incubation and the three bags that had undergone pepsin incubation were used for chemical analysis; the remaining four bags were mixed and used for the mobile bag experiment.

Experimental treatment and mobile bag procedure

The experiment lasted a total of 27 days. The first 7 days were allowed for diet adjustment, and the following 20 days were used for the mobile bag experiment. The 20-day experimental period was divided into 4 subperiods, with each sub-period consisting of 2 intestinal incubation days and 3 rest days for recovery of intestinal function and animal condition. The mobile bags were made from nylon sheets with a pore size of 53 μm. To test the influence of bag size and SS:SA ratio on intestinal digestion, the experiments were performed with 2 bag sizes with external dimensions of 20×20 mm (small bag size; SBS) and 30×30 mm (large bag size; LBS) and 2 SS:SA, i.e., 5.5 mg/cm^2 (low ratio; LR) and 11.0 mg/cm^2 (high ratio; HR). The four experimental treatments were as follows: SBS-LR, SBS-HR, LBS-LR, and LBS-HR.

Eight mobile bags per treatment were prepared during each experimental subperiod. The mobile bags were heat-sealed and soaked in 37°C water for 10 min prior to intestinal incubation. Eight bags/animal were inserted through the duodenal cannula at 15-min intervals, 30 min after the morning feeding. The bags were collected from the feces, and the time to excretion was recorded. The bags were collected until 48 h after the last bag was inserted into the duodenal cannula. The collected bags were immediately washed with iced water and stored in at 0°C until all bags were collected. Once collected, all bags were rinsed again and dried at 60°C for 48 h.

The forage and residues obtained from the incubated samples were analyzed for DM and CP contents using the methods described by AOAC (1995). The forage sample was also analyzed for NDF content according to Van Soest et al. (1991). The intestinal digestibility of DM and rumen undegraded CP was calculated as the amount of DM and CP extracted from the bag divided by the amount of DM and CP in the bag before intestinal passage.

Statistical analysis

The experimental design was a 4×4 Latin square with a 2×2 factorial arrangement. The size of the mobile bag and the ratio of the sample size to bag surface area were the main effects, and the animal and period were random effects. The mixed model used was:

$$Y = \mu+A_i+P_j+T_k+e_{ijk}+BS_l+RSB_m+BS\times RSB_{lm}+f_{ijklm}$$

where μ is the overall mean; A and P are the random

Table 1. Influence of bag size and ratio of the sample size to bag surface area on transition time and intestinal digestibility of dry matter (DM) and crude protein (CP)

	SBS-LR[1]	SBS-HR	LBS-LR	LBS-HR	Pooled SEM	p-level		
						BS[3]	RSB	BS×RSB
Transition time (h)	23.2	25.0	24.1	27.0	0.282	0.691	0.519	0.853
Digestibility within the rumen+pepsin incubation[2]								
DM (g/g)	0.320 (0.009)				-	-	-	-
CP (g/g)	0.497 (0.039)				-	-	-	-
Intestinal digestibility								
DM (g/g)	0.195	0.162	0.179	0.185	0.005	0.883	0.614	0.475
CP (g/g)	0.693	0.614	0.610	0.658	0.052	0.494	0.579	0.052

[1] Bag size-sample size to bag surface area: SBS-LR, 20×20 mm-11 g/cm^2; SBS-HR, 20×20 mm-22 g/cm^2; LBS-LR, 30×30 mm-11 g/cm^2; LBS-HR, 30×30 mm-22 g/cm^2.

[2] The digestibility within the rumen+pepsin incubation is a common value across the treatments. Values in parentheses are standard error.

[3] BS = Effect of the mobile bag size; RSB = Effect of the ratio of the sample size to bag surface area; BS×RSB = Interaction between BS and RSB.

effects of animal and period, respectively; T is the main effect of the bag treatment; BS and RSB are the fixed effects of the bag size and the ratio of the sample size to bag surface area, respectively; e_{ijk} and f_{ijklm} are the error terms. All statistical procedures were performed using the JMP software ver. 5.0.1 (SAS Institute Inc., 2002)

RESULTS

The recovery rate of the mobile bags was 0.77 (99/128 bags) within 48 h of insertion into the duodenal cannulae. In one of the ewes under LBS-LR treatment, most of the bags were not excreted within 48 h because the first bags blocked the duodenal cannula.

Transition time of the mobile bags and the DM and CP digestibility in the intestine are shown in Table 1. There was no significant difference observed in the transition time for bags of the various treatments (BS, p = 0.6907; RSB, p = 0.5185; and BS×RSB, p = 0.853). The digestibility within the rumen+pepsin incubation was 0.320±0.009 for DM and 0.497±0.039 for CP. The intestinal digestibility of DM was not different between different treatments (BS, p = 0.883; RSB, p = 0.614; and BS×RSB, p = 0.475). Differences in the intestinal CP digestibility with different bag sizes (BS, p = 0.494) and with different sample SS:SA (RSB, p = 0.579) were again not significant. However, SBS-LR treatment yielded slightly higher digestibility than other treatments (BS×RSB, p = 0.052).

Influence of transition time of the mobile bag on the intestinal digestibility is shown in Figure 1. The intestinal digestibility of DM increased slightly with longer transition times of the mobile bag (r = 0.335, p<0.001), although this increase in rate was extremely small (0.29%/h). With respect to CP digestibility, transition time of the mobile bag did not affect the intestinal digestibility (r = 0.037, p = 0.710).

DISCUSSION

The hypothesis was that increased size would slow the movement of the bags in the intestine, thereby extending transition time and improving intestinal digestibility.

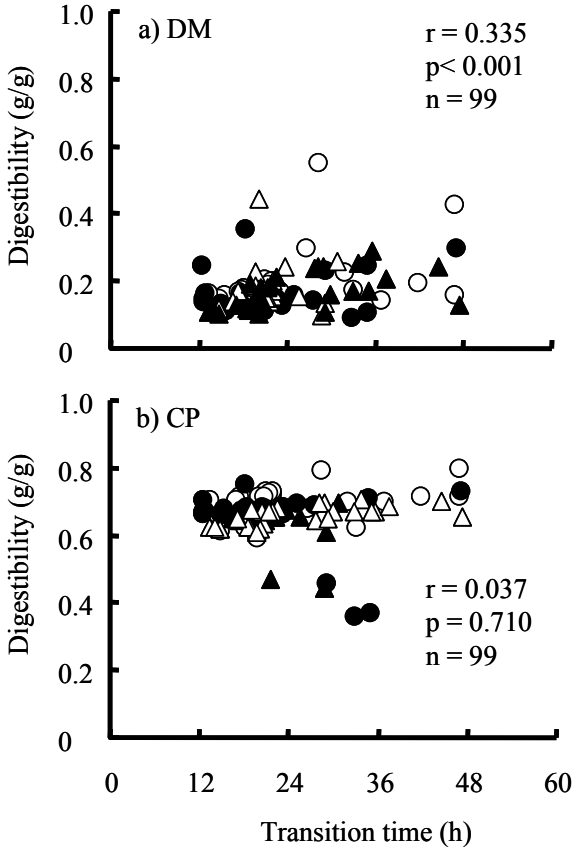

Figure 1. Influence of transition time of each mobile bag on intestinal digestibility of a) DM and b) CP. Bag size–sample size to bag surface area: SBS-LR, 20×20 mm -11 g/cm^2 (○); SBS-HR, 20×20 mm -22 g/cm^2 (●); LBS-LR, 30×30 mm -11 g/cm^2 (Δ), LBS-HR, 30×30 mm-22 g/cm^2 (▲).

However, it was found that the size of the mobile bag had no influence on intestinal digestibility. This is logical since bag size had very little influence on the bag transition time (Table 1). In other reports, the mean transition time of the mobile bag from the duodenum to the feces ranged from 11.6 h to 21.0 h in cattle and sheep (Voigt et al., 1985; Varvikko and Vanhatalo, 1990; Van Straalen et al., 1993; Beckers et al., 1996; Haugen et al., 2006). However, no relationship was observed between the transition time and the size of the bag. This is consistent with the observations in the present study that bag size does not influence the bag transition time or intestinal digestibility in sheep.

Furthermore, the results indicate that the transition time of a bag had little or no effect on the intestinal digestibility of the contents, even when the bags were retained for up to 48 h in the intestine (Figure 1). Van Straalen et al. (1993) similarly found that the bag transit time did not affect either DM or CP digestibility in cow intestines. In contrast, Voigt et al. (1985) reported that in cows, bag transit time influenced intestinal DM digestibility, although not CP digestibility. The experimental setup deviated somewhat from Voigt et al. (1985), since the feed samples in the latter study were not incubated in the rumen before eventual intestinal incubation. This procedure did not reflect the common digestive process and therefore the DM digestibility increased with increased time spent in the large intestine. Therefore, it is concluded that even if the bag size affects transition time, the intestinal digestibility may not change in sheep until the material is digested for at least 48 h.

With the nylon bag technique standardized by Nocek (1988) and Vanzant et al. (1998), the SS:SA ratio might be expected to influence ruminal and also intestinal digestibility. However, no correlation was found between the SS:SA ratio and the intestinal digestibility of the sample (Table 1) when SS:SA of 5.5 and 11.0 mg/cm^2 were tested. These ratios are lower than those recommended in the nylon bag technique for estimating ruminal digestibility (10 to 20 mg/cm^2) (Nocek, 1988; Vanzant et al., 1998), suggesting that this ratio did not have a marked impact on enzyme binding or microbial attachment to the feed samples. The bulkiness of low-quality forage used in this study restricted the maximum weight of food sealed in each bag, thus allowing us to test only the lower range of the ratio. However, many studies have adopted higher ratios of sample size to bag surface area (from 9.7 to 22.9 mg/cm^2 in forages) (Voigt et al., 1985; Jarosz et al., 1994; Vanhatalo and Ketoja, 1995) than those in the present study. Thus, further investigation is required to determine the influence of the ratio of the sample size to bag surface area on intestinal digestibility for different types of forages.

In conclusion, the size of the mobile bag and the ratio of the sample size to bag surface area do not influence intestinal digestibility in sheep. Thus, using larger bags and proportionally larger sample sizes is recommended in mobile bag experiments so that large quantities of material can be harvested for relevant analytical protocols.

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REFERENCES

AFFRC. 1996. Agriculture Forestry Fisher Research Council. Japanese Feeding Standard for Sheep. The Ministry of Agriculture, Forestry and Fisheries, Japan Livestock Industry Association, Tokyo, Japan. (in Japanese)

AOAC. 1995. Official Methods of Analysis. 16th edn. Association of Official Analytical Chemists, Arlington, Virginia.

Beckers, Y., A. Théwis and B. Maudoux. 1996. Intestinal digestibility of rumen undegraded N of concentrates measured by the mobile nylon bag technique. Anim. Feed Sci. Technol. 61:305-323.

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

González, J., J. Faría-Mármol, C. A. Rodríguez and M. R. Alvir. 2001. Effects of stage of harvest on the protein value of fresh lucerne for ruminants. Reprod. Nutr. Dev. 41:381-392.

Haugen, H. L., S. K. Ivan, J. C. MacDonald and T. J. Klopfenstein. 2006. Determination of undegradable intake protein digestibility of forages using the mobile nylon bag technique. J. Anim. Sci. 84:886-893.

Hvelplund, T., M. R. Weisbjerg and L. S. Andersen. 1992. Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique. Acta Agric. Scand., Sect. A, Animal Sci. 42:34-39.

Jarosz, L., T. Hvelplund, M. R. Weisbjerg and B. B. Jensen. 1994. True digestibility of protein in the small intestine and hind gut of cows measured with the mobile bag technique using ^{15}N-labelled roughage. Acta Agric. Scand., Sect. A, Animal Sci. 44: 146-151.

Nocek, J. E. 1988. *In situ* and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci. 71: 2051-2069.

SAS Institute Inc. 2002. JMP User's guide: version 5.0.1. SAS Institute Inc, Cary, North Carolina.

Vanhatalo, A. and E. Ketoja. 1995. The role of the large intestine in post-ruminal digestion of feeds as measured by the mobile-bag method in cattle. Br. J. Nutr. 73:491-505.

Van Soest, P. J., J. B. Robertson and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Van Straalen, W. W., F. M. H. Dooper, A. M. Antoniewicz, I. Kosmala and A. M. Van Vauren. 1993. Intestinal digestibility

in dairy cows of protein from grass and clover measured with mobile nylon bag and other methods. J. Dairy Sci. 76:2970-2981.

Vanzant, E. S., R. C. Cochran and E. C. Titgemeyer. 1998. Standardization of *in situ* techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76:2717-2729.

Varvikko, T. and A. Vanhatalo. 1990. The effect of differing types of cloth and of contamination by non-feed nitrogen on intestinal digestion estimates using porous synthetic-fiber bags in cow. Br. J. Nutr. 63:221-229.

Voigt, J., H. Piatkowski, H. Engelmann and E. Rudolph. 1985. Measurement of postruminal digestibility of crude protein by the bag technique in cows. Arch. Tierernähr. 35:555-562.