A comparison of parameters of the passage of nylon capsules and digesta calculated from faecal excretion data obtained in lactating cows

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ABSTRACT: The aim of the study was to compare parameters of passage of nylon capsules and digesta represented by Cr-labelled maize silage through the digestive tract of dairy cows. The capsules were made of nylon cloth (42 μm pore size, 10 mm outside diameter) and applied orally. The evaluation was carried out in dairy cows with milk yield of 19.0 kg/day. The diet (17.6 kg DM/day) consisted of maize silage, lucerne hay and concentrate. Total mean retention time (TMRT), delay time (τ), summarised compartmental mean retention time (CMRTS), and mean individual compartmental retention times (CMRT1 and CMRT2) were calculated. TMRT, τ, CMRTS, CMRT1 and CMRT2 values of nylon capsules and Cr-labelled silage were 36.2 and 45.4 h ($P < 0.01$), 16.2 and 8.3 h ($P < 0.01$), 20.1 and 37.2 h ($P < 0.01$), 7.8 and 8.5 h, 12.2 and 28.7 h ($P < 0.05$), respectively. The calculated mean retention time of nylon capsules in the reticulo-rumen (CMRTS) was shorter and in the intestines (τ) was longer than that of digesta. For this reason the estimation of digestibility using the nylon capsule method can be questionable.

Keywords: nylon capsule; Cr-labelled silage; rate of passage; dairy cow

In ruminants the digestion of feed depends, among others, on the rate of passage through the gastrointestinal (GI) tract (Faichney, 1975). For this reason the rate of passage of nylon capsules can influence digestibility results obtained by the nylon capsule method which is described below. Nylon capsules (42 μm pore size, 10 mm outside diameter) were developed for the determination of feed digestibility in ruminants (Třináctý et al., 1995). They are filled with a sample of feed and a load. The load (steel balls) helps keep them at the rumen bottom to prevent their rumination. The optimum load weight allowing the highest recovery of capsules in faeces was determined in tests carried out by Třináctý et al. (2002). The capsules are administered *per os* and the main advantage is that no cannulas are required in the experimental animals. The nylon capsule method was used to measure the whole tract digestibility of neutral sugars (Třináctý et al., 1995), crude protein (Třináctý et al., 1999), minerals (Třináctý et al., 2000) and to estimate roughage crude protein and dry matter digestibility (Třináctý et al., 2003). For the correct interpretation of digestibility results determined using the nylon capsule method it was important to calculate and compare the parameters of the passage of nylon capsules with those of digesta (represented by Cr-labelled silage).

To describe the passage of digesta Pond et al. (1988) developed a non-linear mathematical model

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using simultaneously gamma and exponential functions for fitting passage curves. This model divides the curve into two compartments and a delay time. Generally, this model had a good fit of data (Quiroz et al., 1988). However Pond et al. (1988) reported that when the number of data in the first part was low, uncertain distribution of retention times between compartments was obtained. Moreover, the non-linear model mentioned above assumes that the flow of digesta is a continuous process, but as the Faichney (1975) stated, defaecation is obviously discontinuous. For these reasons Mambrini and Peyraud (1997) suggested a simple mathematical method which showed a lower sensitivity to input data and to discontinuity of the digesta flow. These authors used the combination of a summative method (Thielemans et al., 1978) and a graphic method (Udén, 1984) with the visually determined point of the first marker appearance (delay time). With regard to pulse excretion and scarceness of points in the ascending part of the passage curve for nylon capsules, the method of compartment analysis according to Mambrini and Peyraud (1997) was more suitable than the previously mentioned non-linear method.

Chromium bound to fibre is often used as a marker of digesta flow in ruminants (Udén et al., 1980). Usual content of Cr in Cr-labelled fibre is between 2% and 4% Cr in DM (Moore et al., 1992; Huhtanen and Kukkonen, 1995). Ehle (1984) and Firkins et al. (1998) reported a potential bias in estimates of passage rate if high Cr concentrations were applied in the solution used for labelling. Firkins et al. (1998) noted an interaction between the type of Cr-labelled feed and the outflow rate and found out that the influence of Cr-content in feed with higher initial functional specific gravity (FSG) on the change of passage rate was low. In addition, Wattiaux et al. (1991) reported that silages generally had higher FSG than the other forages. With regard to these above-mentioned reports Cr-labelled maize silage with standard Cr-content can represent the flow of the majority component of TMR (maize silage) through the GI tract with low error.

The aim of the study was to compare the passage rates of nylon capsules and digesta (represented by Cr-labelled maize silage) through the individual compartments of digestive tract of lactating cows. The corresponding passage parameters were calculated from faecal excretion curves using the mathematical method according to Mambrini and Peyraud (1997) validated by these authors for this purpose. The data analysed in this paper originate from published articles (Třináctý et al., 2002, 2003) and partly from unpublished experiments.

MATERIAL AND METHODS

Animals

The evaluation was carried out in seven experiments. Each of them had a preparatory period (14 days) and two experimental periods (2 × 4 days) with a three-day pause. The experiments were performed with crossbred dairy cows (Red Holstein > 75% × Friesian-Holstein < 25%). All the experiments were conducted with two dairy cows, and different animals were used in individual experiments. The cows were 60–200 days in lactation and their weight ranged from 580 kg to 715 kg (mean 644 kg, s.e. 11.1). Average milk yield and measured nutrient intake are presented in Table 1. In all the experiments the diet consisted of maize silage (9.91 kg DM/day; s.e. 0.39), lucerne hay (3.67 kg DM/day; s.e. 0.10) and feed mixture (3.97 kg DM/day; s.e. 0.44). The mixture consisted of 32% maize meal, 19% rapeseed expellers, 13% wheat meal, 10% barley meal, 10% oat meal, 8% yeast, 5% flax meal, 2.5% mineral mixture and 0.5% sodium chloride. The diet was calculated according to the performance of cows (Sommer, 1994), and the ration components were fed separately. The diet was fed twice a day (at 5.00 a.m. and 3.00 p.m.) with a maximally 10% of refusals (in DM).

Nylon capsule method and Cr-labelled silage

The capsules of lenticular shape with outside and inside diameters of 10 and 8 mm, respectively,

Table 1. Daily milk yield and intake

Parameter	Amount	s.e.
Milk yield (kg/day)	19.0	0.83
Dry matter intake (kg/day)	17.6	0.43
Crude protein intake (kg/day)	2.36	0.06
NEL[1] intake (MJ/day)	114	3.2
PDIN[1] intake (kg/day)	1.52	0.04
PDIE[1] intake (kg/day)	1.55	0.04

[1]NEL (net energy of lactation), PDIN and PDIE (units of PDI system) calculated according to Sommer (1994)

were made of nylon cloth (Uhelon, Hedva Moravská Třebová, Czech Republic) with 42 μm aperture (Třináctý et al., 1999). They contained feed and a load (two stainless steel balls 2 and 3 mm in diameter, respectively) and their mean functional specific gravity (FSG) was 1.58 g/cm (Třináctý et al., 2002). The capsules with these uniform parameters (diameter, load, FSG) were used in all the experiments. They were administered orally in the form of paper bolus. Each feed was ground through a 1-mm screen and the digestibility of nutrients was measured and published (Třináctý et al., 2002; Třináctý et al., 2003). The weighing of feed into the capsules was done in batches. Each batch consisted of 100 capsules. To calculate the quantity of feed in one capsule, total amount of feed used for one batch was divided by the total number of capsules per batch. Cows in the experiments received 500 (experiments 1, 2), 300 (experiment 3) or 100 (experiments 4, 5, 6, 7) capsules. All the capsules were marked with an ineffaceable colour, each cow received capsules of different colour. Experimental periods were 4 days (96 hours). Excrements were collected in 6-hour intervals during the first 18 hours, then in 3-hour intervals, and in 6-hour intervals again during the last 48 hours. Separately for each cow and collecting interval the capsules were recovered from faeces by rinsing through a 4-mm screen every 24 hours. Cr-labelled material was prepared according to the procedure described by Udén et al. (1980), using maize silage. Samples of faeces from individual cows and intervals were dried 48 hours at 60°C and ground through a 1-mm sieve. Cr content was determined according to Williams et al. (1962).

Calculations

Twenty-eight sets of passage data (Figure 1) were obtained from individual animals in total, four sets were excluded due to a high percentage (more than 10%) of refusals and low recovery of capsules. The nylon capsule data was processed according to Mambrini and Peyraud (1997). In order to obtain comparable results the same method was used in the case of Cr-labelled silage.

Firstly, two levels of Cr were used in the experiments. We tested whether this factor influenced the obtained results (represented by the outflow rate k). The parameter k was calculated by the compartmental analysis according to Udén (1984). Natural logarithms of Cr concentrations in faeces were plotted against the time and the passage curve was visually divided into the ascending and descending part. The slope of the descending part (k) of the curve was calculated by a regression analysis (QC Expert, version 2.5, Czech Republic).

Secondly, the parameters of whole database passage curves were evaluated in two steps:

In the first step, recovery, total mean retention time (TMRT), delay time (τ) and summarised compartmental mean retention time (CMRTS) were

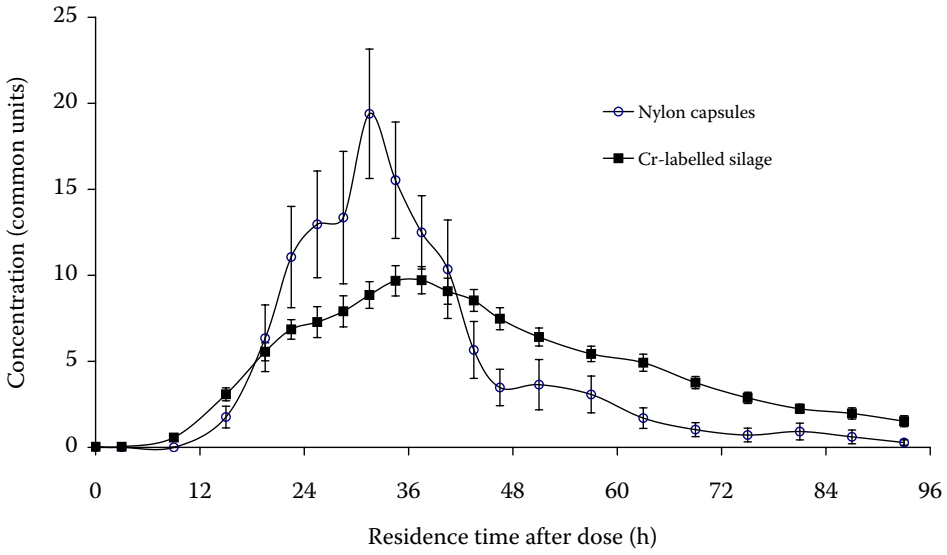

Figure 1. Faecal excretion patterns of nylon capsules and Cr-labelled silage expressed as means of 24 measurements with confidence intervals ($P < 0.05$; vertical bars). Concentrations are expressed in common units: number × kg/DM/number of nylon capsules recovered and in g × kg/DM/g Cr recovered

calculated from individual curves of the passage of nylon capsules and Cr. TMRT was calculated according to a modified procedure described by Faichney (1975), using the following equation:

$$TMRT = \sum n_i t_i / \sum n_i$$

where: n_i = the number of capsules or the quantity of markers excreted during the i-th interval

t_i = the time interval between the dosing and the mid-point of each time interval i of faeces collection

The delay time τ was expressed as a time interval between the dosing and the mid-point of the interval when the capsules (markers) appeared for the first time in faeces (according to Mambrini and Peyraud, 1997). CMRTS was calculated by subtracting τ from TMRT. The pulse characteristic of nylon capsule excretion did not allow to divide the passage curves into the ascending and descending part correctly. For this reason in the second step parameters k, CMRT2 (descending part of passage curve) and CMRT1 (ascending part) were calculated using pooled data (separately for nylon capsules and Cr-labelled silage) from all experiments providing 24 points for each interval (Figures 2 and 3). Before pooling the data of faecal passage curves were transformed into comparable common units as follows. For the capsules it was: number/kg of dry matter/number of total recovery, and for Cr it was: g of Cr/kg of dry matter/g of total recovery. Natural logarithms of the above-mentioned units were pooled within each interval, plotted against the time and visually divided into the ascending and descending parts (Figures 2 and 3). The slope of the descending part (k) of the curve with confidence interval was obtained by regression analysis (QC Expert, version 2.5). CMRT2 was calculated from the parameter k using the equation CMRT2 = $1/k$. CMRT1 was calculated by subtracting CMRT2 from CMRTS (mean value obtained from individual passage curves). Calculations of CMRT1 and CMRT2 and their confidence intervals ($P < 0.05$) were performed on the basis of error propagation law (Taylor, 1997), using Monte Carlo simulation (QC Expert, version 2.5). These propagated confidence intervals were used for the statistical comparison of the obtained values.

Statistical Analysis

Firstly, the effect of two levels of Cr in Cr-labelled silage on outflow rate (k) was analysed according to a nested design with missing cells (four curves excluded) using the following model:

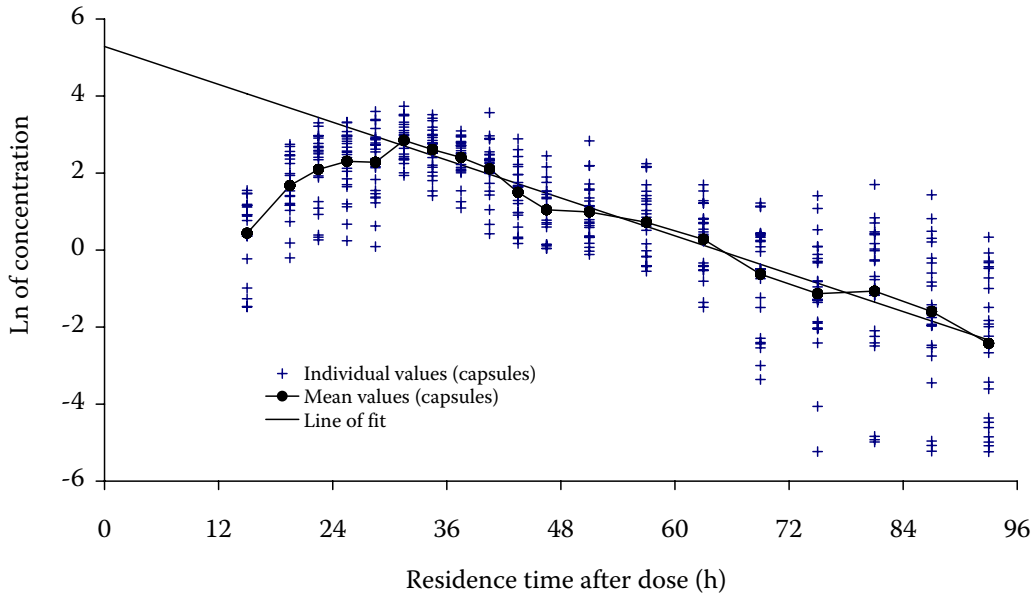

Figure 2. Faecal excretion patterns of nylon capsules from 24 passage curves expressed as logarithms of concentration with fitted slope as the coefficient k

[Ln of concentration] = 5.28 – 0.082 × [Residence time after dose]; $R^2 = 0.66$

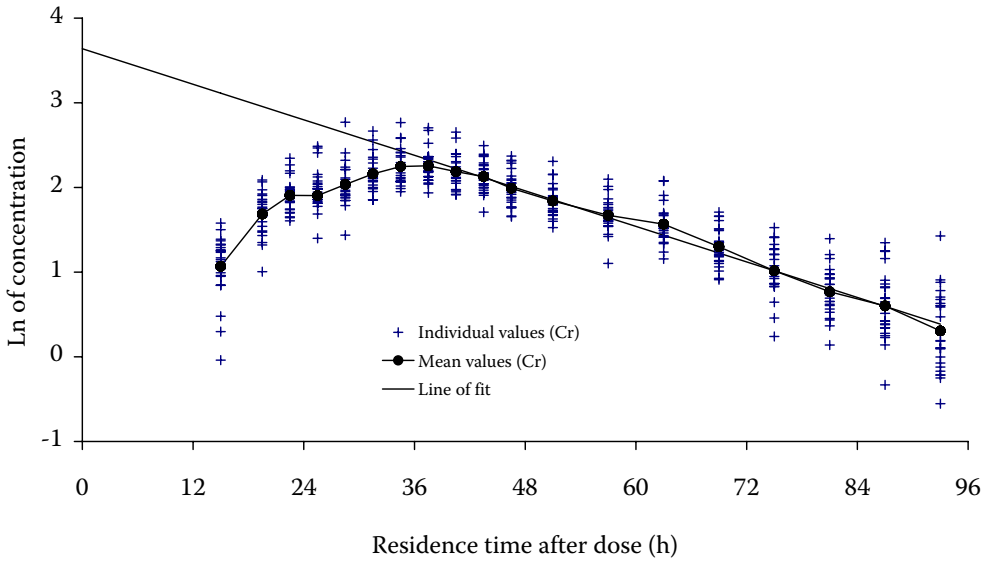

Figure 3. Faecal excretion patterns of Cr-marked silage from 24 passage curves expressed as logarithms of concentration with fitted slope as the coefficient *k*

[Ln of concentration] = 3.64 – 0.035 × [Residence time after dose]; R^2 = 0.84

$$Y_{ijk} = \mu + a_i + c_j(a_i) + e_{ijk}$$

where: μ = the overall mean

a_i = the content of Cr in labelled silage (i = 2)

c_j = the effect of the animal (j = 13)

e_{ijk} = the error term and period effect (k = 2)

The effect of Cr content was tested with c_j as an error term. The analysis was performed using the GLM procedure (Systat, version 10.2).

Secondly, means of recovery, TMRT, τ and CMRTS of nylon capsules and of Cr were compared using the paired comparison *t*-test (QC Expert, version 2.5). Means of CMRT1 and CMRT2 of nylon capsules and Cr were compared by *t*-test (using their propagated confidence intervals).

RESULTS

Firstly, we tried to find out whether the different Cr contents in labelled silage (% of DM), i.e. 2.39 (n = 9) and 3.26 (n = 15), could influence the calculated outflow of digesta from the rumen (k). The respective k values for the lower and higher content of Cr were 3.38 (s.e. 0.21) and 3.79 (s.e. 0.18) %/h. Because the difference was small (0.41%/h) and insignificant (P > 0.05), both sets of data were pooled for subsequent processing.

Secondly, the parameters of whole database passage curves were evaluated. The determined recoveries of capsules and Cr were 90% and higher (Table 2). The passage rate of nylon capsules through the GI tract was higher than for Cr, and for TMRT a large difference was determined (9.2 hours; P < 0.01). The values of delay time indicated that the nylon capsules appeared in faeces 16.2 hours after the administration. For the Cr-labelled silage the corresponding time interval was only 8.3 hours. It means that the passage of Cr-labelled silage through the intestines was faster. The estimated CMRTS values for nylon capsules and labelled silage were 20.1 and 37.2 hours (P < 0.01), respectively. Assuming that in particular CMRTS represents the time of retention in the reticulo-rumen (Wylie et al., 2000), we can state that Cr-labelled particles remained there for a longer period of time than the nylon capsules. It can likely be explained by the high specific gravity of the nylon capsules (Třináctý et al., 2003).

In the second step, the outflow rates k were calculated using the descending part of the passage curve of nylon capsules and Cr-labelled silage (Figures 2 and 3). Regression equations indicated that respective k values for nylon capsules and Cr-labelled silage were 8.2%/h (R^2 = 0.66) and 3.5%/h (R^2 = 0.84). The values of CMRT2 (Table 3) subsequently calculated from the corresponding outflow rates indicated a large difference, 12.2 and 28.7 h for nylon capsules and Cr-labelled silage, respectively (P < 0.05). On the other hand, an insignificant difference of 0.7 h (P > 0.05) was found out between the CMRT1 mean values obtained from the passage

Table 2. Recovery, total mean retention time (TMRT), delay time (τ) and summarised compartmental mean retention time (CMRTS) calculated from the curves of passage of nylon capsules and Cr-labelled silage (n = 24), s.e. in brackets

Parameter	Nylon capsules	Cr-labelled silage	Difference
Recovery (%)	92.2 (1.07)	90.0 (1.35)	2.24
TMRT (h)	36.2 (1.05)	45.4 (0.61)	–9.20*
τ (h)	16.2 (0.50)	8.3 (0.41)	7.94*
CMRTS (h)	20.1 (0.97)	37.2 (0.69)	–17.14*

*P < 0.01

Table 3. Parameters CMRT1 and CMRT2 of the curves of passage of nylon capsules and Cr-labelled silage calculated from pooled data (n = 24), confidence intervals for P < 0.05 are given in brackets

Parameter	Nylon capsules	Cr-labelled silage	Difference
CMRT1 (h)	7.8 (2.18)	8.5 (2.08)	–0.7
CMRT2 (h)	12.2 (0.95)	28.7 (1.47)	–16.5*

*P < 0.05

curves of the nylon capsules and from the Cr-labelled silage.

DISCUSSION

Ramanzin et al. (1991) reported that the higher level of Cr in labelled material increased its specific gravity, which accelerated the rate of passage of the labelled material. A similar, but insignificant finding was reported by Ehle (1984). In our experiments no significant effect of different Cr contents in labelled silage on the passage rate was observed. These contents of Cr (2.39 and 3.26% of DM) are currently used in published papers dealing with the evaluation of digesta passage. As mentioned above, in silages the influence of current Cr contents on the change of their FSG is small and that is why Cr labelled silage can represent the parameters of passage of this feed component through the digestive tract. According to Wattiaux et al. (1991) maize silage contained more than 50% of particles with FSG ranging between 1.15 and 1.30 g/cm^3 and more than 30% between 1.00 and 1.15 g/cm^3. Siciliano-Jones and Murphy (1991) found out the value 0.92 g/cm^3 for the initial specific gravity of maize silage. The above-mentioned values of FSG are lower than those for nylon capsules (1.58 g/cm^3).

TMRT values of Cr-labelled silage (45.4 h), as determined in our experiments, correspond well with published TMRT data, obtained in studies of roughage passage through the digestive tract of lactating dairy cows. Udén (1984) found the TMRT value 48.8 h, Colucci et al. (1990) 46.7 h, Udén and Sutton (1994) 47.0 h and Mambrini and Peyraud (1997) reported 51.7 hours. The lower TMRT values, observed for nylon capsules (36.2 h), were similar to data reported on the passage rate of concentrates: Udén (1984) 44.5 h, Colucci et al. (1990) 35.9 hours, Mambrini and Peyraud (1997) 40.6 h, and Rothfuss et al. (1997) 28.3 hours. The TMRT of nylon capsules was comparable with that of concentrates. We suppose that the main cause of these findings was the higher specific gravity (1.58 g/cm^3) of nylon capsules (Třináctý et al., 2003) in comparison with the above-mentioned values for maize silage.

Mean values of τ, as calculated from Cr-labelled silage passage curves (8.3 h), were similar to data mentioned in the literature for roughage and concentrates; Mambrini and Peyraud (1997) published the respective values of 9.5 and 8.3 h, and Poore et al. (1991) reported 7.2 h for labelled sorghum grain. The τ value of nylon capsules (16.2 h) was almost twice as high as that of Cr-labelled silage. This can be explained by the size and relatively high specific gravity of nylon capsules (Třináctý et al., 2000).

Campling and Freer (1962), Ehle and Stern (1986) and Kaske and Engelhardt (1990) stated that the passage of larger particles of higher specific gravity through the intestines was slower. Wylie et al. (2000) found out that the retention time of ingested and masticated hay in the intestines (measured by a direct method in cannulated animals) accounted for 74.4% and 94.9% of τ value (calculated from faecal samples), respectively. Using the data published by Mambrini and Peyraud (1997) we could calculate that the corresponding values were 75.8% (feeding hay) and 84.8% (ground hay). Using the data for sorghum grain reported by Poore et al. (1991), we calculated the τ value of 95.8%. Based on these reported results we were able to express the retention time of nylon capsules and of Cr-marked silage in the intestines using the τ value obtained from faeces.

CMRT2 (called also ruminal mean retention time) is derived from the ruminal outflow rate (Colucci et al., 1990). The biological interpretation of this parameter is relatively reliable and according to Grovum and Williams (1973), Pond et al. (1988) and Mambrini and Peyraud (1997) it may be allocated with confidence to the reticulo-rumen. Our value of CMRT2 of labelled silage (28.7 h) corresponded with the values published by Udén and Sutton (1994) and Mambrini and Peyraud (1997), who reported similar values (26.3 and 25.3 h, resp.) for dairy cows with similar milk performance. In contrast to the results obtained for the intestine (τ), CMTR2 of capsules was substantially shorter (12.2 h) than that of labelled silage (28.7 h) and despite of the large size of capsules it was more similar to those published for concentrates. For soybean meal, Colucci et al. (1990) published the values ranging from 12.3 to 16.0 hours. Rothfuss et al. (1997) reported 14.8 h and Mambrini and Peyraud (1997) 22.9 hours. This discrepancy is caused by the higher FSG of capsules (1.58 g/cm^3) in comparison with the above-mentioned FSG of maize silage. Comparable results were obtained in the study of Kaske and Engelhardt (1990). The authors confirmed that the large particles (10 mm) with high SFG (1.44 g/cm^3) spent a shorter time in the reticulo-rumen than those of 1 mm in size and FSG of 1.03 g/cm^3.

CMRT1 represents a sum of retention times of several mixing sub-compartments. One of them is situated in the rumen (pool of large light particles with their rumination) and the others in the caecum and proximal colon (Pond et al., 1988; Mambrini and Peyraud, 1997). The distribution of retention times between sub-compartments depends on the properties of the material under evaluation. Prange et al. (1982) calculated that the particles remained for 40% of the total CMRT1 value in the caecum and in the proximal colon. According to Mambrini and Peyraud (1997) particles of ingested hay and concentrate remained in the intestinal mixing compartment for 3.5 hours. For hay and concentrate, these values accounted for 20.7% and 37.2% of the total CMRT1 value, respectively. CMRT1 of labelled silage, as calculated on the basis of our data (i.e. 8.5 h), was shorter than that of labelled hay (16.9 h) but similar to the value of labelled concentrate 9.4 h (Mambrini and Peyraud, 1997). It was also in agreement with the value (8.8 h) published by Udén and Sutton (1994). CMRT1 values of nylon capsules were not different from CMRT1 of labelled silage.

The sum of CMRT1 and CMRT2 results in CMRTS. According to Wylie et al. (2000), due to a mixing flow in the forestomach digesta CMRTS amounted to 94.2% of that for the entire gastrointestinal tract. From the results of the detailed compartmental analysis mentioned above it stands to reason that the shorter mean retention time of nylon capsules in the reticulo-rumen (CMRTS) was caused in particular by the short CMRT2 (descending part of the passage curve), thus by the high outflow rate (k) of nylon capsules from the reticulo-rumen (see different faecal excretion patterns of nylon capsules and Cr-labelled silage in Figure 1. The influence of CMRT2 was strong and the mean retention time of nylon capsules in the GI tract (TMRT) was shorter than that of Cr-labelled silage although the nylon capsules remained in the intestines for a longer time period (τ).

CONCLUSION

The calculated mean retention time of nylon capsules in the reticulo-rumen was shorter and in the intestines it was longer than that of digesta represented by Cr-labelled maize silage. The short mean retention time of nylon capsules in the reticulo-rumen was the main cause of the shorter total mean retention time of these ones. For this reason the estimation of digestibility using the nylon capsule method can be questionable. For more authentic interpretation of the results, parameters of the passage of nylon capsules obtained from cannulated animals are necessary.

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