

Comparison of apparent and true digestibility of nutrients determined in dairy cows either by the nylon capsule or *in vivo* method

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ABSTRACT: In this study the values of true digestibility of DM, OM, CP, NDF and starch determined either by the nylon capsule method or conventional *in vivo* method were compared. Four intact crossbred dairy cows (mean milk yield 21.9 kg/d) were used in two experimental periods. TMR consisted of maize silage, lucerne hay and concentrate. Nylon capsules (external diameter 10 mm) were made of nylon cloth (pore size 42 µm). Capsules were filled with TMR and at the beginning of the *in vivo* trial they were administered orally as a paper bolus into the cows. The values of true digestibility (after washing loss correction) of DM and OM, as determined by means of the nylon capsule method were lower than those estimated by the *in vivo* method. The respective differences were –6.9 and –7.3% ($P < 0.05$). Insignificant differences were found in CP, NDF and starch.

Keywords: cow; true digestibility; nylon capsule; dry matter; organic matter; crude protein

To maintain physiological functions of the rumen it is necessary to take into account that fibre is an important part of the ration. For this reason it is not easy to determine digestibility of feeds with low content of fibre in a conventional *in vivo* trial. This problem can be eliminated when using a combined *in situ* method, called the mobile bag method (Aronen et al., 1991; Varvikko and Vanhatalo, 1993), in which the whole tract digestibility is a sum of ruminal and intestinal digestibility. However, cannulated animals are required when using the above-mentioned method. In contrast, the new nylon capsule method (Třináctý et al., 1999, 2002) uses intact animals. The nylon capsule method is based on the application of nylon capsules of 10 mm in diameter, which contain a sample of feed and a load (two stainless steel balls; 2 and 3 mm). The capsules are kept by this load at the bottom of the rumen and their rejection and rumination

are hindered. Capsules are administered orally into animals and after their passage through the whole tract they are separated from faeces.

For the reason of different microbial contamination the comparison of total tract digestibility values determined by an *in situ* or *in vivo* (apparent digestibility) method is questionable (Varvikko and Vanhatalo, 1992). In order to determine true digestibility a simple method for direct elimination of microbial and endogenous matter using a neutral detergent solution was developed (Van Soest et al., 1966). This procedure was verified by Mason (1969), Mason and Frederiksen (1979) and used by Mulligan et al. (2001).

In situ methods show a typical phenomenon, namely the tested material is washed out through the cloth, which leads to overestimation of digestibility values (Varvikko and Vanhatalo, 1990). Procedures have been developed to calculate digest-

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ibility corrections for washing losses (Weisbjerg et al., 1990).

The aim of the study was to determine the values of (apparent and true) digestibility of DM, OM, CP, NDF and starch by the nylon capsule and *in vivo* method, and to compare the results of both methods.

MATERIAL AND METHODS

Animals

Four intact crossbred dairy cows (Red Holstein × Black Friesian) were used as experimental animals. These cows were 60–180 days in lactation. The values of nutrient intake are presented in Table 1. The diet consisted of maize silage with 38.4% DM (9.66 kg DM/d), lucerne hay (3.52 kg DM/d) and concentrate (5.29 kg DM/d). The concentrate consisted of 35% maize meal, 15% rape cakes, 15% barley meal, 12% wheat meal, 12% oat meal, 5% extracted soybean meal, 3% flax meal, 2.5% mineral mixture and 0.5% sodium chloride. The diet was formulated to meet nutritional requirements of lactating dairy cows. Contents of PDIN, PDIE and NEL in the ration were calculated according to Sommer (1994), in agreement with principles set up by INRA (1988). The diet was provided as total mixed ration (TMR), an allowable refusal level was 10% DM of diet. The animals were fed twice a day (at 5.00 and at 15.00 hours).

Nylon capsule method and *in vivo* trial

Digestibility of nutrients in TMR was measured either by the nylon capsule method (Třináctý et al., 1999, 2003) or by the *in vivo* method (Schiemann, 1981). Both experimental methods were performed simultaneously using the same animals. The trial consisted of a preparatory period (2 months) and two experimental periods (2 × 5 days) with a two-day break. The preparatory period was used to stabilise daily feed consumption and milk yield and to manufacture nylon capsules.

The nylon capsules were made of nylon cloth (Uhelon, Hedva Moravská Třebová, Czech Republic) with a pore size of 42 µm. The capsules were of lenticular shape and their external and internal diameters were 10 mm and 8 mm, respectively. Samples of TMR used for the filling of capsules

were prepared as follows: except the concentrate which was used without processing, samples of individual TMR components were dried overnight at 60°C, ground through a 2-mm screen and mixed in the same ratio as TMR fed to dairy cows. The weighing of samples into the capsules was done in batches. Each batch consisted of 150 capsules (16 batches corresponded to 2 400 capsules). To calculate the amount of the sample in one capsule, the total amount of sample used in one batch was divided by the total number of capsules per batch. An average sample weight in one capsule is given in Table 3. Two stainless steel balls (2 and 3 mm) were inserted into each capsule as load. In each period, 300 capsules were inserted into each cow orally as a paper bolus in the morning of the first day of the *in vivo* trial.

Maize silage was labelled with chromium (Cr) according to Udén et al. (1980), the material contained 32.6 g Cr/kg DM. Cr-mordanted silage (100 g of material) was applied just before the feeding mixed with a small amount of TMR.

Collection of faeces was carried out as follows: in 6 h intervals for the first 18 h, thereafter in 3 h intervals for 30 h and again in 6 h intervals for the last 72 h. Faeces were collected in containers and preserved with chloroform (Schiemann, 1981). For the purpose of analyses (Cr and nutrients) a small amount of faeces was dried at 60°C and ground through a 1 mm sieve.

Capsules were recovered from faeces every 24 h by rinsing on a 4 mm screen. After the recovery, they were washed (3 × 4 min) in an automatic washing machine (time of washing according to Madsen and Hvelplund (1994)). The recovered capsules were cut by hand with scissors and the steel balls with capsule residue were separated from the feed residue under running water on a 1.9 mm sieve. Feed residues were slightly squeezed through a 42 µm nylon cloth and dried overnight at 60°C. Capsules damaged (marked) by the teeth of cows were considered as chewed (regurgitated).

Analyses

The samples of feeds, nylon capsule residues and faeces were analysed for DM (105°C), ash, CP and crude fat contents (AOAC, 1984; No. 7.009, 7.021, 7.060). Neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991). NDF was determined without sodium sulphite, with α-

amylase and expressed without residual ash. The true digestibility of DM, OM and CP was estimated by using a neutral-detergent solution to remove endogenous and microbial matter from faeces (Van Soest et al., 1966; Mulligan et al., 2001; Mason and Frederiksen, 1979). This process is identical to that used for NDF determination (Van Soest et al., 1991). According to the above-mentioned authors the material removed using the neutral-detergent treatment represents endogenous and microbial nutrients. Neutral-detergent insoluble crude protein in faeces (NDI-CP) was determined according to Licitra et al. (1996), starch in feeds and faeces according to Wester et al. (1992), who modified the procedure described by MacRae and Armstrong (1968) by adding a step of α -amylase treatment in order to obtain a higher yield of starch from samples. Cr was determined according to Williams et al. (1962).

Calculations and statistical evaluation

Total mean retention time (TMRT) of digesta and of capsules in the digestive tract was calculated according to Mambrini and Peyraud (1997) using the following equation (in hours):

$$\text{TMRT} = \sum (Ni \times ti) / \sum (Ni)$$

where: Ni = the amount of Cr (and/or the number of capsules) collected in faeces during the interval

ti = the time elapsed from dosing to the midpoint of the collection interval

The apparent digestibility (%) of nutrients (DM, OM and CP) was calculated according to the following equation (intake and output of nutrients in kilograms):

apparent nutrient digestibility = $(1 - (\text{faecal nutrient} / \text{total nutrient intake})) \times 100$

The true digestibility (%) of nutrients was calculated according to the following equations (intake and output of nutrients in kilograms):

true DM digestibility = $(1 - ((\text{faecal NDEA} + \text{faecal starch}) / \text{total DM intake})) \times 100$

true OM digestibility = $(1 - ((\text{faecal NDF} + \text{faecal starch}) / \text{total OM intake})) \times 100$

true CP digestibility = $(1 - (\text{faecal NDI-CP} / \text{total CP intake})) \times 100$

NDF digestibility = $(1 - (\text{faecal NDF} / \text{total NDF intake})) \times 100$

starch digestibility = $(1 - (\text{faecal starch} / \text{total starch intake})) \times 100$

where: NDEA = NDF not corrected for ash

The neutral-detergent solution with α -amylase removes residual feed starch from faeces besides endogenous and microbial matter (Van Soest, 1994). For this reason the content of faecal starch was included in the calculation of the true DM and OM digestibility.

The apparent and true digestibility values (DV (g/g)) determined by means of the nylon capsule method were corrected for washing loss using the equation (Weisbjerg et al., 1990):

$$\text{CDV} = 100 \times \{(\text{DV} - \text{SPL} \times [1 - (\text{DV} - (\text{SPL} + \text{WSL}))]) / [1 - (\text{SPL} + \text{WSL})]\}$$

where: CDV (%) = the corrected digestibility value

SPL (g/g) = solid particle loss and WSL (g/g) water-soluble loss. SPL was calculated by subtracting WSL from total washing loss (TWL)

To determine TWL, nylon bags 5×10 cm (internal size) were made of the same cloth as the nylon capsules. Into each of quadruplicated bags 1 g of sample was weighed, and the bags were washed in a washing machine (3×4 min). WSL was determined by the washing of quadruplicate samples through folded filter paper (No. 595^{1/2}, Schleicher and Schuell, Germany). All washed bags and filter paper residues were dried for 48 h at 55°C. Thereafter contents of nutrients were determined.

The results were statistically analysed using a mixed model analysis of variance (fixed treatment effects were “methods”, random effects were “animals”) with interaction and replication (Snedecor and Cochran, 1973) using the Microsoft Excel 97 according to the following equation:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

where: Y_{ijk} = dependent variable for methods i , animals j and replications k

μ = overall mean

α_i = effect of method ($i = 1$ to 2)

β_j = effect of animals ($j = 1$ to 4)

$(\alpha\beta)_{ij}$ = effect of interaction

ϵ_{ijk} = random residual ($k = 1$ to 2)

RESULTS

The *in vivo* trial and the experiments with the nylon capsule method were performed on dairy

Table 1. Intake of nutrients, protein and energy units

Parameter	Amount	s.e.
Dry matter (kg/d)	18.27	0.44
Organic matter (kg/d)	17.11	0.27
Crude protein (kg/d)	2.40	0.04
NDF (kg/d)	6.59	0.067
Starch (kg/d)	5.32	0.05
Fat (kg/d)	0.68	0.009
PDIN ¹ (kg/d)	1.63	0.02
PDIE ¹ (kg/d)	1.67	0.03
NEL ¹ (MJ/d)	123	2.7

¹values calculated according to Sommer (1994)

cows producing on average 21.9 kg/d (s.e. 0.82) of milk, of the average body weight 649 kg (s.e. 19). Consumption of nutrients is presented in Table 1. Differences between the contents of nutrients in TMR fed in *in vivo* trials and samples of TMR weighed into the capsules are presented in Table 2. Contents of OM and CP were nearly identical and small differences were found in NDF and starch. The results of nylon capsule method (Table 3) showed that only a small number of capsules was rejected and subsequently regurgitated (5.3%) and that the recovery of capsules (94.2%) and Cr (94.9%) was high. TMRT of nylon capsules and of Cr-mordanted silage was different (39.6 vs. 51.7 h, $P < 0.01$).

A quantitative classification of faecal nutrients is presented in Table 4. It was found out that contents of OM, CP, NDF and starch in faeces of the *in vivo* trial and of the nylon capsule method (in the rest of sample in capsules after their passage through

the digestive tract) were different ($P < 0.01$) almost in all the cases (except OM in the composition of endogenous and microbial matter). For total faecal nutrients the largest difference was found in CP. In the *in vivo* trial, CP content in faeces was more than three times higher than in experiments with nylon capsules (120 vs. 36.9 g/kg, respectively).

Contents of faecal DM, OM and NDF of feed origin (residue after neutral-detergent washing) were higher ($P < 0.01$) in the nylon capsule method than those estimated in the *in vivo* trial. On the contrary, contents of CP (17.2 vs. 24.2 g/kg DM) and of starch (19.8 vs. 89.5 g/kg DM) were lower ($P < 0.01$) in the latter case. The values of faecal nutrients from feed for DM and OM were obtained by adding faecal starch content to the ND-residue and NDF, respectively, because residual starch is in particular of the feed origin. On the other hand, faecal fat is mostly of microbial origin (Sniffen et al., 1992) and that is why it was not added to the ND-residue and NDF.

Contents of endogenous and microbial DM and OM in faeces obtained by the nylon capsule method reached only one third of the values determined in the *in vivo* trial, for CP this ratio was even higher (19.7 vs. 95.8 g/kg DM, respectively).

As for the composition of endogenous and microbial matter a significant difference ($P < 0.01$) between both methods was found only in CP (Table 4).

TWL determined by the nylon capsule method (Table 5) ranged from 11.4% (NDF) to 76.8% (starch). WSL and SPL for DM and OM had similar values, for CP WSL was substantially higher (34.8%) than SPL (25.4%). On the contrary, in the

Table 2. Comparison of the content of nutrients in TMR (total mixed ration) fed in the *in vivo* trial and weighed into the capsules

Nutrient	Content of nutrients in TMR of cows (g/kg DM)	Content of nutrients in TMR sample weighed into nylon capsules (g/kg DM)
Organic matter	936	937
Crude protein	131	129
NDF	361	388
Starch	291	245

Table 3. Weight of sample in one capsule, regurgitation, recovery of capsules and Cr, TMRT (total mean retention time) of nylon capsules and of Cr-mordanted maize silage

	Value	s.e.
Sample/capsule (mg)	16.2	0.06
Regurgitation of capsules (%)	5.3	1.12
Recovery of capsules (%)	94.2	0.80
Recovery of Cr (%)	94.9	1.63
TMRT of nylon capsules (h)	39.6 ^a	1.75
TMRT of Cr-mordanted silage (h)	51.7 ^b	0.53

Values marked by various letters are significantly different ($P < 0.01$)

Table 4. Quantitative classification of faecal nutrients as proportions of faecal DM (g/kg DM) in the nylon capsule method and the *in vivo* trial, endogenous and microbial nutrients expressed as percentage of total nutrients and as proportion of dry matter intake (DMI)

Nutrient and method	Total ¹ faecal nutrient (g/kg DM of faeces)		Faecal nutrient of feed origin (g/kg DM of faeces)		Endogenous and microbial faecal nutrient (g/kg DM of faeces)		Composition of endogenous and microbial matter (g/kg endogenous and microbial DM)	
	value	s.e.	value	s.e.	value	s.e.	value	s.e.
Dry matter								
Nylon capsule	1 000	–	920 ^a	4.0	80 ^a	4.0	1 000	–
<i>In vivo</i> trial	1 000	–	673 ^b	5.0	327 ^b	5.0	1 000	–
Organic matter								
Nylon capsule	959 ^a	0.6	884 ^a	5.5	75 ^a	5.5	928	45.5
<i>In vivo</i> trial	907 ^b	0.8	644 ^b	6.4	263 ^b	5.9	803	9.5
Crude protein								
Nylon capsule	36.9 ^a	0.44	17.2 ^a	0.35	19.7 ^a	0.38	248 ^a	9.9
<i>In vivo</i> trial	120 ^b	2.3	24.2 ^b	0.48	95.8 ^b	2.06	293 ^b	4.5
Neutral detergent fibre								
Nylon capsule	–	–	864 ^a	5.4	– ²		–	
<i>In vivo</i> trial	–	–	555 ^b	7.5	–		–	
Starch								
Nylon capsule	–	–	19.8 ^b	1.46	– ²		–	
<i>In vivo</i> trial	–	–	89.5 ^a	3.58	–		–	

The values of one nutrient shown in the nylon capsule method or in the *in vivo* trial in one column marked by various letters differ ($P < 0.01$)

¹sum of nutrients of feed origin and of endogenous and microbial nutrients

²not determined

case of starch WSL was smaller (1.33%) than SPL (75.4%). A relatively low value of SPL was estimated for NDF (11.4%).

The values of apparent digestibility of nutrients are presented in Table 6. Washing correction mark-

edly changed the results obtained. Apparent digestibility values determined by means of the nylon capsule method before washing correction were higher ($P < 0.05$) than those obtained in the *in vivo* trial, the differences ranged from 9.3% (OM) to

Table 5. Total washing loss from bags (TWL), water-soluble loss measured as loss after washing on filter paper (WSL) and calculated solid particle loss (SPL = TWL – WSL) of nutrients. Each value is the mean of four replications

Nutrient	TWL		WSL		SPL (%)
	(%)	s.e.	(%)	s.e.	
Dry matter	45.2	0.17	22.7	0.09	22.5
Organic matter	43.8	0.34	20.6	0.12	23.2
Crude protein	60.2	0.05	34.8	0.85	25.4
NDF	11.4	0.36	0.0	0.00	11.4
Starch	76.8	0.45	1.33	0.03	75.4

Table 6. Apparent digestibility determined in the *in vivo* trial or by the nylon capsule method with or without washing correction (%)

Nutrient	Nylon capsule method, values before washing correction		Nylon capsule method, values after washing correction		<i>In vivo</i> trial		Diff. A–C	Diff. B–C
	A	s.e.	B	s.e.	C	s.e.		
Dry matter	77.3	0.58	68.0	0.82	66.5	1.01	10.8*	1.5
Organic matter	76.8	0.60	67.2	0.85	67.5	0.97	9.3*	–0.3
Crude protein	93.5	0.19	89.4	0.31	69.4	0.95	24.1*	20.0*

*the difference was significant ($P < 0.05$)

Table 7. True digestibility determined in the *in vivo* trial or by the nylon capsule method with or without washing correction (%)

Nutrient	Nylon capsule method, before washing correction		Nylon capsule method, after washing correction		<i>In vivo</i> trial		Diff. A–C	Diff. B–C
	A	s.e.	B	s.e.	C	s.e.		
Dry matter	79.1	0.56	70.5	0.79	77.4	0.63	1.7	–6.9*
Organic matter	78.6	0.58	69.7	0.82	77.0	0.64	1.6	–7.3*
Crude protein	97.0	0.11	95.0	0.18	93.8	0.24	3.2*	1.2
NDF	49.4	1.37	42.9	1.55	48.5	1.55	0.9	–5.6
Starch	98.2	0.14	92.2	0.60	89.7	0.45	8.5*	2.5

*the difference was significant ($P < 0.05$)

24.1% (CP). After washing correction, the values of apparent digestibility for DM and OM were similar, the differences were insignificant (1.5 and –0.3%, respectively). The difference in CP remained to be high (20.0%), the washing correction had a small effect in this case.

The values of true digestibility (except starch) before washing loss correction showed an apparent agreement between both methods (Table 7). The differences in true digestibility of DM, OM and NDF were insignificant (from 0.9 to 1.7%; ($P > 0.05$)), higher differences ($P < 0.05$) were found for CP (3.2%) and starch (8.5%). The values of true digestibility determined by the nylon capsule method decreased after washing loss correction, differences between both methods were significant for DM and OM ($P < 0.05$), insignificant for NDF, starch and CP ($P > 0.05$).

DISCUSSION

TMRT of the nylon capsules and Cr-mordanted silage (39.6 and 51.7 h, respectively) was in agree-

ment with our previous results (Třináctý et al., 1999) as well as with results estimated in lactating cows and reported in the literature. Mambrini and Peyraud (1997) determined 51.7, 45.6 and 40.6 h with mordanted hay, ground hay and concentrate, respectively. Udén (1984) reported a range from 43 to 67 h (determined with mordanted hay in dairy cows). TMRT of nylon capsules is mainly influenced by the weight of load (steel balls) regardless of the kind of feed inside the capsules (Třináctý et al., 2002). Large particles of high specific gravity (similarly to our nylon capsules) are retained in the rumen for a shorter period of time and in the intestines for a longer period of time than the particles of digesta (Ehle and Stern, 1986; Kaske and Engelhardt, 1990). On the basis of these findings we assume that the difference (12.1 h) between TMRTs of nylon capsules and Cr-mordanted silage can be explained mainly by a shorter stay of nylon capsules in the rumen.

In the literature, excretion of endogenous and microbial nutrients is often expressed as grams per kg of dry matter intake (DMI). Van Soest (1966)

reported that in cattle faecal endogenous and microbial excretion of DM ranged from 130 to 180 g per kg DMI. In our *in vivo* trial a slightly lower value was determined (109.7 g/kg DMI). This difference probably originated from the higher DMI (dairy cows) in this experiment. With the nylon capsule method this value was only 18.2 g/kg DMI (in the case of the nylon capsule method, DMI was calculated from the amount of feed DM weighed into the capsules and therefore it has not any biological basis). The excretion of endogenous and microbial OM determined in our *in vivo* experiment (88.2 g/kg DMI) was similar to data reported by Udén (1984), who found 90–130 g/kg DMI.

Mulligan et al. (2001) determined the values of endogenous and microbial N content in faeces of sheep, ranging from 11.60 to 12.78 g/kg DM (72.5–79.9 g CP/kg DM). These values were lower than the values we obtained in dairy cows (95.8 g/kg DM). The percentage of endogenous and microbial CP in total CP content in faeces was 79% according to Mason (1979). Mason and Frederiksen (1979) reported 84.8%, our result was similar (79.8%). Using g N per kg DMI Mulligan et al. (2001) reported a range of 4.72–5.99 g N/kg DMI (29.5–37.4 g CP/kg DMI), Mason and Frederiksen (1979) determined the value of 6.0 g N/kg DMI (37.5 g CP/kg DMI). Both the values were determined in sheep and were comparable with those estimated in our *in vivo* trial, i.e. 31.9 g CP/kg DMI.

According to Russell et al. (1992) bacteria consist of 956 g OM per 1 kg of DM, in our study the nylon capsule method and the *in vivo* method provided the values of 928 g and 803 g, respectively ($P > 0.01$). A higher content of OM observed when using the nylon capsule method complies with results reported by Martin et al. (1994). These researchers found a higher content of OM in bacteria attached to digesta particles than in bacteria contained in the liquid phase (783 vs. 603 g/kg DM). We assume there were higher counts of bacteria attached to particles of the residue in the nylon capsule method, and on the contrary, in faeces there were higher counts of bacteria in the liquid phase.

CP content in microbial and endogenous matter determined in the present study in the residue by the nylon capsule method and in faeces by the *in vivo* method (248 vs. 293 g/kg DM, respectively) was similar to values calculated from data reported by Russell et al. (1992) and it ranged from 188 to 313 g/kg DM.

Varvikko and Vanhatalo (1990) reported that washing losses could increase digestibility values

determined by the mobile bag method and we indicated that the results of nylon capsule method could also be influenced by this factor. For barley whole crop Jarosz et al. (1994) reported total washing loss of DM amounting to the value of 31.1%, including 29.8% of WSL and 1.3% of SPL. A similar composition of washing losses was described by López et al. (1995) with grass silage. In our study the SPL value was substantially higher (22.5%) and we deduced that this fact could be caused by high SPL of starch (75.4%). Philippeau and Michalet-Doreau (1998) mentioned SPL values of starch from ensiled maize grains (3-mm grinding) ranging from 35 to 63% according to the genotype.

When the values of apparent digestibility determined by both methods used in this study were compared, it was obvious that a large difference in the values of digestibility was found mainly for CP (24.1%, $P < 0.05$). After washing loss correction the difference decreased but remained significant (20.0%, $P < 0.05$). This finding corresponds with the results of Varvikko and Vanhatalo (1993), who concluded that a high content of endogenous and microbial CP in faeces led to a considerable underestimation of CP digestibility determined *in vivo* in comparison with the mobile bag method.

Table 7 (column A–C) shows that almost all the values of true digestibility before washing loss correction (except CP and starch) determined by the nylon capsule method were in agreement with values obtained in the *in vivo* trial. After washing loss correction (column B–C) insignificant differences were obtained in CP and starch. Despite the washing loss correction, the difference in NDF digestibility values between nylon capsule method and *in vivo* method remained insignificant, probably due to the higher standard errors (variability between animals). On the other hand, significant differences ($P < 0.05$) were obtained in DM and OM.

Digestibility of starch in cows can be influenced by a type of diet, method of feed processing and rate of passage. Poore et al. (1993) found values ranging from 83 to 98% depending on the method of feed processing. We found similar values: the values 92.2% and 89.7% were determined by the nylon capsule method and in the *in vivo* trial, respectively. Philippeau and Michalet-Doreau (1998) described a considerable influence of particle size on starch digestibility, determined by an *in situ* method. For the nylon capsule method feed was ground through a 2-mm sieve. However we found a substantial number of whole maize grains in faeces.

For this reason the values of starch digestibility determined by both methods are not comparable and their agreement is only apparent. The washing loss correction seems to be overestimated. Starch represents a substantial part of washing loss in the case of DM and OM. We assume that the values of DM and OM true digestibility were influenced (underestimated) by this factor.

CONCLUSION

The comparison of the apparent digestibility of nutrients in total mixed ration (TMR) determined either by the nylon capsule method or in the *in vivo* trial showed that digestibility was significantly higher with the nylon capsule method for CP, insignificantly for DM and OM. A large difference for CP confirmed that the nylon capsule (*in situ*) method in respect of a lower level of microbial contamination provides values of true digestibility while the conventional *in vivo* method gives values of apparent digestibility.

The comparison of the true digestibility of nutrients showed that digestibility was significantly lower with the nylon capsule method for DM and OM, insignificantly for CP, NDF and starch.

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