Use of the mobile nylon bag technique to determine digestible energy in pig diets

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The experiment was conducted to compare the mobile nylon bag technique (MNBT) with conventional digestibility techniques for the estimation of digestible energy (DE) in pig diets. Factors investigated were the effect of washing after retrieval, predigestion time, particle size, retention time of the bags, and the effect of the basal diet. Retention time and predigestion time had no effect on DE. DE values obtained with unwashed bags (13,57 MJ/kg) were highly significantly ($P \leqslant 0{,}01$) lower than either DE values obtained with washed bags (15,01 MJ/kg) or values obtained with the conventional technique (14,87 MJ/kg). Although particle size appeared to play an important role, DE values obtained with washed bags, for the different particle sizes, differed non-significantly from the reference DE value (14,87 MJ/kg). DE values from material with the 3-mm particle size (14,52 MJ/kg) were significantly ($P \leqslant 0{,}01$) lower and DE values, with an 800-μm particle size (15,49 MJ/kg), significantly ($P \leqslant 0{,}05$) higher, than the DE value obtained with the 1-mm particles (15,02 MJ/kg). The 1-mm particles correlated best with the reference DE value and differed from it by only 1,0%. Washed bags, with or without predigestion, which were milled through a 1-mm sieve size correlated best with the reference DE value. According to an analysis of correlation between DE versus particle size using the MNBT method, a mean diameter of 1,16 mm should give the same predicted DE value as that obtained by the conventional technique. The basal diet appeared to influence the DE value obtained with the MNBT, in that a high crude fibre level (8%) in the diet was associated with a lower DE value (14,53 MJ/kg) than that (15,17 MJ/kg) obtained with a low fibre (2%) diet, although these differences were not significant. With high tannin feed samples in the bags, the DE value obtained with a low-tannin basal diet (14,20 MJ/kg) appeared to be higher than the DE value obtained with a high-tannin basal diet (13,81 MJ/kg).

Die studie is uitgevoer om die mobiele-kunsveselsakkietegniek (MNBT) met konvensionele verteringstegnieke, vir die bepaling van die verteerbare-energie(VE)-inhoud van varkvoere, te vergelyk. Ondersoek is ingestel na die invloed van was/ongewas, verteringstyd, partikelgrootte, retensietyd, en die basisdieet op die VE-waarde. Retensietyd en die lengte van die tyd van voorafvertering het geen betekenisvolle invloed op VE gehad nie. Die VE-inhoud van ongewaste sakkies (13,57 MJ/kg) was hoogsbetekenisvol ($P \leqslant 0{,}01$) laer as die VE-inhoud van gewaste sakkies (15,01 MJ/kg) en ook laer as die VE-inhoud van die monster soos bepaal met konvensionele studies (14,87 MJ/kg). Partikelgrootte het 'n belangrike rol gespeel en hoewel die VE-waarde vir gewaste sakkies vir die verskillende partikelgroottes nie betekenisvol van die verwysings-VE-waarde (14,87 MJ/kg) verskil het nie, was die VE-waarde van die 3mm-monsters (14,52 MJ/kg) hoogsbetekenisvol ($P \leqslant 0{,}01$) laer as by 1 mm (15,02 MJ/kg), en 800 μm (15,49 MJ/kg) betekenisvol ($P \leqslant 0{,}05$) hoër as by 1 mm. Die 1mm-partikel het die beste ooreenkoms met die verwysings-VE-waarde getoon en het met slegs 1,0% verskil. Gewaste sakkies, met voermonsters gemaal deur 'n 1mm-sifgrootte het, ongeag voorafvertering, dus die beste korrelasie met die verwysings-VE-waarde getoon. Volgens 'n grafiek van VE teenoor die partikelgrootte van die monsters in die sakkies, sal 'n partikelgrootte van 1,16 mm die presiese VE-waarde, soos bepaal in die konvensionele studie, weergee. Die samestelling van die basisdieet het die MNBT VE-waardes beïnvloed. Met 'n ruveselinhoud van 8% in die basisdieet is 'n VE-waarde van 14,53 MJ/kg verkry, terwyl 'n VE-waarde van 15,17 MJ/kg met 'n lae-ruvesel (2%) basisdieet verkry is. Met hoë-tannien voermonsters in die sakkies, was die VE-waardes onderskeidelik 14,20 en 13,81 MJ/kg vir 'n lae- en hoë-tannien basisdieet.

Keywords: Cannula, digestible energy, mobile nylon bag technique, pigs, retention time

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Introduction

Determination of the digestible energy (DE) content of pig diets by conventional methods requires a large quantity of feed, many animals and is time consuming and expensive. Sauer, Jorgensen & Berzinz (1983) developed a mobile nylon bag technique (MNBT) to expedite the measurement of DE content in small samples of feedstuffs. Several authors, however, found that the MNBT compared to conventional methods, underestimates dry matter (DM) and energy digestibility (Petry & Handlos, 1978; Taverner & Campbell, 1985; De Lange, Sauer, Den Hartog & Huisman, 1987; Sauer, 1986). Factors such as sample size, degree of fineness of grind (Cherian, 1985; De Lange *et al.*, 1987), basal diet (Taverner & Campbell, 1985) and the possibility of faecal contamination of residues in the bags (Graham, Amon, Newman & Newman, 1985), were also found to influence the results. However, significant correlations have been found between energy digestibilities obtained by MNBT and conventional methods. Digestibility values were found to be of the same order of magnitude, despite the application of these different methods (Taverner & Campbell, 1985; De Lange *et al.*, 1987). This experiment was therefore conducted to determine the effect of predigestion, retention time, particle size, washing, and composition of the basal diet on the DE values obtained by the MNBT. The MNBT was also calibrated against the conventional total collection of faeces technique.

Experimental Procedures

Digestion trials

Two digestion trials to determine the DE content of grain sorghum (PNR 8469), milled through a 1-mm and a 3-mm sieve, respectively, were carried out. In the first trial, five boars of Large White origin having a mean livemass of 40 kg were used as experimental animals. A daily amount of 1500 g of air-dry meal (100% grain sorghum plus a commercial mineral and vitamin mixture) was fed to each pig in two equal portions at 08h00 and 13h00. The procedures followed with the total collection of faeces were described in detail by Kemm & Ras (1971). In the second trial seven boars of Large White origin (*ca.* 40 kg) were used. During this trial, maize was substituted at a rate of 75% sorghum in a maize meal control diet (Table 1). Pigs were fed the different diets at a rate of 1500 g of air-dry meal in two equal portions at 08h00 and 13h00 per day. The analysis and procedures were the same as described by Siebrits & Ras (1981). The diets fed were analysed for DM by the standard AOAC method (AOAC, 1984) and energies of the feed and faeces were determined using a adiabatic bomb calorimeter.

Table 1 Maize-meal based control and substituted diets*

Ingredient	Maize meal control diet	Substituted diet
Maize meal	100%	25%
Sorghum	–	75%
Minerals and vitamins	Added	Added

* On an air-dry basis.

Rate of passage study

Duodenal fistulae, fitted with flexible cannulas, were created in six 40 kg boars (Large White) as described by Sauer *et al.* (1983). Following surgery, the pigs were allowed a 14-day recuperation period. During this time they had free access to a 18% protein diet. Thereafter, they were adapted to a 15% protein sorghum diet (see Table 2), at a rate of two times the maintenance requirement supplied twice daily, at 8h00 and 13h00, for seven days. Thereafter, 100 g of feed, marked with ^{51}Cr-mordant (Udén, Colucci & Van Soest, 1980) was placed in the duodenum of each pig. Faeces were collected consecutively every 4 h for seven successive days. Radio-activity in excreta was determined using a Gamma counter and expressed in cpm ^{51}Cr per g dry matter. The mean retention time ($R\bar{t}$) of marked particles was calculated from the total excretion curve from the time the marker first appeared in the faeces using the following equation (Pienaar & Roux, 1984):

$$R\bar{t} = \frac{1}{N} \Sigma \{ n [(t^1 + t) \div 2] \}$$

where n = ^{51}Cr counts excreted between times t and t^1, representing the sum of these counts for successive intervals until n = 0. N represents the total number of marker counts excreted. The mean retention time of mobile nylon bags was determined using the same procedure, where n and N represent the corresponding number of bags.

Table 2 15% Protein sorghum diet*

Ingredient	
Sorghum**	64,4%
Wheaten bran	22,0%
Fish-meal	9,3%
Lucerne meal	2,3%
Fine salt	1,0%
Feed lime	0,83%
Minerals and vitamins	0,2%
Synthetic lysine	0,08
Composition (calculated)	
Protein	15%
Lysine	0,8%
Methionine and cystine	0,5%
Triptophan	0,2%
DE	13 MJ/kg
Crude fibre	4,5%
Fat	3,7%
Ca	0,8%
P	0,7%

* On an air-dry basis.

** High-tannin sorghum (1,24% tannin) was used in one of the basal diet studies.

MNBT study

Calibration of the MNBT with total collection of faeces

A factorial design (2 × 3 × 3) with 15 bags per treatment was used to determine the effect of washing, time of predigestion, and particle size of the feed samples on the DE values as determined by the MNBT. Two hundred and seventy polyester bags (25 mm × 40 mm) with a 53-μm mesh (manufactured by Swiss Silk, CH 9425, Thal SG, Switzerland, and supplied by Rhologan Engineering, P.O. Box 84158, Greenside, Johannesburg), were machine-sewn using polyester thread. Grain sorghum (PNR 8469) samples (1 g), milled through a Wiley mill with sieve sizes of 800 μm, 1 mm, and 3 mm, were placed into the bags, sewn-closed and sealed with a contact adhesive. Prior to insertion, the bags with the samples were predigested in a water-bath at 37°C (agitated at 90 oscillations / min) with 0,01N HCl and 1 g l^{-1} pepsin (2500 I.U/g) for either 0, 2,5, or 4 h, and stored in a deep-freeze at 0°C. Five bags were randomly inserted through the duodenal cannula of each of three pigs on a daily basis, while the pigs were eating (these pigs were subjected to the same treatments as those in the rate of passage study). An interval of 5 min was allowed between bags. The bags were collected in the faeces at 4-h intervals. Half of the retrieved bags were immediately washed in cold water to remove contaminating faecal material while the other half remained unwashed. Thereafter, all bags were immediately frozen and freeze-dried. Following this, the bags were opened and the DM and gross energy (GE) content (using an adiabatic bomb calorimeter) of the residue in each bag was determined.

Effect of fibre and tannin in the basal diet on DE values
Two diets with 2% and 8% crude fibre content respectively (Table 3) were used to determine the effect of fibre in the basal diet on the MNBT DE value. In two further treatments, pigs were fed a high and a low tannin diet (see Table 2) while a high tannin sorghum sample was placed in the bags. The procedure followed in this study, which yielded the most accurate results for washing the bags after retrieval, in which the test material was milled through a 1-mm sieve, but with no pretreatment applied and which gave the best correlation when compared to the conventional collection of faeces, was used. Twenty-one bags per diet were used. The residues of every three bags used with a specific basal diet were pooled prior to analysis. Three cannulated boars (*ca.* 40 kg) were adapted for seven days on the different basal diets prior to the insertion of the bags. (The same pigs were used in succession for the four treatments.) Further experimental procedures followed were as described previously in this study.

Table 3 High and low crude fibre grain sorghum diets*

Ingredient	High crude fibre diet	Low crude fibre diet
Sorghum	53,6%	83,07%
Wheaten bran	22,0%	1,09%
Lucern meal	14,7%	-
Fish-meal	7,7%	12,0%
Fine salt	1,0%	1,0%
Feed lime	0,5%	1,2%
Minerals and vitamins	0,2%	0,2%
Synthetic lysine	0,8%	0,01%
Composition (calculated)		
Protein	15,0%	15,0%
Lysine	0,8%	0,8%
Methionine and cystine	0,4%	0,4%
Triptophan	0,15%	0,15%
DE	12 MJ/kg	12 MJ/kg
Crude fibre	8,0%	2,0%
Fat	1,0%	1,0%
Ca	0,8%	0,8%
P	0,6%	0,6%

* On an air-dry basis.

Results and Discussion

Digestion trials

Digestion data for the 3-mm and 1-mm sorghum diets fed during the digestion trials are summarized in Table 4. The DE value for the 3-mm sorghum was calculated according to the substitution method described by Siebrits & Ras (1981). DE content determined with 3-mm sorghum (14,87 MJ/kg) was significantly lower ($P \leq 0,01$) than the DE content of the 1-mm sorghum (16,76 MJ/kg). Data of Owsley, Knabe & Tanksley (1981) also indicated that reducing sorghum particle size caused higher digestibility values.

Rate of passage

The excretion curves of the bags and the pooled ^{51}Cr-mordant particles are presented in Figures 1 and 2. It is clear from the excretion curves that the bags and ^{51}Cr-mordanted particles followed similar patterns. The bags were excreted over a wider time span than the ^{51}Cr-mordanted particles, although differences in the mean retention times were non-significant. The mean retention times for ^{51}Cr-mordanted particles and the polyester bags were 47,0 ± 6,9 and 48,4 ± 20,9 h, respectively, which was in agreement with the results of Sauer *et al.* (1983) (38—48 h) and Graham *et al.* (1985) (23—69 h). It was found that retention time of these bags had no significant effect on the DE values. Graham *et al.* (1985) also found that retention time did not influence the extent of degradation in the bags whereas Hvelplund (1985) observed that bag passage time, in experiments with ruminants, did not correlate with the disappearance of nitrogen from the bags.

Table 4 Means ± *SD* for energy digestion of the maize meal control, 75% sorghum-substituted, and 100% sorghum (1 mm) diets

	Diet			
Measurement***	Control (Maize)	75% Sorghum (3 mm)	100% Sorghum (1 mm)	Sorghum* (3 mm)
DM intake, g/day	1308	1254	1310	–
DM digestibility, %	98,0 ± 0,1	84,8 ± 0,1	89,67 ± 1,31	–
Energy digestibility, %	97,9 ± 0,2	83,0 ± 0,8 **	89,63 ± 1,82 **	–
DE content, MJ/kg DM	18,56 ± 0,03	15,79 ± 0,14	16,76 ± 0,30	14,87

* DE value calculated according to the substitution method (Siebrits & Ras, 1981).
** DE values differ significantly ($P \leq 0,01$).
*** On a DM basis.

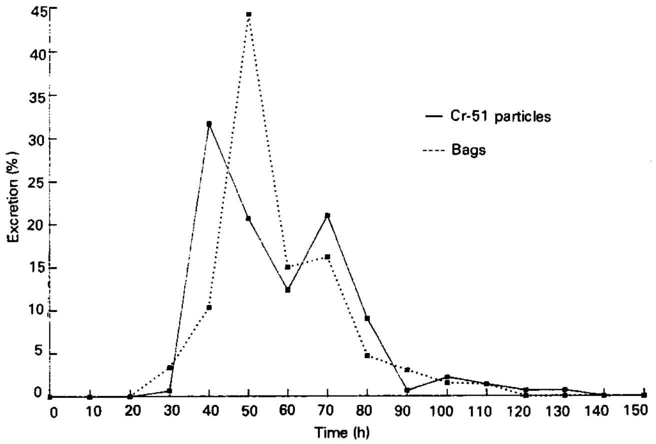

Figure 1 Excretion curves of ^{51}Cr-mordanted particles and polyester bags.

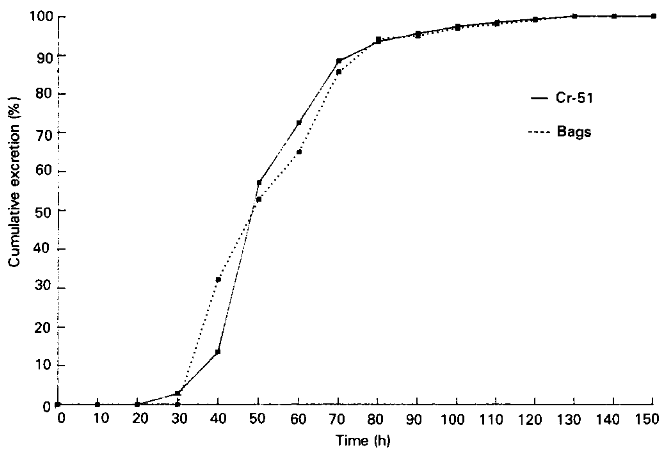

Figure 2 Cumulative excretion curves for Cr-51 mordanted particles and polyester bags.

MNBT study

Calibration of the MNBT

DE values of grain sorghum (PNR 8469), determined with the MNBT, are presented in Table 5. The DE values of the different treatments were compared with the DE value of sorghum milled through a 3-mm sieve size, since sorghum, when milled for pig diet inclusion, is conventionally milled through a 3-mm sieve.

In all treatments predigestion (2,5 or 4 h) did not significantly affect the DE value, therefore values were pooled. This is in accordance with reports from Graham *et al.* (1985) and Rooke (1985) (with cattle), who found that pepsin pretreatment did not influence intestinal digestion in the bags. Cherian, Sauer & Ozimek (1985), however, found that without pretreatment the digestion of protein was underestimated. It could, however, have been predicted that predigestion would not influence digestibility, when the large capacity of the caecum and colon is considered (Graham *et al.*, 1985), as well as the time the bag spends in the large intestine. The retention time for ^{51}Cr-mordanted particles was *ca.* 20,7 h up to the ileum and *ca.* 65,4 h over the whole digestive tract (Brand, Badenhorst, Siebrits & Hayes, 1989). The bags, therefore, were exposed for a considerable time to microbial degradation in the large intestine. Just, Jorgensen & Fernandez (1981) found that crude protein, a complete balanced diet, maize starch and potato starch, when infused into the caecum and colon of the pig, were digested nearly to the same extent as over the total digestive tract. Their work therefore confirmed the above findings.

Table 5 Effect of washing on the mean DE values assessed by the MNBT obtained with feed samples with different particle sizes

Particle size	Washed*	Unwashed*
800 μm	15,49^{c} ± 0,14	13,91^{c} ± 0,15
1 mm	15,02^{b} ± 0,17	13,77^{c} ± 0,18
3 mm	14,52^{a} ± 0,20	12,95^{a} ± 0,20
Mean ± *SD*	15,01 ± 0,11	13,57 ± 0,11

Statistical differences for different particle sizes in different columns:
[a] Differ significantly ($P \leq 0,05$) from [b].
[a] Differ highly significantly ($P \leq 0,01$) from [c].
* Values in different rows differ highly significantly ($P \leq 0,01$).

Washing had a highly significant ($P \leq 0,01$) effect on DE content. The mean DE values of the washed and unwashed bags were 15,01 ± 0,11 and 13,57 ± 0,11 MJ/kg DM, respectively. The DE values found with the unwashed bags (13,57 MJ/kg) were significantly ($P \leq 0,01$) lower than the DE values determined by means of the conventional study (3-mm sorghum) (14,87 MJ/kg). The major cause of this difference probably lies in contamination of the residues in the bags with faecal material, as also suggested by Graham *et al.* (1985). Mehrez & Ørskov (1977) observed that washing, with the *in sacco* technique for the determination of digestibility values in the rumen, contributed to a 1,2% difference in DM digestibility. The above data indicate that the influx of digesta into the bags resulted in an underestimation of the DE value.

Data on the effect of particle size are presented in Table 5. In all the different trials namely, washed and unwashed bags, DE values determined with 3-mm particles (respectively 14,52 and 12,95 MJ/kg) were highly significantly ($P \leq 0,01$) lower than DE values obtained with 800-μm particles (respectively 15,49 and 13,91 MJ/kg). In the case of the unwashed bags, the DE values calculated with the 3-mm particles (12,95 MJ/kg) differed significantly ($P \leq 0,01$) from those values obtained with 1-mm particles (13,77 MJ/kg). In the case of the washed bags, the DE values for 1-mm (15,02 MJ/kg) and 3-mm particles (14,52 MJ/kg) were significantly different ($P \leq 0,05$). Changing from 800-μm to 1-mm particles caused small but non-significant effects (15,49 vs. 15,02 and 13,91 vs. 13,77 MJ/kg for the washed and unwashed bags, respectively), although there was a tendency for coarser particles to have a lower DE value. Correlation coefficients for particle size versus DE values were respectively –0,91 and –0,99 for washed and unwashed bags. These findings agreed with results from Erwin & Ellison (1959) and Mohamed & Smith (1977) with *in sacco* studies on ruminants. However, these results differ from those of Van Keuren & Heineman (1962) and Lowrey (1970) who also reported with *in sacco* studies that mesh size of the mill sieve had little effect on the digestion of the sample incubated in the bag. Our results, however, suggested that too small a mesh size of the mill sieve could lead to excessive losses of particles from the bag and an overestimation of digestibility, while too big a particle would be degraded more slowly, which could lead to an underestimation of digestibility. Thus there must be a compromise between particle size and bag aperture size. Determined in accordance with the logarithmic normal distribution for milled food (Waldo, Smith, Cox, Weinland & Lucas, 1971), there is little if any loss of sorghum particles, milled with a 1-mm sieve, through a 53-μm mesh size. DE values determined with unwashed bags represented all the particle sizes (13,91, 13,77, and 12,95 MJ/kg for the 800-μm, 1-mm, and 3-mm particle sizes respectively) were highly significantly ($P \leq 0,01$) lower than the DE value determined by the total collection of faeces technique (14,87 MJ/kg). In the case of the washed bags, the differences between the DE values determined with different particle sizes (respectively 15,49, 15,02, and 14,52 MJ/kg for the 800-μm, 1-mm, and 3-mm particle sizes) and the total collection method of faeces were non-significant. DE values for 800-μm and 1-mm particles were 4,2 and 1,0% higher, respectively, while the DE values for the 3-mm particles were 2,4% lower than the reference DE value. In this study the washed bags, with feed samples milled through a 1-mm screen, gave the best agreement with the reference DE value. Figure 3 graphically illustrates MNBT DE values obtained with the washed bags versus different particle sizes and with DE values obtained with the conventional method at 1- and 3-mm particle sizes. From Figure 3, it is clear that sorghum grain milled through a 1,16-mm mesh would give a DE value nearest to that obtained with the conventional total collection method, when determined on a conventional sample milled through a 3-mm sieve.

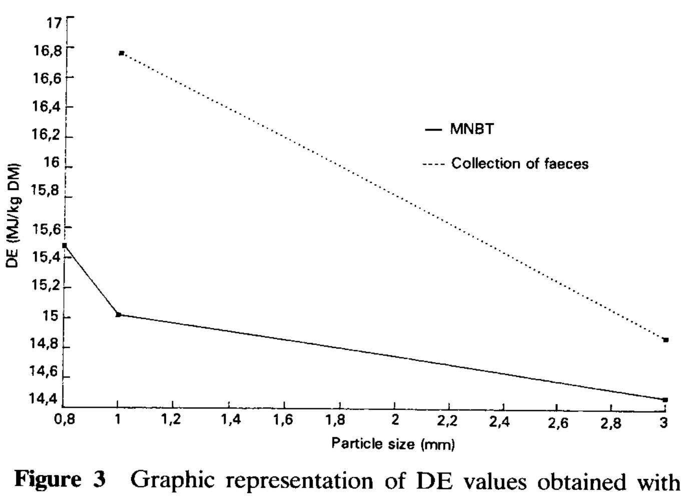

Figure 3 Graphic representation of DE values obtained with the MNBT and total collection of faeces technique for different particle sizes.

Effect of the basal diet

Table 6 presents the DE values obtained with different basal diets. The DE values were not significantly influenced by the basal diets. Nevertheless, data given in Table 6 suggest that the high-fibre diet tended to depress the DE value obtained with the MNBT while low-fibre diet had the opposite effect. DE values of 15,17 and 14,53 MJ/kg DM were obtained with crude fibre levels of 2 and 8% respectively in the basal diets. When the values obtained with the calibration study were included in the data (4,5% crude fibre level in the diet and a DE value of 15,02 MJ/kg DM), the crude fibre content of the basal diet appeared to correlate well ($r^2 = 0,98$) with MNBT DE values despite the lack of statistically significant differences between the determined DE values. This agreed with results from Taverner & Campbell (1985) who found that the digestibility of samples was lower when determined in the company of a diet that was higher in fibre and thus itself less digestible. The DE value obtained for a high-tannin sorghum sample when it was accompanied by a high-tannin basal diet (13,81 MJ/kg DM) was lower than the value obtained when it was accompanied by a low-tannin basal diet (14,20 MJ/kg).

Table 6 DE values obtained with the MNBT when different basal diets were used

Feed sample	Basal diet	DE(MJ/kg DM)
PNR 8469	Low fibre	15,17 ± 0,66
PNR 8469	High fibre	14,53 ± 0,85
High tannin	High tannin	13,81 ± 0,50
High tannin	Low tannin	14,20 ± 0,65

Conclusion

The polyester bags used with the MNBT had about the same mean retention time as ^{51}Cr-mordanted particles and the DE values obtained were not affected by retention time. It is clear from this study that no predigestion is necessary and that the best results would be obtained with test samples milled through a 1,16-mm sieve size and washed after reclamation. The basal diet may affect DE values and must therefore be carefully standardized. The MNBT lends itself excellently for the rapid evaluation of grain, especially where many samples have to be evaluated. It can be used with unpalatable diets as well as high fibre components which cannot be included at high levels of diets. However, it may be necessary to evaluate the MNBT for different types of grain, because the individual characteristics of different grains could affect DE values. That also concerns different types of diets. For evaluating protein digestibility and amino acid digestibility it is possible that another standard treatment is necessary.

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