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Evaluation of reliability of solubility in potassium hydroxide solution as an in vitro method for predicting organic matter digestibility of ruminant feeds

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ABSTRACT

The applicability of the alkaline extraction method using a 5% potassium hydroxide (KOH) solution to predict organic matter digestibility (DOM) of ruminant feeds was examined. The predictive value of the assay was estimated using 110 feeds including dried grass (70), grass silages (33) and compounds (7) of known DOM determined on sheep (DOM_v).

A fairly close linear relationships for all samples and forages between the *in vitro* organic matter digestibility (DOM_k, X, %) and DOM_v (Y, %) estimates were found. The regression equation for all samples (n = 110) was $Y = 0.934\ X + 5.74$, $R^2 = 0.75$ ($P < 0.001$), residual standard deviation RSD 3.65, for all forages (n = 103) $Y = 0.903\ X + 7.67$, $R^2 = 0.67$ ($P < 0.001$), RSD 3.62. The regression of DOM_v on DOM_k for compounds (n = 7) was insignificant ($R^2 = 0.30$, RSD 3.74).

The relationship between DOM_v (determined values) and DOM_{vk} (predicted from the equation for all samples) was linear. The predicted values could account for 75% of the variance in determined DOM_v; RSD = 2.52. The residuals ($DOM_v - DOMv_k$) were correlated with DOM_v ($r = 0.51$, $P < 0.001$). DOM_{vk} tended to be overestimated for samples of DOM ranging 50-60% (negative residuals only) and underestimated for that of DOM exceeding 80% (positive residuals only). The most even distribution of the residuals was in the DOM range of 70-80%.

KEY WORDS: feeds, organic matter, *in vitro* digestibility, alkaline extraction, potassium hydroxide

INTRODUCTION

The content of digestible nutrients, particularly organic matter digestibility (DOM) is one of the main determinants of the metabolizable energy (ME) value of ruminant feeds. The DOM may be accurately determined *in vivo* on sheep in balance trials, providing the reliability of animals has been tested beforehand (Daccord and Schneeberger, 1986). However, such trials are costly, considering the maintenance and careful selection of animals as well as feed, labor and time consumption. Therefore, for routine feed examination, particularly in comparing the quality of varieties and maturity stages of plants, or different batches of concentrate ingredients etc. various *in vitro* methods have been developed. Also several models have been designed to calculate DOM and ME of feeds from their chemical composition (Weiss et al., 1992).

Most *in vitro* methods simulate, to some extent, gut digestion, because feeds are incubated with buffered rumen fluid or/and different enzyme systems (Tilley and Terry, 1963; Kirchgessner and Kellner, 1978; McLeod and Minson, 1982). A very simple method, based on solubilization of feed while boiled with a KOH solution was proposed by Kesting (1978). The method proved more accurate than incubation with cellulase for the prediction of DOM of dried grass and red clover in the work of Kosmala and Brzóska (1982). However, Vencl and Flam (1981) found that laboratory estimates of *in vivo* DOM were better using a two-stage method of Tilley and Terry (1963) than extraction with 5% KOH according to Kesting (1978).

Considering the simplicity and potential applicability of the method of Kesting (1978) for routine screening of DOM of ruminant feeds, further evaluation of this method seemed advisable. This paper shows the results of examining the accuracy of predicting DOM of hays, grass silages and compound feeds of known DOM determined *in vivo* on sheep.

MATERIAL AND METHODS

Feeds

Feed samples of known DOM determined *in vivo* (DOM_v) on sheep were obtained from three laboratories in the Institute of Animal Production in Melle-Gontrode (Belgium), and the Research Institute of Animal Production in Poland. The type and number of feeds and their protein and fibre contents are given in Table 1.

TABLE 1

Description of samples used in evaluation of KOH extraction procedure

		Crude protein		Crude fibre (g/kg DM)		NDF	
Sample	n	Min	Max	Min	Max	Min	Max
All samples	110	74	233				
Dried grass	70	74	198	206	425		
Grass silages	33	90	208	193	393		
Compounds	7	162	233			155	301

In vitro analysis

Extraction of feeds with potassium hydroxide solution (5%) according to the Kesting (1978) procedure was conducted using a Fibretec M6 apparatus (Tecator, Sweden). The residues were ashed at 600°C.

Statistical analysis

The values of OM digestibility of samples used for validation of the chemical method were evaluated as to their distribution around the centroid (a mean point in the multidimensional space) using Mahalonobis distances (H), in order to define outliers in the population (Shenk and Westerhaus, 1991).

The results of DOM_v and that obtained by the chemical method (DOM_k) were subjected to analysis of variance and the relationship between the *in vivo* and *in vitro* values was evaluated using linear regression and correlation coefficients. The regression equations of DOMv in relation to DOMk were calculated for all samples and separately for specific groups, e.g. all forages, dried grass, grass silages and compounds. The predictive ability of the regression equations was characterized by standard errors of b (SE) and residual standard deviations (RSD) which were calculated as a measure of variation in DOM_v that could not be accounted for by the differences in DOM_k. The values of DOM *in vivo* predicted based on in vitro observations and the regression equation (DOM_{vk}) were compared to DOM_v values using linear regression. The distribution of residuals i.e. the determined minus predicted values, in relation to the determined DOM_v was analysed. All calculations and analyses were done using Microsoft Statistica® software.

RESULTS

The distribution of Mahalanobis distances (H) in comparison with the expected normal distribution is shown in Figure 1. Most of the samples fit the

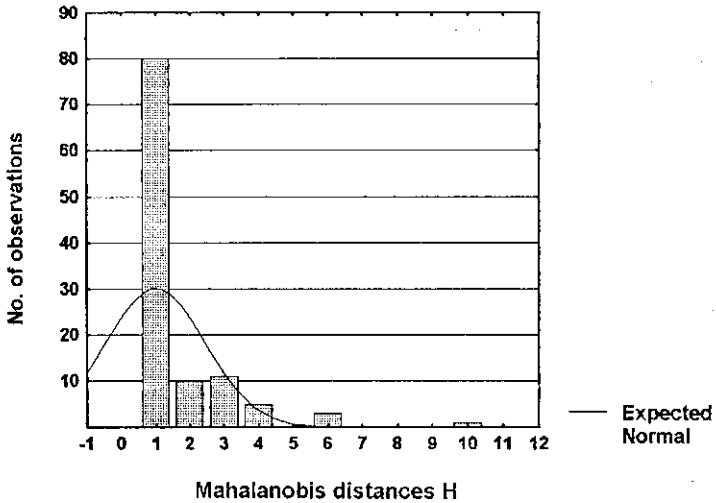

Figure 1. The distribution of Mahalanobis distances H in the values of in vivo organic matter digestibility DOM_v of 110 feed samples (observations); H>3 indicates structural outliers

within maximum of normal distribution. Out of 110, only 9 samples – 4 hays of relatively low DOM_v (56-64%), 4 compounds and 1 silage of extremely high DOM_v (82.6%) were characterized by H>3 and could be considered as structural outliers.

The descriptive statistics for the DOM_v and DOM_k values are given in Table 2. The means and ranges of the values from both *in vivo* and *in vitro* methods were similar – differences ranged from 0.6 to 3.5%. However, standard deviations in the DOM_k values were slightly lower, which may be a consequence of either lower sensitivity of the *in vitro* method or a higher error on *in vivo* estimates.

TABLE 2

Descriptive statistics of the digestible organic matter content determined in sheep (DOM_v) and using KOH extraction (DOM_k)

Sample Variable	n	Mean	Min	Max	SD[a]	g/kg OM
All samples	DOM_v	110	705	548	887	71.9
	DOM_k	110	694	545	898	66.6
Dried grass	DOM_v	70	674	548	801	61.0
	DOM_k	70	661	545	791	59.0
Grass silages	DOM_v	33	741	658	826	37.3
	DOM_k	33	736	660	813	32.7
Compounds	DOM_v	7	849	763	887	40.9
	DOM_k	7	829	790	898	37.2

[a]SD – standard deviation

TABLE 3

Statistical parameters of linear regression between digestibility of organic matter determined *in vivo* on sheep (Y, %) and *in vitro* by solubility in KOH solution (X, %); model Y = a + bX

Sample	n	R^2	RSD[a]	a	b	SE[b] of b	significance of b
All samples	110	0.75	3.65	5.74	0.934	0.053	P < 0.001
Dried grass	70	0.61	4.02	7.91	0.901	0.096	P < 0.001
Grass silages	33	0.53	2.59	12.96	0.831	0.140	P < 0.001
Compounds	7	0.30	3.74	34.88	0.603	0.410	P = 0.20

[a]RSD – residual standard deviation
[b]SE – standard error

The regression equations between DOM_v (Y, %) and DOM_k (X,%) for the combined and individual groups of samples are given in Table 3. The relationship was significant ($P < 0.001$) for the combined set of samples ($r = 0.86$, $n = 110$), all forages ($r = 0.82$, $n = 103$), dried grass ($r = 0.75$, $n = 70$) and grass silages ($r = 0.73$, $n = 33$). However, the relationship was insignificant ($r = 0.55$, $P = 0.2$) for compounds, due to either the low number of feeds evaluated ($n = 7$) or the relatively narrow range of DOM_k values what made it impossible to determine coefficient b with high accuracy. The regression of DOM_v on DOM_k explained from 30 to 75% of total variation in the organic matter digestibility of various feed categories determined *in vivo*.

The relationship between DOM_v (Y, %) and DOM_k (X, %) values is presented in Figure 2. All samples, regardless of their nature and origin appear to fit the

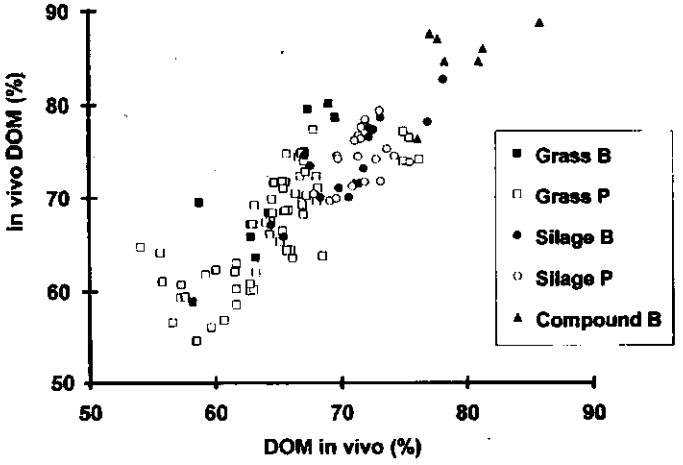

Figure 2. The relationship between the digestibility of organic matter *in vivo* (Y) and *in vitro* (X) for 110 feed samples: $Y = 0.934 X + 5.47$, R^2 0.75, RSD 3.65, $P < 0.001$. In the legend B indicates samples from Belgium, P from Poland

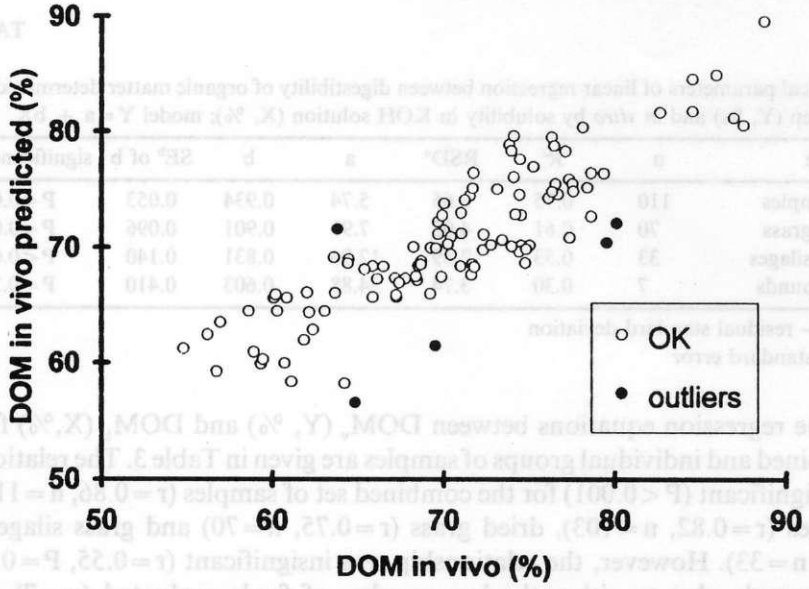

Figure 3. The relationship between the digestibility of organic matter (DOM) *in vivo* DOM_v determined on sheep (observed) and predicted from DOM *in vitro* based on the relationship: $Y = 0.934\ X + 5.47$ (DOM_{vk}). Open circles indicate samples having residuals within the range ± 2 sigma; closed circles indicate outliers (residuals beyond the range $\pm$ 2 sigma)

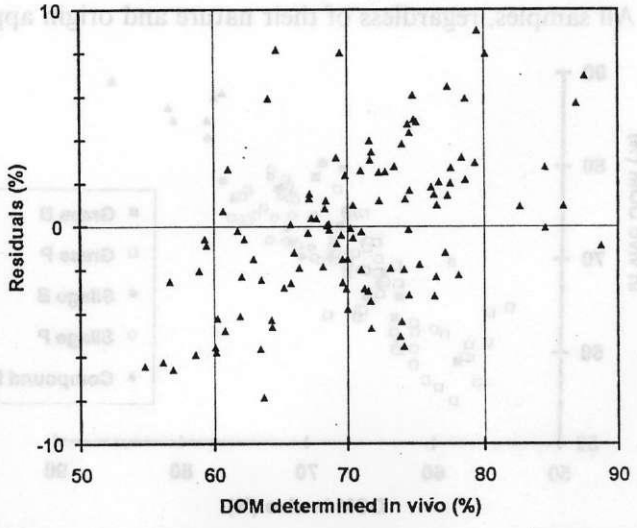

Figure 4. The relationship between the residuals i.e. ($DOM_v - DOM_{vk}$) and DOM_v: $r = 0.51$, $P < 0.001$. DOM_v denotes the digestibility of organic matter (DOM) determined on sheep; DOM_{vk} denotes DOM predicted from DOM *in vitro* (X) based on equation $Y = 0.934\ X + 5.47$

same equation relatively well (Table 3): $Y = 0.934\ X + 5.74$, standard error of b $SE_b = 0.05$, significance of coefficient a $P = 0.05$, $SE_a = 3.74$.

The relationship between DOM_v (determined values) and DOM_{vk} (predicted from the equation for all samples) is shown in Figure 3. The predicted values could account for 75% of variance in determined DOM_v; RSD = 2.52%. There were only 5 outliers, defined by residuals $> +2$ sigma, actually having the residuals higher than 7.8.

Analysis of residuals ($DOM_v - DOM_{vk}$) in relation to the DOM_v is shown in Figure 4. The residuals were correlated with DOM_v ($r = 0.51$, $P < 0.001$). It may be seen from the scatterplot that DOM_{vk} values tend to be overestimated for samples of DOM ranging between 50-60% (negative residuals only) and underestimated for those exceeding 80% (positive residuals only). The most even distribution of the residuals was in the DOM range of 70-80%.

DISCUSSION

The distribution of H values revealed that about 80% of samples are centered around $H = 1$. According to Shenk and Westerhaus (1991), probably part of these samples could have been eliminated from calculation of the regression equation for DOM prediction without significant loss of accuracy. However, our set was at least 10 times less numerous than the set they used for nutrient content prediction by near infrared spectroscopy. Therefore, any of these samples was eliminated. Neither data for the identified outliers (showing $H > 3$) removed from the calculation of the regression of DOM_v on DOM_k, in order to obtain a robust prediction equation.

In our study, the extraction with boiling KOH solution could account for 75% of the variability in DOM of 110 samples in wide range with a CP and CF content (RSD 2.6-4.0). The relationship was definitely poorer than that showed by Kesting (1978) who indicated the method could account for 88% of variation in DOM in 109 samples including forages and concentrates. However, the prediction in our study was better than found by Vencl and Flam (1981): $R^2 = 17\%$, $n = 56$. The latter authors showed a relatively good *in vivo-in vitro* relationship for maize silage ($R^2 = 58\%$, $n = 7$) and pelleted forages ($R^2 = 74\%$, $n = 8$). In contrast to the results of Kesting (1978) and that found in the present study, Vencl and Flam (1981) were not able to predict DOM_v of various samples using the same equation. However, the total number of samples used by the latter authors was only half of that used in our study and in the work of Kesting (1978).

DOM_v of ruminant feeds, particularly forages, can be precisely predicted by the rumen inoculum-pepsin method of Tilley and Terry (1963). In the study of Vencl and Flam (1981), this method proved slightly better than the alkaline

extraction in the prediction of DOM_v ($R^2 = 31$, 55 and 83%, respectively, for all 56 feeds, hays and silage samples). Kosmala and Brzóska (1982) comparing *in vitro* methods using KOH, cellulase or rumen fluid to predict DOM_v of grass and clover forage ($n = 17$) found the best results with the chemical method ($r = 0.74$). Narasimhalu (1985) also compared the rumen inoculum-pepsin method (Tilley and Terry, 1963) with various cellulase procedures and found that the coefficients of determination in the prediction of DM digestibility were 75 and 24-58%, respectively. In case of using a two-stage cellulase procedure, the variation accounted for by the regression increased from 24 to 69% when lignin content was included as the second independent variable in the prediction equation (Narasimhalu, 1985).

Essentially, the results of enzymatic methods using fungal cellulases depended on the kind of samples evaluated and procedure used. In most experiments, the coefficients of determination R^2 for forages (dried grass and legumes, silages) ranged from 50 to 72-88% and RSDs were between 2 and 4 digestibility percentage units (Dowman and Collins, 1977; McLeod and Minson, 1982; Valdes and Jones, 1987). Thus, the accuracy of the enzymatic method was not always better than that of the alkaline extraction shown in the present study. The results of the latter method are also better than those obtained using the technique of gas production during incubation with buffered rumen fluid: R^2 72%, RSD 2.4-7.9 depending on sample set (Menke and Steingass, 1987).

The method of alkaline extraction has an advantage compared with methods using different inocula: its conditions are very standarized so that it is not necessary to analyze standard feeds in each series to allow for the changes in cellulolytic activity of the incubation medium. However, the method is very unspecific.

In feed evaluation laboratories that do not keep cannulated animals as donors of rumen fluid, the KOH method or enzymatic techniques may be used. Fungal cellulases resemble to some extent the action of ruminal cellulolytic enzymes and therefore the enzymatic methods seem more specific. However, considering its simplicity and very low cost of reagents, and taking into account a small RSD, the method using the alkaline extraction may be used in comparative works. It may introduce systematic errors as far as the feeds of $DOM < 60\%$ and of $DOM > 80\%$ are concerned. Its accuracy could probably be improved by adding lignin as a second independent variable in the regression.

CONCLUSIONS

The measurement of organic matter digestibility by extracion with boiling 5% KOH solution is a cheap, simple, rapid and fairly accurate procedure to evaluate

ruminant feeds. More research is needed to improve the accuracy of the method by introducing further independent variable (i.e. lignin) to the regression equation.

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STRESZCZENIE

Ocena przydatności rozpuszczalności w roztworze wodorotlenku potasu jako metody *in vitro* szacowania strawności masy organicznej pasz dla przeżuwaczy

Badano przydatność metody ekstrakcji przy pomocy 5% roztworu wodorotlenku potasu (KOH) do szacowania strawności masy organicznej (DOM) pasz dla przeżuwaczy. Zdolność predykcyjną

metody oceniano dla 110 pasz, w tym: suszone trawy (70), kiszonki z traw (33) i mieszanki treściwe (7) o znanej DOM oznaczonej na owcach (DOM_v).

Stwierdzono dość ścisłą zależność pomiędzy strawnością masy organicznej *in vitro* DOM_k (X, %) i DOM_v (Y, %) przy porównywaniu danych dla wszystkich prób i pasz objętościowych. Równania regresji wynosiły: dla wszystkich prób (n = 110) $Y = 0{,}934\ X + 5{,}74$, $R^2 = 0{,}75$ ($P < 0{,}001$), błąd szacowania RSD 3,65; dla pasz objętościowych (n = 103) $Y = 0{,}903\ X + 7{,}67$, $R^2 = 0{,}67$ ($P < 0{,}001$), RSD 3,62. Regresja DOM_v względem DOM_k dla mieszanek treściwych (n = 7) była nieistotna ($R^2 = 0{,}30$, RSD 3,74).

Stwierdzono liniową zależność pomiędzy DOM_v (wartości zmierzone) i DOM_vk (szacowane na podstawie równania dla wszystkich prób). Wartości szacowane pozwalały na przewidywanie do 75% zmienności stwierdzonej w DOM_v; RSD 2,52. Różnice $DOM_v - DOM_vk$ były skorelowane z DOM_v ($r = 0{,}51$, $P < 0{,}001$). Ujawniła się tendencja do szacowania wartości DOM_vk za wysoko przy DOM_v w zakresie 50-60% (różnice < 0) i za nisko przy $DOM_v > 80\%$ (różnice > 0). Najbardziej równomierny rozkład różnic obserwowano w zakresie DOM_v 70-80%.