

**Variability of cow-specific organic matter digestibility within lactation and across cows based on NIRS scans from fecal samples**

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**ABSTRACT:** Near infrared reflectance spectroscopy (NIRS) provides an opportunity to measure cow-specific organic matter digestibility (OMD) from fecal samples. In this study individual digestibility measurements from a trial with 47 cows were investigated. Digestibility increased when lactation progressed. The repeatability estimates between different lactation stages were low which suggests that several fecal collections during lactation are needed to measure cow-specific OMD. Higher repeatability estimates of cow-specific composite samples compared to lower repeatability estimates between individual samples indicate that collecting of composite samples is preferable. The variability of OMD and moderate repeatability estimates give some evidence that genetic improvement of dairy cattle digestibility might be possible, but to make the method practically feasible, further studies on the measurement protocol are still needed.

**Keywords:** dairy cattle; feed efficiency; organic matter digestibility

### **Introduction**

Feed efficiency of dairy cattle is dependent on the amount of intake and the efficiency with which nutrients are digested, absorbed, and metabolized to maintenance, growth and milk production. It is influenced by diet, environmental factors, genetic capacity and physiological state of the cow to utilize nutrients for milk yield. Selection for feed efficiency traits in dairy cattle has up to now been indirect due to the difficulties in trait definition and costs associated with measuring feed efficiency in large populations.

Digestibility is one part of the feed efficiency of a cow with large economic value. The effects of feeding level and diet composition on digestibility are well identified (Nousiainen et al. (2009); Huhtanen et al. (2009)), but there are gaps in knowledge on the variability of digestibility within lactation and across cows fed on the same diet. Previously it was presented that little variation exists among cows in their ability to digest a given diet, particularly when intakes are standardized (Korver (1988); Veerkamp and Emmans (1995)). More recent studies however indicate differences in digestibility among cows (Berry et al. (2007)).

Diet digestibility has been traditionally determined by total fecal collection method or by using different internal markers, e.g., acid-insoluble ash (AIA) (Van Keulen and Young (1980)) or natural odd carbon-

chain n-alkaline pentatriacontane (Dillon (1993)). Unfortunately, these methods are too expensive and time-consuming to be used routinely on-farms. Near infrared reflectance spectroscopy (NIRS) can be used to determine diet digestibility of cows (Nyholm et al. (2009)). This provides an opportunity to obtain digestibility predictions from on-farm samples, but fecal sampling needs to be minimized to make the method practically feasible.

The objective of this study was to quantify the variability of organic matter digestibility (OMD) within lactation and across cows based on NIRS scans from fecal samples and to obtain key parameters for assessing whether it is possible to develop an on-farm measurement protocol.

### **Materials and Methods**

**Data.** A trial with 47 cows was conducted at MTT Maaninka research farm during 2012-2013. All 32 Holstein cows (14 primiparous) and 15 Nordic Red dairy cows (2 primiparous) were fed ad libitum a grass silage based TMR with a concentrate level of either low (L) or high (H). During the dry period all cows were given the same diet.

The trial was designed to quantify cow-specific diet digestibility at different stages of lactation by samples taken during calendar weeks where a cow was approximately 50, 150 and 250 days in milk (DIM), and once during the dry period. During each sampling week fecal samples were collected for five consecutive days every morning and every evening. Feces were collected directly from the rectum of the cows. For all 47 cows composite samples were made from these samplings using 100 g of feces per each sampling time over the whole 5-day period. These 10 samples were composed to one composite sample (one from every lactation stage), resulting in four observations for each cow. From each of the 10 individual collections also other 400 g feces were retained from a subset of 21 cows. Thus these 21 cows had also 10 observations from every lactation week (40 observations per cow in total). All samples were immediately frozen at -20 °C and kept until drying at 60 °C and milling through a 1-mm screen.

The composite samples collected from lactation stages 50, 150 and 250 DIM were analyzed by acid-insoluble ash (AIA) as an internal marker, which served as reference method, and from all four lactation

stages by NIRS. The individual samples from the 21 cow subset were analyzed by NIRS only. The NIRS analyses were performed at Valio Ltd. laboratory by scanning duplicate cuvettes for each sample between 400-2500 nm in 2 nm increments using a FOSS NIRSystems 6500 spectrometer. Applied NIRS prediction equations for OMD were developed from a reference data including 221 samples collected from previous MTT trials.

The reference values obtained by the AIA method were used to assess the reliability of NIRS scans by comparing the means and standard deviations and calculating the correlation between these methods.

To study the optimization of sampling protocol and differences between individual samples the NIRS scan data from 21 cows with daily individual morning and evening measurements were used.

**Statistical analyses.** In the first step, variability of cow-specific OMD within lactation and across cows was assessed. Therefore, both OMD predictions based on AIA and NIRS from the composite samples of the 47 cows were modeled by a linear mixed model, which included the fixed effects of feeding level, lactation stage, feeding level  $\times$  lactation stage interaction, and week of collection  $\times$  lactation stage interactions. Unstructured covariance matrix for the error term was used to model the correlation between measurements made from the same cow at different stages of lactation and to allow the variance of measurements to change during lactation.

Next, OMD predictions by NIRS and AIA from the composite samples of the 47 cows were modeled by a linear mixed model to assess the repeatability between lactation stages. The applied statistical model was fitted by lactation stage pairs and included feeding level, lactation stage and week of collection as fixed effects and animal as a random effect. Repeatability estimates were calculated as:

$$r_a = \frac{\sigma_{animal}^2}{\sigma_{animal}^2 + \sigma_{\epsilon}^2}$$

The repeatability over the 21 cows' individual OMD predictions between lactation stages was modeled by a linear mixed model, which was fitted by lactation stage pairs and included feeding level, lactation stage and day of collection as fixed effects, and animal and animal  $\times$  lactation stage  $\times$  sampling time interaction as random effects. Repeatability estimate was calculated as:

$$r_a = \frac{\sigma_{animal}^2}{\sigma_{animal}^2 + \sigma_{interaction}^2 + \sigma_{\epsilon}^2}$$

The repeatability over individual OMD predictions within a lactation stage was modeled by a linear mixed model, which included feeding level, lactation

stage, day of collection and sampling time (morning or evening) as fixed effects, and animal and animal  $\times$  sampling time as a random effect. Repeatability estimates were calculated as:

$$r_s = \frac{\sigma_{animal}^2 + \sigma_{interaction}^2}{\sigma_{animal}^2 + \sigma_{interaction}^2 + \sigma_{\epsilon}^2}$$

The analyses were performed using the MIXED and VARCOMP procedures (Littell et. al. (1996)) in SAS/STAT software version 9.3 (SAS Institute Inc., Cary, NC, USA).

## Results and Discussion

In total there were 185 composite samples (47, 47, 47 and 44 from lactation stage 50, 150, 250 DIM and dry period, respectively) analyzed by NIRS. The mean OMD predicted by NIRS remained constant as the lactation progressed (average of all 740 g/kg). In the data analyzed by AIA there were in total 114 composite samples (42, 41 and 31 from lactation stage 50, 150 and 250 DIM, respectively). The mean OMD predicted by AIA increased as the lactation progressed (average of all 724 g/kg). Standard deviation was greatest in early lactation analyzed by both methods (NIRS 28.5 g/kg and AIA 23.5 g/kg).

The correlation between OMD predicted by AIA and NIRS over the whole lactation was 0.49. The correlation between methods was strongest in 150 DIM (0.66) and weakest in 250 DIM (0.42).

In total 762 individual samples were collected from the 21 cow subset (199, 186, 191, and 186 from lactation stage 50, 150, 250 DIM and dry period, respectively). In this data the increase in OMD was more obvious than in the composite samples data when the lactation progressed. There were no differences between means and standard deviations of morning and evening samples.

Feeding level  $\times$  lactation stage interaction was statistically significant ( $P < 0.001$ ) at lactation stages 150 and 250. Cows on L had on average 20.1 g/kg and 22.1 g/kg higher OMD than cows on H. There were no statistically significant differences between diets at lactation stages 50 and during the dry period. The present data agrees with the results of Nousiainen et al. (2009) and Huhtanen et al. (2009) who reported that increased concentrate feeding depressed total diet OMD. This is due to decreased ruminal fiber digestion caused by decreased ruminal pH and digesta retention time. Also the effect of week of collection was significant in all lactation stages ( $P < 0.001$ ), which is due to change in basal silage composition. No linear relationship between OMD and breed or parity was found in this small data set.

Variability of OMD at different stages of lactation based on NIRS scans and AIA is presented in Table 1. The largest variability in OMD based on NIRS was

found in early lactation and as the lactation progressed, variability decreased. This result is in agreement with Berry et al. (2009) who also found the largest residual variances in early lactation. Slightly larger coefficients of variation were found when OMD was based on AIA.

**Table 1. Variability of organic matter digestibility (g/kg) at different stages of lactation based on NIRS and AIA.**

| Lactation stage | NIRS<br>$\sigma^2_{\epsilon}$ | AIA<br>$\sigma^2_{\epsilon}$ | NIRS<br>CV | AIA<br>CV |
|-----------------|-------------------------------|------------------------------|------------|-----------|
| 50 DIM          | 150.8                         | 263.5                        | 1.67       | 2.26      |
| 150 DIM         | 157.3                         | 272.9                        | 1.71       | 2.27      |
| 250 DIM         | 86.7                          | 297.3                        | 1.26       | 2.36      |
| Dry period      | 112.2                         |                              | 1.40       |           |

$\sigma^2_{\epsilon}$  = Residual variance

CV = Coefficient of variation

A repeatability estimate of 0.24 for OMD analyzed by NIRS, and 0.34 analyzed by AIA was found using observations from composite samples of 47 cows of all lactation stages (Table 2 and 3). When the data with individual samples of 21 cows was used, the repeatability estimates of OMD between different stages of lactation were lower than the estimates calculated from the composite samples data (Table 4). Table 5 shows that also the repeatability estimates of individual samples within lactation stage were quite low. However, these estimates were greater than the estimates between lactation stages. Higher repeatability estimates of composite samples indicate that collecting composite samples of feces from successive days may be preferable. Estimates for OMD at early lactation stage (DIM 50) analyzed by AIA and in dry period based on NIRS showed lowest repeatability. This indicates that it is preferable to collect digestibility measurements from mid-lactation.

**Table 2. Repeatability estimates (r) of organic matter digestibility (OMD, g/kg) of composite samples of 47 cows based on NIRS between different stages of lactation.**

|                         | $\sigma^2_{\text{animal}}$ | $\sigma^2_{\text{error}}$ | r    |
|-------------------------|----------------------------|---------------------------|------|
| OMD                     | 37.87                      | 119.79                    | 0.24 |
| OMD <sub>50, 150</sub>  | 56.91                      | 110.50                    | 0.34 |
| OMD <sub>150, 250</sub> | 42.00                      | 100.76                    | 0.29 |
| OMD <sub>250, 350</sub> | 11.18                      | 113.46                    | 0.09 |

**Table 3. Repeatability estimates (r) of organic matter digestibility (OMD, g/kg) of composite samples of 47 cows based on AIA between different stages of lactation.**

|                         | $\sigma^2_{\text{animal}}$ | $\sigma^2_{\text{error}}$ | r    |
|-------------------------|----------------------------|---------------------------|------|
| OMD                     | 109.5                      | 216.1                     | 0.34 |
| OMD <sub>50, 150</sub>  | 8.4                        | 317.7                     | 0.03 |
| OMD <sub>150, 250</sub> | 216.3                      | 102.1                     | 0.68 |

**Table 4. Repeatability estimates (r) of organic matter digestibility (OMD, g/kg) of individual samples of 21 cows between different stages of lactation.**

|                         | $\sigma^2_{\text{animal}}$ | $\sigma^2_{\text{interaction}}^{\S}$ | $\sigma^2_{\text{error}}$ | r    |
|-------------------------|----------------------------|--------------------------------------|---------------------------|------|
| OMD                     | 12.66                      | 195.55                               | 370.66                    | 0.02 |
| OMD <sub>50, 150</sub>  | 12.15                      | 189.48                               | 422.12                    | 0.02 |
| OMD <sub>150, 250</sub> | 56.21                      | 40.79                                | 370.00                    | 0.12 |
| OMD <sub>250, 350</sub> | 53.97                      | 20.59                                | 318.22                    | 0.14 |

$\S$  Variance of animal  $\times$  lactation stage  $\times$  sampling time

**Table 5. Repeatability estimates (r) of organic matter digestibility (OMD, g/kg) of individual samples of 21 cows within different stages of lactation.**

| Lactation stage | $\sigma^2_{\text{animal}} + \sigma^2_{\text{interaction}}^{\S}$ | $\sigma^2_{\text{error}}$ | r    |
|-----------------|---|---------------------------|------|
| 50 DIM          | 85.14   | 479.19                    | 0.15 |
| 150 DIM         | 71.18   | 331.98                    | 0.18 |
| 250 DIM         | 90.62   | 386.22                    | 0.19 |
| Dry period      | 82.90   | 220.22                    | 0.27 |

$\S$  Variance of animal  $\times$  sampling time

## Conclusion

NIRS can be used to assess variability of OMD between cows. The variability found in this study was small and this result is in agreement with the literature. Correlations between OMD predictions based on NIRS and AIA reference values were moderate, which may indicate that predictions by NIRS should be more accurate to quantify small differences in OMD between cows. To calculate genetic parameters significantly more data is required, but repeatability estimates indicated that improving dairy cattle digestibility by animal breeding may be possible. However, further effort has to be made to develop a reliable measurement protocol applicable on commercial farms.

## Literature cited

- Berry, D.P., Horan, B., O'Donovan, M., et al. (2007). J. Dairy Sci. 90: 4835-4845.
- Dillon, P. (1993). PhD Thesis. Natl. Univ. Ireland, Dublin.
- Huhtanen, P., Rinne, M., and Nousiainen, J. (2009). J. Dairy Sci. 92:5031-5042.
- Korver, S. (1988). Livest Prod Sci. 20: 1-13.
- Littell R.C., Milliken G.A., Stroup W.W., et al. (1996). SAS System for Mixed Models. SAS Institute Inc, Cary, NC. 633 pp.
- Nousiainen, J., Huhtanen, P., and Rinne, M. (2009). J. Dairy Sci. 92: 5019-530.
- Nyholm, L., Nousiainen, J., Rinne M., et al. (2009). Proc 7th ISRP, p. 300-301.
- Van Keulen, J., and Young, B.A. (1977). J. Anim. Sci. 44:282-287.
- Veerkamp, R.F., and Emmans, G.C. (1995). Livest Prod Sci. 44: 87-97.