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# **JOURNAL OF ANIMAL SCIENCE**

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# Comparison of fecal crude protein and fecal near-infrared reflectance spectroscopy to predict digestibility of fresh grass consumed by sheep<sup>1</sup>

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**ABSTRACT:** Organic matter digestibility (OMD), an essential criterion for the evaluation of the nutrition of ruminants, cannot be measured easily at pasture. Therefore, the objective of this study was to test and compare 2 methods of OMD prediction based on the fecal CP content (CPf) or near infrared reflectance spectroscopy (NIRS) applied to feces. First, published equations derived from fecal N (Eq. 1<sub>CP</sub>, n = 40) and from fecal NIRS (Eq. 1<sub>NIRS</sub>, n = 84) were used to predict OMD of an independent validation data set from which in vivo OMD, ranging from 58 to 74%, was measured for 4 regrowth stages of *Digitaria decumbens*. Second, to establish equations usable in grazing situations and to improve the efficiency of the predictions, new equations were calculated from a large data set (n = 174) using CPf (Eq. 2<sub>CP</sub>) or fecal NIRS (Eq. 2<sub>NIRS</sub>). By applying the CPf method, Eq. 2<sub>CPf</sub> (OMD, % = 88.4 - 263.9/CPf, % of OM; residual SD = 2.92, r<sup>2</sup> = 0.63) showed similar statistical parameters (P < 0.01) when compared with Eq. 1<sub>CP</sub> (OMD, % = 86.6 - 266.2/CPf, % of OM; residual SD = 2.95, r<sup>2</sup> = 0.79). When using fecal NIRS, Eq. 2<sub>NIRS</sub> showed decreased SE of calibration (SEC = 1.48) and of cross-validation (SECV =

1.75) and greater coefficient of determination of cross-validation (R<sup>2</sup><sub>CV</sub> = 0.85) than the previously published Eq. 1<sub>NIRS</sub> (SEC = 1.78, SECV = 2.02, R<sup>2</sup><sub>CV</sub> = 0.77). The validation of the 4 equations on the validation data set was satisfactory overall with an average difference between the predicted and the observed OMD ranging from 0.98 to 2.79 percentage units. The Eq. 2<sub>NIRS</sub> was nevertheless the most precise with a decreased residual SD of 2.53 and also the most accurate, because the SD of the average difference between predicted and observed OMD was the lowest. Therefore, fecal NIRS provided the most reliable estimates of OMD and is thus a useful tool to predict OMD at pasture. However, an adequate number of reference data are required to establish good calibration. Indeed, better calibration statistics were obtained by increasing the data set from 84 (Eq. 1<sub>NIRS</sub>) to 174 (Eq. 2<sub>NIRS</sub>). In contrast, using fecal N on a set of 84 or 174 points did not improve the prediction. Both methods are useful for predicting OMD at pasture in certain circumstances, using fecal NIRS when a large data set (n = 84 and n = 174) is available and fecal CP with smaller data sets (n = 40).

**Key words:** digestibility, fecal crude protein, near infrared reflectance spectroscopy, sheep, tropical grass

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## INTRODUCTION

Digestibility of OM is one of the most important characteristics used to evaluate feed nutritional quality. For grazing animals, OM digestibility (OMD) cannot be determined directly, unlike for stall-fed animals, where direct determination by quantitative measurements of ingested forage and fecal excretion can be accomplished. Therefore, several indirect assessment methods have

been developed from forage or fecal samples. Methods based on forage samples such as in vitro or in sacco degradability of hand-plucked herbage may introduce bias in digestibility estimation because hand-plucked herbage may not be representative of the herbage grazed because of diet selection by grazing animals (Baumont et al., 2000; Schlegel et al., 2000). Methods based on esophageal fistula samples have then been used to overcome the inaccuracy of hand-plucked samples (Le Du and Penning, 1982). However, these methods require the surgical alteration of experimental animals, which is impractical in production situations and can be undesirable from an animal welfare point of view.

Methods to predict OMD based on fecal profiling, including regression with fecal CP (CPf) content (Wehausen, 1995; Boval et al., 2003) and near infrared reflectance spectroscopy (NIRS) applied to feces (Coates,

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1999; Landau et al., 2006), have provided consistent estimates of OMD. Therefore, the aim of this study was to examine the potential of fecal indices based on fecal CP or fecal NIRS to predict OMD in grazing situations. A 2-step procedure was employed. First, existing published equations derived from fecal CP (Boval et al., 2003) and from fecal NIRS (Fanchone et al., 2007) were used to predict OMD of a small independent validation data set. Second, a larger calibration data set was created to further assess the applicability of these 2 fecal-based methods to predict OMD in a variety of Pangola grass pastures.

## MATERIALS AND METHODS

Care and use of animals were performed according to the Certificate of Authorization to Experiment on Living Animals issued by the French Ministry of Agriculture, Fishing, and Feeding.

### *Validation Data Set*

The validation data set (**VDS**,  $n = 23$ ) came from an independent trial carried out in 1996 at the animal experimental station of the Institut National de la Recherche Agronomique (**INRA**) in the French West Indies (Guadeloupe, latitude  $16^{\circ}16'$  N, longitude  $61^{\circ}30'$  W). This trial was designed to evaluate variations in nutritive values of Pangola grass (*Digitaria decumbens*) according to various stages of regrowth (Archimède et al., 2000). This data set was retained for validation because of its large range in values for OMD. Six adult Martinik rams ( $40.8 \pm 0.6$  kg of BW) were fed 14-, 28-, 42-, and 56-d-old fresh regrowth of Pangola grass during 4 successive experimental periods. The regrowth stages of 14, 28, 42, and 56 d were used during periods 1, 2, 3, and 4, respectively. The plots intended to be used at 14, 28, 42, and 56 d of regrowth were divided into 15, 30, 30, and 30 subplots respectively. The first of the 15, 30, 30, and 30 subplots had been cut 15, 29, 43, and 57 d before the beginning of the experimental period 1, 2, 3, and 4, respectively. One subplot was cut per day, so that each subplot had 1 d more than the subplot cut the day before and 1 d less than the subplot cut the following day. Consequently, the regrowth stage of the subplot intended to be harvested daily was exactly 14, 28, 42, and 56 d in period 1, 2, 3, and 4, respectively. For the 4 stages of regrowth (14, 28, 42, and 56 d), the concentration (% of DM) of OM was 84, 89, 90, and 88; CP was 13.0, 7.9, 7.2, and 5.7; NDF was 74, 78, 79, and 79; and ADF was 38, 43, 44, and 44, respectively (Archimède et al., 2000). In the 56-d treatment, 1 ram having a very low intake level (30% less than the average intake of the group) was removed. Each experimental period consisted of 14 d of adaptation to the diet, followed by 5 d of intake and total-tract digestibility measurements. The grass was cut daily, early in the morning, and chopped (to a 5-cm length) before being offered. The amount of forage provided was 1.15

$\times$  animal voluntary intake estimated during the adaptation period. Digestibility was calculated per animal by weighing the daily amounts of forage offered, the refusals, and feces excreted. Dry matter contents of fresh forage and refusals were determined daily by drying for 72 h at  $60^{\circ}\text{C}$  (Cochran and Galylean, 1994). A representative subsample of feces excreted was obtained by pooling 10% of the daily amount of feces excreted per animal. Subsamples of feces were stored at  $-20^{\circ}\text{C}$  until DM content determination. Dry matter content of fecal subsamples was determined in similar conditions as described previously for fresh forage and refusals. Samples were ground to a 0.75-mm particle size using a cross beater mill SK 100 (Retsch, Haan, Germany). Ground samples were then stored in closed plastic containers before chemical analyses. Organic matter content of forage and fecal samples was measured after an 8-h pyrolysis at  $550^{\circ}\text{C}$  to estimate OMD according to the reference procedure of Cochran and Galylean (1994). Nitrogen concentration of feces was determined using the Dumas method (AOAC, 1990). Crude protein content of feces was calculated by multiplying the N concentration by 6.25. Approximately 2.5 g of ground fecal sample was packed in a ring-cup sample cell with a near infrared, transparent, quartz cover glass (Foss, 2000). Cells were scanned 32 times using a scanning reflectance monochromator (NIRSystem 6500 Inc., Silver Springs, MD). Reflectance energy [ $\log(1/R)$ , where  $R$  = reflectance] was measured and averaged over the 32 scans. The average spectra of absorbance were recorded at 2-nm intervals over the wavelength range 700 to 1,100 and 1,100 to 2,500 nm. Only the near infrared region was used for calibration.

### *Calibration Data Set*

A large data set (**LDS**,  $n = 174$ ) was made from 4 digestibility trials carried out in stalls from 1997 to 2000. All trials were conducted at the animal experimental station at INRA in Guadeloupe (French West Indies). The effects tested through the different trials were mainly the leaf-stem proportion of the ration, the regrowth stage of the herbage, or the physiological stage of the animals (H. Archimède, unpublished data). In all trials, adult Martinik rams ( $45.1 \pm 0.31$  kg of BW) were fed fresh Pangola.

Determination of OM content of forage and feces, estimation of in vivo OMD, calculation of CPf, as well as recording of the absorbance spectra of fecal samples were as described previously for the VDS.

### *Calculations and Statistical Analysis*

Existing predicting equations of OMD, derived from CPf (**Eq. 1<sub>CP</sub>**; Boval et al., 2003) and from fecal NIRS (**Eq. 1<sub>NIRS</sub>**; Fanchone et al., 2007), were used to predict OMD of the VDS. New equations, based on CPf (**Eq. 2<sub>CP</sub>**) or fecal NIRS (**Eq. 2<sub>NIRS</sub>**), were developed from the LDS and were used to predict OMD of the

VDS. The equation Eq. 2<sub>CP</sub> was calculated according to a hyperbolic model ( $OMD = a - b/CPf$ ) as proposed by Boval et al. (2003), because it has been observed to be more precise than linear or quadratic models. The MIXED procedure (SAS Inst. Inc., Cary, NC) was used to account for the fixed effects of experiment (4 experiments) and the random effect of rams within each experiment (4 to 6 rams per experiment). The stage of regrowth was added as a covariate. The intercept and the slope of Eq. 2<sub>CP</sub> were compared with that of Eq. 1<sub>CP</sub> using the Neyman-Pearson test.

Before calibration, absorbance spectra of the LDS were transformed using standard normal variate and detrend scatter correction and 2 mathematical pretreatments: 1.4.4. and 2.5.5. using ISI software (Infra-software International, Port Matilda, PA), where the first number is the order of derivatization of spectral data, the second number is the gap over which the derivative is to be calculated, and the third number is the smoothing factor. The mathematical treatment 2.5.5. yielded superior calibration statistics, namely reduced SE of calibration (**SEC**), decreased SE of cross-validation (**SECV**), greater multiple coefficient of determination ( $R^2$ ), and greater  $R^2$  of cross-validation ( $R^2_{CV}$ ), and this treatment was retained rather than treatment 1.4.4. The SEC represents the variability in the difference between predicted values and reference values when the equation was developed from the calibration data set. The SECV represents the variability in the difference between predicted and reference values when the equation is applied sequentially to subsets of data from the calibration data set (Landau et al., 2006). Cross-validation is often employed when an independent validation set is unavailable or when removal of samples from a calibration set results in too few samples for effective equation development. Briefly, this process involves removing a certain number of samples during the calibration procedure; for example, 25%, and predicting these with the remaining 75%. This step is then repeated until all have served as validation samples. Cross-validations were based on splitting the sample population into 6 groups to select the optimum number of terms (i.e., principal components or eigenvectors) without over-fitting. The combined SE for each of these steps is the SECV. The equation Eq. 2<sub>NIRS</sub> to predict OMD was derived by processing pretreated fecal spectra of the LDS using modified, partial, least squares regression (ISI, 1999) because this technique has proven superior to other methods (principal component regression or stepwise multiple linear regression) in earlier research (Shenk and Westerhaus, 1991; Park et al., 1997, 1998).

The predicted values of OMD for the VDS, starting from the 4 equations described above (Eq. 1<sub>CP</sub>, Eq. 1<sub>NIRS</sub>, Eq. 2<sub>CP</sub>, and Eq. 2<sub>NIRS</sub>), were compared with the observed values. The precision of estimation was evaluated from the absolute difference (**Dpo**) between the predicted OMD and the observed OMD. Factorial analyses of variance was computed to determine the influ-

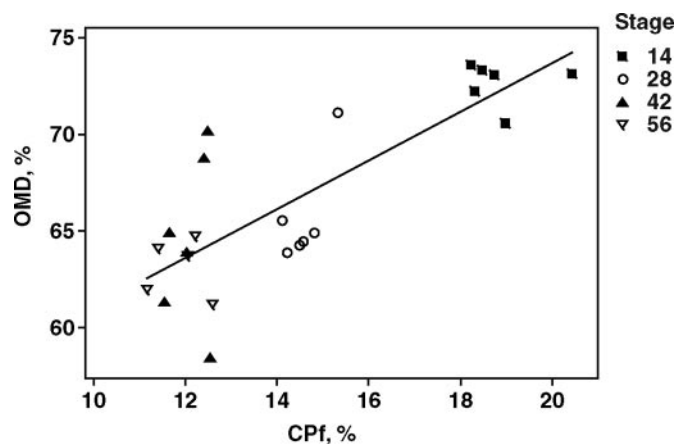
ence on Dpo of the main factors of variation in the VDS (Archimède et al., 2000), the regrowth stage (14, 28, 42, and 56 d), and animals using the GLM procedure (SAS Inst. Inc.). The SECV of the fecal NIRS equations and the SD of the different equations were compared using a Fisher test.

## RESULTS

In the VDS, OMD and CPf variation (about 26 and 84%, respectively) was mainly due to regrowth stage of the grass used in the study (Figure 1; Table 1). In this set of data, OMD and CPf varied over a broader range (15 and 9%, respectively) compared with the data used to establish Eq. 1<sub>CP</sub> (which varied by 12 and 6% for OMD and CPf, respectively). In addition, variation in OMD and CPf in the VDS was less than in the other 2 data sets used to establish Eq. 1<sub>NIRS</sub> and Eq. 2<sub>NIRS</sub>. The LDS used to establish Eq. 2<sub>NIRS</sub> presented the most significant variation for the 2 variables (a range of approximately 28% for OMD and 12% for CPf).

For CP equations, experiment, animal, and stage of regrowth were not significant ( $P > 0.10$ ) for the equation Eq. 2<sub>CP</sub> ( $P > 0.10$ ; Table 2). The a and b values of Eq. 2<sub>CP</sub> were not significantly different ( $P > 0.05$ ) from those of the equation Eq. 1<sub>CP</sub> based on the Neyman-Pearson test. Similar residual SD were achieved for both equations ( $P > 0.05$ ), whereas  $r^2$  was less for Eq. 2<sub>CP</sub>. For fecal NIRS equations derived from the LDS, better calibration and cross-validation statistics (decreased SEC and SECV,  $P < 0.05$ ; greater  $R^2$  and  $R^2_{CV}$ ; Table 3) were obtained for Eq. 2<sub>NIRS</sub> than for Eq. 1<sub>NIRS</sub>.

Using CPf equations to predict OMD from the VDS, we obtained a residual SD numerically greater for Eq. 1<sub>CP</sub> compared with Eq. 2<sub>CP</sub> (Table 4; Figure 2). The differences between the observed and predicted values were numerically less with Eq. 1<sub>CP</sub> compared with Eq. 2<sub>CP</sub>. Similarly, the SD of the difference was proportionally larger for Eq. 1<sub>CP</sub> compared with Eq. 2<sub>CP</sub>. The ef-



**Figure 1.** Evolution of OM digestibility (OMD) with fecal CP (CPf) content (% of OM) for sheep fed with Pangola grass at various stages of regrowth (14, 28, 42, and 56 d) in the validation data set:  $OMD = 48.4 + 1.26 CPf$ ,  $r^2 = 0.64$ .

**Table 1.** Descriptive statistics for OM digestibility (OMD) and fecal CP (CPf) in the validation data set, the data set of Boval et al. (2003), the data set of Fanchone et al. (2007), and the large data set

| Data set                           | n   | OMD, %         |              | CPf, % of OM   |              |
|------------------------------------|-----|----------------|--------------|----------------|--------------|
|                                    |     | Mean $\pm$ SD  | Range        | Mean $\pm$ SD  | Range        |
| Validation data set                | 23  | 66.6 $\pm$ 4.6 | 58.3 to 73.6 | 14.5 $\pm$ 2.9 | 11.1 to 20.4 |
| Eq. 1 <sub>CP</sub> <sup>1</sup>   | 40  | 63.0 $\pm$ 1.8 | 59.0 to 71.0 | 11.7 $\pm$ 0.4 | 9.8 to 16.0  |
| Eq. 1 <sub>NIRS</sub> <sup>2</sup> | 84  | 68.7 $\pm$ 7.4 | 59.9 to 82.3 | 14.3 $\pm$ 2.6 | 10.0 to 20.3 |
| Large data set                     | 174 | 67.1 $\pm$ 4.9 | 53.9 to 82.3 | 12.8 $\pm$ 2.6 | 7.9 to 20.3  |

<sup>1</sup>Eq. 1<sub>CP</sub> = equation of Boval et al. (2003):  $OMD = 86.6 - 266.2/CPf$ .

<sup>2</sup>Eq. 1<sub>NIRS</sub> = fecal near infrared reflectance spectroscopy equation of Fanchone et al. (2007).

fects of regrowth stage and animal were significant ( $P < 0.05$ ) on the Dpo (Table 4) for the 2 equations Eq. 1<sub>CP</sub> and Eq. 2<sub>CP</sub>. Using fecal NIRS to predict OMD of the VDS, we obtained a greater residual SD with Eq. 1<sub>NIRS</sub> compared with Eq. 2<sub>NIRS</sub> ( $P < 0.09$ ; Table 4 and Figure 3). For Eq. 1<sub>NIRS</sub>, the mean difference between predicted OMD and observed OMD was decreased compared with Eq. 2<sub>NIRS</sub>, but the SD was proportionally greater with Eq. 1<sub>NIRS</sub> compared with Eq. 2<sub>NIRS</sub> (Table 4). The effect of both stage of regrowth and animal was highly significant ( $P = 0.02$  and  $P = 0.003$  for the effect of stage of regrowth and animal, respectively) for Eq. 1<sub>NIRS</sub>, whereas only the animal effect was significant on Dpo for the Eq. 2<sub>NIRS</sub> ( $P = 0.009$ ). Considering the SECV of Eq. 2<sub>NIRS</sub> (Table 3) and the numerically less SD of the equation predicting OMD by using Eq. 2<sub>NIRS</sub>, this equation is more precise.

## DISCUSSION

### Fecal CP Equations

Fecal CP equations allow precise prediction of in vivo OMD. Residual SD obtained using Eq. 2<sub>CP</sub> was numerically less than the residual SD obtained with other methods aimed at predicting in vivo OMD from forage (from 3.2 to 5.1%, Kitessa et al., 1999; from 2.4 to 5.0%, Gosselink et al., 2004) and slightly greater than those of CPf equations using hyperbolic models (2.5%, Boval et al., 1996) or exponential models (2.7%, Lukas et al., 2005). By increasing the range of OMD and CPf using the LDS, we expected to increase the predictive ability of Eq. 2<sub>CP</sub> compared with the published Eq. 1<sub>CP</sub> of Boval et al. (2003) but that was not the case. The values of a and b were not significantly different between

the 2 fecal CP equations. The similarity between the 2 CPf equations may be explained by the hyperbolic model retained to derive the 2 equations. This model is assumed to describe the biological relationship between OMD and CPf, as described by Lancaster (1949), and can be used outside of its range of establishment (Lancaster, 1949; Wehausen, 1995; Boval et al., 2003). Thus, although the range of variation of data from Boval et al. (2003) is less than the range of the LDS, Eq. 1<sub>CP</sub> was capable of adequately describing this biological relationship, giving valid a and b values throughout the entire range of OMD and CPf in the VDS. Hence, the similarity of our 2 equations based on CPf means that it is not absolutely necessary to develop local equations for Pangola grass, in a fixed range of variation, as reported by Le Du and Penning (1982) and Armstrong et al. (1989). In fact, different researchers employed linear or quadratic models, which tended to overestimate the OMD for increased values of CPf, whereas the hyperbolic or exponential models used by Wehausen (1995), Boval et al. (1996), and Lukas et al. (2005) better explain the biological relationship between OMD and CPf. Thus, equations established under a fixed range can have a wider range of application, and methods based on CPf may be more powerful than expected.

The prediction could be improved by using additional predictors, although Boval et al. (2003) and Lukas et al. (2005) explored, unsuccessfully, the fiber content of the herbage or feces and the CP content of herbage. Another possible improvement would be to better describe the biological relationship between OMD and CPf. Fecal CP is composed of 2 fractions: 1) the undigested dietary protein and 2) the metabolic fecal protein, including bacterial and endogenous protein (Lancaster, 1949; Wehausen, 1995; Ferri et al., 2003). These

**Table 2.** Predictive regressions of OM digestibility (OMD, %) from fecal CP content per unit of OM (CPf, % of OM), calculated for sheep fed *Digitaria decumbens*

| Item                             | n   | Equation   | Residual SD | r <sup>2</sup> |
|----------------------------------|-----|--|-------------|----------------|
| Eq. 1 <sub>CP</sub> <sup>1</sup> | 40  | $OMD = 86.6_{(\pm 7.3)} - 266.2_{(\pm 83.1)} / CPf$  | 2.95        | 0.79           |
| Eq. 2 <sub>CP</sub> <sup>2</sup> | 174 | $OMD = 88.4_{(\pm 4.72)} - 263.9_{(\pm 64.4)} / CPf$ | 2.92        | 0.63           |

<sup>1</sup>Equation of Boval et al. (2003).

<sup>2</sup>Fecal CP equation derived using the large data set.

**Table 3.** Descriptive statistics of the fecal near infrared reflectance spectroscopy (NIRS) equations to predict OM digestibility (OMD, %)

| Item                               | Statistic <sup>1</sup> |      |      |      |                |      |                   |
|------------------------------------|------------------------|------|------|------|----------------|------|-------------------|
|                                    | n                      | Mean | SD   | SEC  | R <sup>2</sup> | SECV | R <sup>2</sup> cv |
| Eq. 1 <sub>NIRS</sub> <sup>2</sup> | 84                     | 68.8 | 4.12 | 1.78 | 0.81           | 2.02 | 0.77              |
| Eq. 2 <sub>NIRS</sub> <sup>3</sup> | 174                    | 67.0 | 4.48 | 1.48 | 0.89           | 1.75 | 0.85              |

<sup>1</sup>SEC = SE of calibration; SECV = SE of cross-validation; R<sup>2</sup>cv = coefficient of cross-validation.

<sup>2</sup>Eq. 1<sub>NIRS</sub>: Equation of Fanchone et al. (2007).

<sup>3</sup>Eq. 2<sub>NIRS</sub>: Fecal NIRS equation derived using the large data set.

authors state that the biological relationship between OMD and CPf is linked to the metabolic fecal protein fraction. However, the opinions are divided concerning the respective role of bacterial and endogenous fractions and even on the proportion of these fractions in metabolic fecal protein. Therefore, an evaluation of the various sources of CPf and their respective relationship with OMD should improve the ability to predict OMD via fecal-based calibrations. Further experiments are required to better understand the relationship between the different CPf fractions.

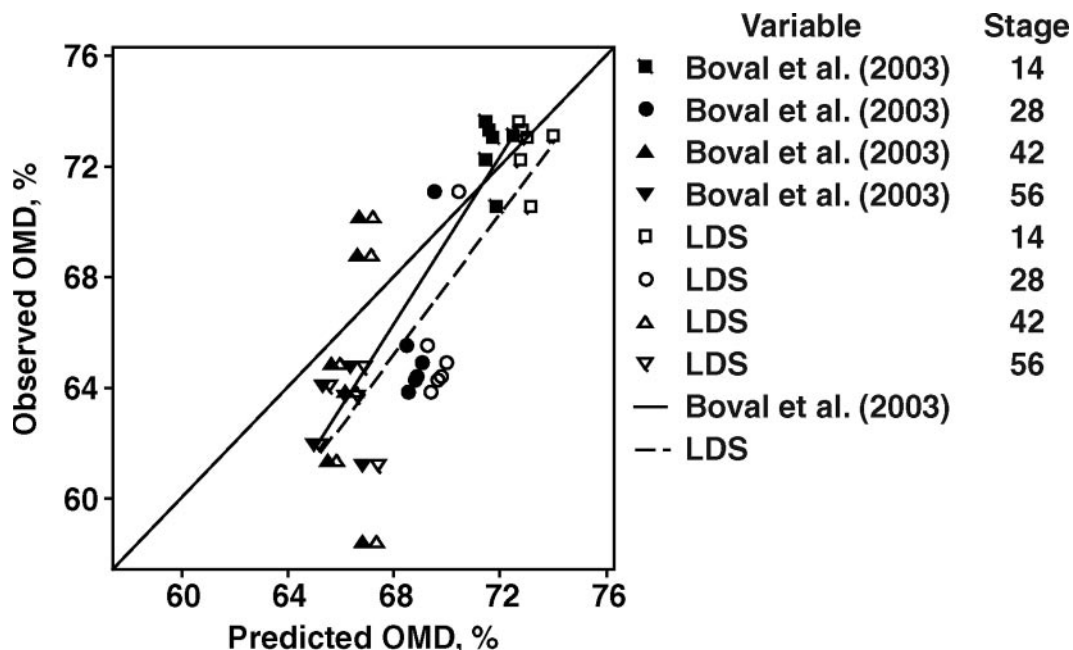
### Fecal NIRS Equations

The prediction of OMD by fecal NIRS using the LDS was as expected and confirmed the potential of this indirect method to assess in vivo OMD for grazing animals. Contrary to CPf equations, the precision of fecal NIRS equations increased by using a larger data set. The Eq. 2<sub>NIRS</sub> calculated from the LDS was more precise than Eq. 1<sub>NIRS</sub> derived from a smaller data set. Enlarging the data set increased variability in spectral proper-

ties, which led to an increase in precision of prediction. In addition, using the LDS expanded the prediction potential of the equation, particularly for low OMD values (<0.60). For NIRS to be used successfully, it is essential that the sample and reference data cover all sources of variation likely to be encountered in routine analysis (Kitessa et al., 1999). Statistics of calibration for Eq. 2<sub>NIRS</sub> were thus better than those previously published. Indeed, SEC of 2.26 and 2.2, and R<sup>2</sup> of 0.94 and 0.72 were reported, respectively, by Krachounov et al. (2000) for sheep fed an array of forage, and Boval et al. (2004) for cattle fed tropical grasses.

### Validation

The 2 CPf equations provided good estimates of in vivo OMD of the VDS. The precision of prediction using Eq. 1<sub>CP</sub> or Eq. 2<sub>CP</sub> on the VDS were close, although Eq. 2<sub>CP</sub> presented a slightly smaller residual SD. The variation of the difference between predicted and observed values was less for Eq. 1<sub>CP</sub> compared with Eq. 2<sub>CP</sub>, indicating a more robust prediction. However, both



**Figure 2.** Observed and predicted OM digestibility (OMD) by fecal CP equations of Boval et al. (2003,  $OMD = -34.1 + 1.47 OMD_{Eq. 1CP}$ , and  $r^2 = 0.63$ ) or derived using a large data set (LDS;  $n = 174$ ,  $OMD = -21.2 + 1.27 OMD_{Eq. 2CP}$ , and  $r^2 = 0.63$ ).

**Table 4.** Relationships between OM digestibility observed (OMD, %) in an independent validation set and predicted OMD, using 4 different equations, based on fecal CP (CPf; Eq. 1<sub>CP</sub> and Eq. 2<sub>CP</sub>) or fecal near infrared reflectance spectroscopy (Eq. 1<sub>NIRS</sub> and Eq. 2<sub>NIRS</sub>)

| Prediction of OMD                                   | Residual SD | Dpo <sup>1</sup> | SD   | P-value <sup>2</sup> |       |
|---|-------------|------------------|------|----------------------|-------|
|   |             |                  |      | RS                   | A     |
| OMD = 0.97 × OMD <sub>Eq. 1CP</sub> <sup>3</sup>    | 3.04        | 1.65             | 3.02 | 0.01                 | 0.01  |
| OMD = 0.959 × OMD <sub>Eq. 2CP</sub> <sup>4</sup>   | 2.80        | 2.79             | 2.79 | 0.04                 | 0.009 |
| OMD = 0.983 × OMD <sub>Eq. 1NIRS</sub> <sup>5</sup> | 3.15        | 0.98             | 3.21 | 0.002                | 0.003 |
| OMD = 0.965 × OMD <sub>Eq. 2NIRS</sub> <sup>6</sup> | 2.53        | 2.40             | 2.55 | 0.45                 | 0.009 |

<sup>1</sup>Dpo = difference between predicted and observed OMD.

<sup>2</sup>Factorial ANOVA of Dpo, including the effects of regrowth stage (RS) and animal (A).

<sup>3</sup>OMD<sub>Eq. 1CP</sub> = OMD predicted by the equation Eq. 1<sub>CP</sub> of Boval et al. (2003): OMD = 86.6 - 266.2/CPf.

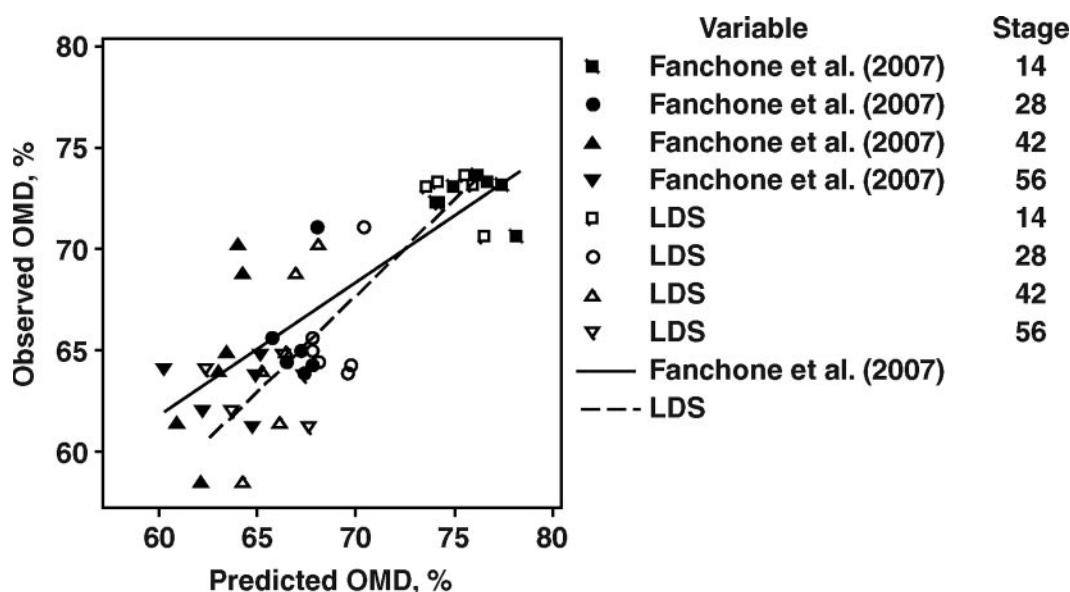
<sup>4</sup>OMD<sub>Eq. 2CP</sub> = OMD predicted by the fecal CP equation Eq. 2<sub>CP</sub> derived using the large data set: OMD = 88.4 - 263.9/CPf.

<sup>5</sup>OMD<sub>Eq. 1NIRS</sub> = OMD predicted by the equation Eq. 1<sub>NIRS</sub> of Fanchone et al. (2007).

<sup>6</sup>OMD<sub>Eq. 2NIRS</sub> = OMD predicted by the equation Eq. 2<sub>NIRS</sub> derived using the large data set.

CPf equations overestimated the decreased values of OMD measured for 42 and 56 d of regrowth. If studies agreed on the existence of a biological relationship between OMD and CPf, they also agreed on the fact that undigested dietary protein adversely affects this relationship (Lukas et al., 2005; Schlecht and Susenbeth, 2006). When digestion of dietary CP is constrained, the undigested dietary protein fraction of CPf increases and induces an artificial increase in the OMD prediction. Particularly, tropical forages are known to be resistant to digestion because they mature rapidly. High fiber content of tropical forage may restrict CP digestion in the rumen leading to an increase of undigested dietary protein. For example, Archimède et al. (2000) reported a decrease in the apparent total-tract CP digestibility from 0.67 to 0.32 for 14- and 56-d regrowth of Pangola

grass, respectively. This decreased CP digestibility for more mature forage can generate increased fractions of undigested dietary protein in feces and an overestimate of the prediction of OMD by the CPf method. Fecal NIRS equations provided more rewarding estimates of in vivo OMD of the VDS than CPf equations. The Eq. 2<sub>NIRS</sub> had the least residual SD of the 4 equations tested and appears to be more precise. This equation was also the most reliable because the Dpo varied to a lesser extent than in the Eq. 1<sub>NIRS</sub> and CPf equations, and only the effect of animal was significant on Dpo. For the other models, both animal and stage of regrowth had a significant effect on Dpo. After increasing the variability in spectral properties and reference data by using the LDS, the entire range of variation of the VDS was covered, resulting in a gain in precision and reli-



**Figure 3.** Observed and predicted OM digestibility (OMD) by fecal near infrared reflectance spectroscopy (NIRS) equations of Fanchone et al. (2007, OMD = 21.7 + 0.665 OMD<sub>Eq. 1NIRS</sub>, and  $r^2 = 0.66$ ) or derived using a large data set (LDS;  $n = 174$ , OMD = 2.59 + 0.928 OMD<sub>Eq. 2NIRS</sub>, and  $r^2 = 0.68$ ).



ability of prediction. The main advantage of the NIRS compared with the CPf technique is its ability to take indirectly into account several predictors in the calibration process, which improves the predictive ability. Although the CPf method takes into account only one chemical component of feces to derive an equation, the NIRS technique provides 700 absorbances of light in wavelengths ranging between 1,100 and 2,500 nm, each one a potential indicator of diet characteristics. Therefore, the NIRS technique can retain absorbances related to CPf, as well as spectral absorbance values for other constituents associated with OMD of the diet. Thus, fecal NIRS takes into account the microbial and endogenous fractions of CPf that are correlated to OMD.

Furthermore, other advantages of the NIRS technique compared with CPf are speed and repeatability of prediction, and the fact that it does not require repeated chemical analyses except for calibration (Stuth et al., 2003). In addition, NIRS calibration permits the estimation of several constituents from the scan of a single sample (Stuth et al., 2003). The main limitation of this approach is the difficulty of obtaining a sufficient number of samples, with values measured in vivo, to develop calibration equations (Deaville and Flinn, 2000). Nevertheless, this drawback is also shared by the CPf method. However, given the similarity between Eq. 1<sub>CP</sub> and Eq. 2<sub>CP</sub> for the VDS, it appears possible to achieve satisfactory predictions with a small set of data. It is necessary, however, that this small data set covers a range of sufficient variation of CPf and that a suitable model, such as hyperbolic or exponential, is used (Lukas et al., 2005). When using NIRS, in contrast to the CPf method, the data set used by Boval et al. (2003) may not allow for consistent calibration because of a narrow range of spectral properties. Also, even if the NIRS has many advantages, the method of CPf can be useful with a small data set to derive reliable predictive equations of OMD. In fact, each of the 2 methods can be useful to estimate digestibility of pasture in different contexts.

This study has shown that CPf content is a reliable index to predict in vivo OMD for sheep. The hyperbolic model first proposed by Lancaster (1949) is of interest because it describes a biological relationship between OMD and CPf that allows reliable estimates using an independent data set with values outside the range of the originally modeled data. However, NIRS applied to fecal samples allows better estimation of in vivo OMD than the CPf method because it can take into account more indicators of digestibility (Andrès et al., 2005). An increase of variability of the reference data improved the precision of the estimated fecal NIRS equation. Varying the forage species, the agronomic treatment of tropical grass, or extending our data set to cool-season forages should be explored to widen the predictive potential of our fecal NIRS equation and to further increase the precision of prediction. When a small data set of reference data ( $n \approx 40$ ) is available, a hyperbolic equation

based on CPf can be suitable to predict the OMD of grazing animals.

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