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ABSTRACT

The neutral detergent fibre (NDF) degradability of corn silage samples, measured *in vitro* (ivNDFd) by a filter bag system, was used to examine (i) the relationship between the ivNDFd and that calculated from acid detergent lignin (L) content (NDFd) and (ii) the impact of ivNDFd variations on the predicted milk yield (MY) of dairy cows fed corn silage based diets. A total of 173 samples of corn silage were collected during a period of three years (2001-03) in different dairy farms of the Po Valley (Northern Italy). Each sample was analysed for chemical composition and was also tested in triplicate for the ivNDFd using the Daisy^{II} incubator (Ankom, Tech. Co., Fairport, NY, USA) with incubation time of 48hs. Moreover, the NDFd of samples was calculated from the L contents, while the measured ivNDFd values were used to estimate the NEl, the potential dry matter intakes (DMI) and to predict the MY of cows.
Corn silage samples of the three years were similar for NDF and starch contents (44.2 and 30.7% DM, on average, respectively) while samples from 2003, in comparison with 2001 and 2002, had lower crude protein (6.9 vs 8.3-8.4% DM, $P<0.01$) and L contents (3.3 vs 3.6-3.9% DM, $P<0.01$) and higher ivNDFd values (53.3 vs 45.6-47.8%, $P<0.01$). The relationship between ivNDFd and NDFd was weak ($R^2=0.09$, not significant). The MY predicted from the NEl content and DMI of corn silage (5.5 MJ/kg DM and 8.9 kg/d) minus the maintenance energy costs, was 11.5 kg/d on average (coefficient of variation 20%). Our simulations indicate that a variation of ivNDFd by $\pm$1.0% changes the NEl of corn silage to have an expected variation in milk yield of $\pm$0.15 kg/d. If the ivNDFd is also used to predict the corn silage DMI then a $\pm$1.0% variation in ivNDFd of corn silage produces an overall $\pm$0.23 kg/d MY variation.
The present results indicate that ivNDFd is highly variable in corn silage populations and differences in this nutritional parameter have an appreciable impact on the predicted milk yield from dairy cows.

Key words: Corn silage, Rumen degradability, NDF, Milk yield.

RIASSUNTO

DEGRADABILITÀ DELL'NDF DEL SILOMAIS E PREVISIONE DELLA PRODUZIONE DI LATTE IN BOVINE

Il lavoro si è proposto l'obiettivo di studiare, su una raccolta di campioni di silomais, i) la correlazione tra la misura della degradabilità in vitro dell'NDF (ivNDFd) e i valori di digeribilità dell'NDF (NDFd) calcolati

sulla base del contenuto di lignina (L) e ii) l'impatto che le variazioni della ivNDFd hanno sulla produzione di latte di bovine ad alta produzione. Centosettantatre campioni di silomais sono stati raccolti durante tre annate consecutive (2001-03) in allevamenti di bovine da latte della Pianura Padana ed analizzati per il contenuto di sostanza secca, ceneri, proteina grezza (PG), estratto etereo, amido, fibra neutro detersa (NDF), acido detersa (ADF) e lignina (L). Per ogni campione è stata effettuata la stima della NDFd sulla base del contenuto di L e la misura in triplo della ivNDFd, a 48 ore di durata della incubazione ed impiegando l'incubatore Daisy^{II} (Ankom, Tech. Co., Fairport, NY, USA). I dati di ivNDFd sono stati utilizzati per stimare l'ENl del silomais, l'ingestione di sostanza secca e la produzione potenziale di latte delle bovine.
I silomais raccolti nelle diverse annate hanno presentato contenuti simili di NDF e amido (in media rispettivamente 44,2 e 30,7% SS); tuttavia i campioni del 2003, rispetto a quelli raccolti nelle annate precedenti, hanno evidenziato contenuti di PG e L significativamente più ridotti (6,9 vs 8,1-8,4% SS, P<0,01, 3,3 vs 3,9-3,6% SS, P<0,01, rispettivamente). I valori di ivNDFd sono risultati più elevati nei campioni del 2003 rispetto agli altri due anni (53,3 vs 45,6 e 47,8%, P<0,01) e, complessivamente, la variabilità entro anno è risultata molto elevata (coefficiente di variabilità del 14%). La ivNDFd è risultata scarsamente correlata con l'NDFd calcolata in funzione del contenuto di L (R^2=0,09). I contenuti di ENl e di ingestione giornaliera del silomais (in media 5,5 MJ/kg SS e 8,9 kg SS/d, rispettivamente) calcolati sulla base dei valori di ivNDFd, ed al netto dei costi di mantenimento, hanno consentito di stimare una produzione potenziale di latte di 11,5 kg/d (±20%). Nelle specifiche ipotesi del lavoro, la variazione di ±1% di ivNDFd del silomais consentirebbe una modifica produttiva stimata pari a ±0,15 Kg/d di latte per il solo effetto sulla concentrazione energetica del silomais. Considerando l'influenza dell' ivNDFd anche sull'ingestione, la medesima variazione, potrebbe modificare la produzione giornaliera di latte di ±0,23 Kg/d.
Utilizzando la specifica raccolta di dati di composizione chimica e nutrizionali di silomais nazionali, il lavoro ha consentito di stimare l'elevato impatto che le variazioni di ivNDFd del foraggio hanno sulle potenziali prestazioni produttive di bovine da latte ad alta produzione.

Parole chiave: *Silomais, Degradabilità ruminale, NDF, Produzione di latte.*

Introduction

The fibre (NDF) of corn silage has a variable rumen degradability, which influences its energetic value and intake by animals (Schwab *et al.*, 2003; Oba and Allen, 2005).

As corn silage is the primary source of fibre in most diets for dairy cattle, several models are now available to predict the milk yield of cows by considering the effect of NDF degradability both on the energetic value and on the intake of corn silage (Schwab *et al.*, 2003; Allen, 2006; Shaver, 2006). These models have been originally proposed to rank corn hybrids for genetic selection (Hartnell *et al.*, 2005), but they are now also used by dairy nutritionists and producers as useful tools to assess dairy diets based on corn silage (Chase *et al.*, 2005; Nelson *et al.*, 2005).

NDF degradability is measured by *in vitro* rumen fermentation techniques (ivNDFd) based on filter bag (Robinson *et al.*, 1999; Adesogan, 2005) and vessel systems (Hall and Mertens, 2008). As an alternative, it is predicted by lignin (L) content of feedstuffs (NDFd; NRC, 2001).

Present study has used the ivNDFd of corn silage samples measured by a filter bag system to examine:

- the relationship between the ivNDFd and NDFd calculated by L content;
- the impact of ivNDFd variations on the predicted milk yield of cows fed corn silage based diets.

Material and methods

Origin of samples and chemical analysis

A total of 173 samples of corn silage were collected during three consecutive years (2001, 2002 and 2003) in dairy farms located in the Po valley (Northern Italy) by two laboratories (2001 and 2002 samples from one lab and 2003 samples from the other). Each laboratory analysed their own samples, which were dried at 60°C, milled and then analysed for dry matter (DM), ash, crude protein (CP), ether extract (EE), (AOAC, 1998). For the analysis of fibre (NDF, ADF and L content, Van Soest *et al.*, 1991) the Ankom^{200} apparatus (Ankom ®, Tech. Co., Fairport, NY, USA) was used. For NDF analysis, samples were treated with α-amylase, ND solution contained sodium sulfite and residues were not corrected for residual ash. Starch (S) content was measured by polarimetric method (Martillotti *et al.*, 1987) and NDF-bound CP (NDICP) and ADF-bound CP (ADICP) were assumed equal to 1.3 and 0.8% DM (NRC, 2001), respectively. The non fibre carbohydrates (NFC) content was calculated as 100-(CP+ash+EE+NDF-NDICP).

In vitro NDF degradability measurement

Each sample was tested in triplicate for the ivNDFd by the Daisy^{II} incubator (Ankom, Tech. Co., Fairport, NY, USA) according to Robinson *et al.* (1999). The *in vitro* rumen incubations were performed by each laboratory in consecutive fermentative runs during the Spring of 2004 and reference forages were used in each run as standard samples. The samples were finely ground (1 mm screen) and an amount of about 250 mg was inserted in filter bags (Ankom F57) and then sealed. Four digestion jars were filled with pre-warmed (39°C) buffer solutions (266 ml of solution A: KH_2PO_4 10 g/l, $MgSO_47H_2O$ 0.5 g/l, NaCl 0.5 g/l, $CaCl_22H_2O$ 0.1 g/l, Urea 0.5 g/l; 1330 ml of solution B: Na_2CO_3 15.0 g/l, Na_2S9H_2O 1.0 g/l) and placed into a Daisy^{II} incubator. Rumen liquor was collected from two rumen fistulated cows fed a forage based diet and 400 ml of filtered liquor was introduced into each jar together with the filter bags. After 48h of incubation, the bags were removed, rinsed thoroughly with cold tap water and immediately analysed for NDF content with the ANKOM^{200} Fiber Analyzer.

Calculations

NDFd – For each sample the NDFd was calculated as function of the L content (NRC, 2001):

$$NDFd=(0.75x((NDF-NDICP)-L)x[1-(L/(NDF-NDICP)^{0.667}])/NDF$$

NEl content- For each sample, the CP, NFC and NDF contents (in % DM) were used to calculate their respective truly digestible (td) amounts as follows (NRC, 2001):

td CP=CP x exp[-1.2 x (ADICP/CP)];
td NFC=0.98 x NFC;

The td NDF was calculated for each sample from both ivNDFd and assuming a fixed NDF degradability value (equal to the average ivNDFd of the year of sample collection, ivNDFdy):

td NDF=(NDF x ivNDFd); (1)
td NDF=(NDF x ivNDFdy); (2)

The digestible energy content at maintenance was calculated as follows (DE_{1X}, NRC, 2001, eq. 2-8a, NFC processing adjustment factor of 0.94, fatty acids (FA) content as (EE-1):

$$DE_{1X}(Mcal/kgDM)=[(tdCP/100x5.6)+(FA/100x9.4)+(tdNDF/100x4.2)+(tdNFC/100x0.94x 4.2]-0.3$$

The two DE values of each sample, differing in td NDF calculation (by equation 1 or 2, respectively), were adjusted for a level of intake of 3 times maintenance (NRC, 2001) and for a dietary total digestible nutrient content (TDN) of 0.74 (NRC, 2001). The two DE values of each sample were the basis of calculation of the two NEl values of each sample, applying the equations 2-10, 2-11 and 2-12 of the NRC (2001). The NEl values, expressed in megacalories, were then converted in megajoules using a multiplying conversion factor of 4.184.

Prediction of corn silage DM intake - The prediction of corn silage DM intake (CSDMI) was based on the following assumptions:

- average total daily NDF intake: 1.2% of live weight (LW, equal to 650 kg) of cows (Mertens, 1987);
- dietary NDF content: 32% DM;
- corn silage content in diets: 50% of dietary NDF;
- ±0.12 kg DM/d of dietary intake (Oba and Allen, 2005) for each percentage of ivNDFd variation. We assumed that the increase in total DMI was from CSDMI (Schwab *et al.*, 2003).

For each sample the CSDMI was calculated according to the following two equations, respectively:

$$CSDMI(kg/d)=[(650*0.012*.50)/(NDF/100)] \quad (3)$$

$$CSDMI(kg/d)=[(650*0.012*.50)/(NDF//100)]+[(ivNDFd–ivNDFdy)*0.12*.50] \quad (4)$$

Prediction of milk yield - The daily milk yield (3.5% fat, 2.93 MJ/kg milk) was predicted according to Schwab *et al.* (2003):

$$MY(kg/d)=(A-B)/2.93 \quad (5)$$

where A is the amount of NEl supplied by corn silage (in MJ/d, i.e. CSDMI x corn silage NEl) and B is the maintenance energy requirement of cows (0.335 MJ/kg $LW^{0.75}$, NRC, 2001) proportioned according to the concentration of corn silage in the diet (e.g. 50% of dietary NDF for diets having 32% DM of NDF).

For each sample three different MY were estimated, each one as a result of a different combination of calculated corn silage NEl and DMI:

- MY1: NEl calculated from td NDF by eq 1 and CSDMI by eq 3;
- MY2: NEl calculated from td NDF by eq 2 and CSDMI by eq 3;
- MY1: NEl calculated from td NDF by eq 2 and CSDMI by eq 4.

Finally, for each sample the following two differences in estimated MY were calculated:

dMY1=MY2-MY1;
dMY2=MY3-MY1.

Statistical analysis

Chemical composition and ivNDFd data were analysed (SAS, 1999) according to the following linear model:

$y_{ij}=\mu+\alpha_i+\varepsilon_{ij}$

where: μ=overall mean; α=fixed effect of year (i=1,3).

The predicted MY differences (dMY1, dMY2) and the NDFd calculated by L content were regressed on ivNDFd according to the following linear mixed model (SAS, 1999):

$$Y_{ij}=B_0+B_1X_{ij}+s_i+e_{ij}$$

where:

B_0 is the overall intercept across years (fixed effect), B_1 is the across years, overall regression coefficient for the linear effect of dMY or NDFd (fixed effects), X_{ij} is the ivNDFd for the ith sample of the jth year, (i=1, …, n_j; j=1, … 3), s_i is the random effect of year i, approximately normal (0, σ^2_s), and e_{ij} is the residual error, approximately normal (0, σ^2_e). Adjusted values for the year effect, calculated according to St-Pierre (2001), were used to generate two dimensional graphs.

Results and discussion

In the present research we examined data of corn silage samples collected during three years to obtain a representative data set from a homogeneous area. The two laboratories involved in the trial, which analysed samples of different years, utilised the same protocols and previously participated in collaborative ring tests regarding the chemical and ruminal fermentative procedures (Getachew *et al.*, 2002; Bovera *et al.*, 2003). Moreover, the main statistical analyses were performed by using a mixed model in which the regressions were examined within the year effect, allowing the removal of any laboratory effect (e.g. year effect confounded with lab).

Chemical composition and *in vitro* degradability of corn silages are shown in Table 1. Corn silages collected in different years were similar in terms of NDF and S content (44.2 and 30.7% DM, on average, respectively) and differed slightly for EE and ash levels. Samples of 2003 had lower CP and L contents with respect to the other two years (6.9 vs 8.1-8.4% DM, P<0.01 and 3.3 vs 3.9-3.6% DM, P<0.01, respectively).

The ivNDFd values were higher for 2003 samples in comparison with the other two years (53.3 vs 45.6 and 47.8%, P<0.01). The within year variability of ivNDFd (14% of CV) was higher than that reported by Wisconsin University Extension lab 8% (Hoffman *et al.*, 2006) and confirmed the wide range of variation found in US populations by Oba and Allen (2005) (36-70%, 30 h incubation) and by Shaver *et al.* (2003) (43-82%, 48 h incubation). Allen (2006) indicates that commercial hybrids (excluding brown midrib types) can differ up to 5% in ivNDFd, when averaged over many growing environments. However, the high variability found in our inventory of samples is the result of the overall genetic, agronomic and harvesting conditions found in the dairy farms located in the considered area.

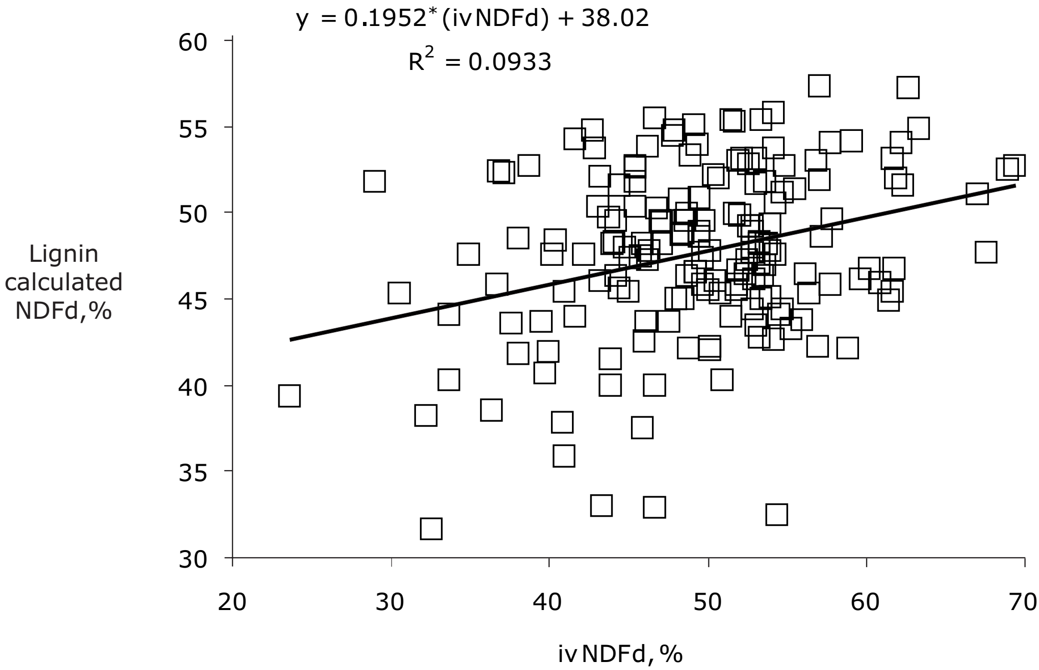
The relationship between ivNDFd and that calculated on the basis of L content was weak (R^2=0.09) as reported in Figure 1. This is in agreement with that found by Shaver (2006) in an inventory of 534 corn silages (R^2=14%) and by Robinson *et al.* (2004) in a study across several categories of feedstuffs (forages, grains, protein meals and by products). However, data obtained

Table 1. Chemical composition and *in vitro* NDF degradability of corn silages.

		Year			RSD
		2001	2002	2003	
Dry matter	g/ kg	345	352	338	48
Ash	g/kg DM	50^B	53^A	45^C	7
Ether extract	"	27^C	28^B	34^A	3
Crude protein	"	81^B	84^A	69^C	7
NDF	"	450	436	441	50
ADF	"	244	238	250	35
ADL	"	39^A	36^A	33^B	9
Starch	"	317	306	298	55
In vitro NDF degradability (ivNDFd)	%	45.6^B	47.8^B	53.3^A	7.0

Means in the same row with different letters differ (P<0.01).

Figure 1. Regression between the calculated NDFd according to the lignin contents (adjusted for year effect) and the *in vitro* measured NDFd of corn silages.



in vitro with the Daisy fermenter are accurate, given the satisfactory relationship between ivNDFd and data obtained *in vivo* (Robinson *et al.*, 2004), *in situ* (Spanghero *et al.*; 2003, 2007) or with other traditional *in vitro* procedures (Adesogan, 2005). The lack of relation between ivNDFd and NDFd predicted by L is surprising as the stover lignification is mainly responsible for the NDF degradability decline with advancing the plant maturity (Traxler *et al.,* 1998). Recently, Masoero *et al.* (2006) measured L content in corn stalk of 5.2, 6.3 and 7.4% DM at the vegetative stages of blister, dough and at the physiological maturity, respectively. An evident proof of the negative role of L on fibre digestibility has been clearly demonstrated by the higher NDF degradability found for the brown midrib mutants, which are selected for low L contents (Hartnell *et al.*, 2005). A possible explanation of the poor NDF degradability prediction by L content could be the unique chemical modification of corn silage with advancing maturity, where the NDF degradability declines and the L content tends to be quite stable, as result of the stover lignification and the starch accumulation, respectively. In addition, the very scarce precision of the analytical measure of L, which was also demonstrated using the filter bag technology (Bovera *et al.*, 2003), can also contribute to increase this variability.

The simple statistics of calculated NEl content, expected corn silage DMI and predicted potential MY of dairy cows are

Table 2. Simple statistics of NEl content, corn silage DM intake and expected milk yield calculated according to different models.

Models used in calculations	Procedure of calculation					
	Equation used (see text[1])	ivNDFd[2] inclusion	Mean	SD	Min	Max
NEl content, MJ/kg DM:						
-NRC, 2001	(1)	No	5.51	0.40	4.54	6.23
- " "	(2)	Yes	5.52	0.48	4.02	6.67
Corn silage DMI, kg/d:						
-Mertens, 1987	(3)	No	8.93	1.01	7.01	12.01
- " " ; Oba and Allen, 2005	(4)	Yes	8.93	0.98	6.69	11.38
Estimated milk yield, kg/d:						
-Schwab *et al.*, 2003	(1, 3)		11.50	2.31	6.71	18.28
- " "	(2, 3)		11.50	2.27	6.02	17.09
- " "	(2, 4)		11.53	2.42	5.71	17.51

[1]*numbers correspond to equations reported in Material and methods section.*
[2]*in vitro measured on each sample.*

shown in Table 2. The average NEl contents in our dataset (eq. 1,3 and eq. 2,4; 5.51-5.52 MJ/kg DM) are lower than those reported by NRC (2001) for a medium maturity (32-38% DM) corn silage (6.07 MJ/kg DM). This is partly due to differences in chemical composition (higher ash and low EE content of corn silages of the considered inventory) and also because the NDFd, calculated by L contents, in the NRC is 59% (Shaver, 2006), which is about 20% higher than the average of our measured data. The variability of NEl content was moderately increased when the calculations included the ivNDFd measured upon each individual sample (standard deviations from ±0.40 to ±0.48 MJ/kg DM).

The MY predicted from the NEl content and DMI of corn silage over the maintenance energy costs, was 11.5 kg/d on average, with a coefficient of variation of about 20%.

The calculated difference in predicted MY (dMY1 and dMY2) varied as function of ivNDFd (%) with the following equations, respectively:

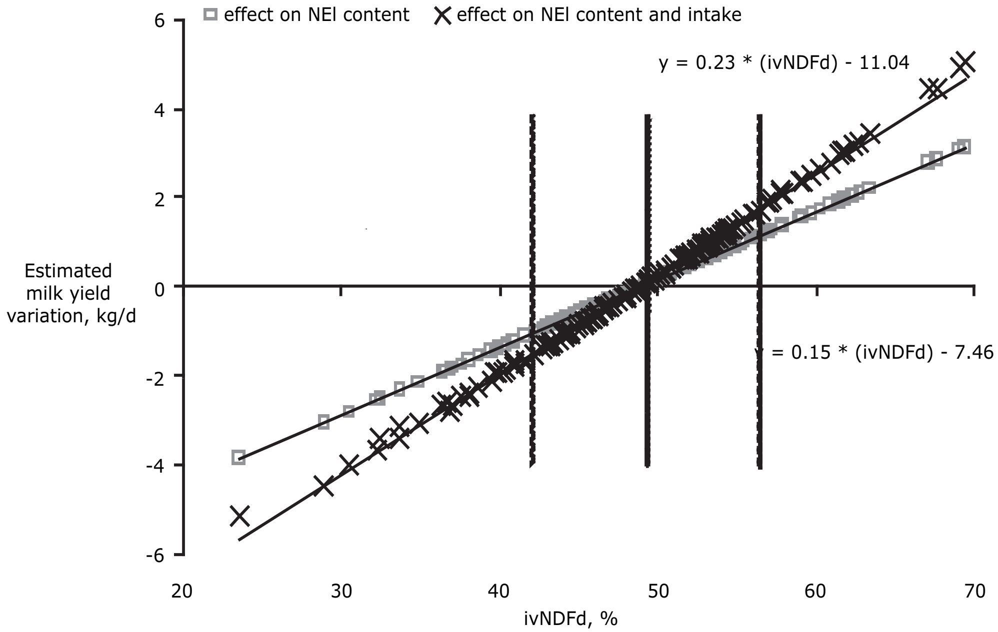
$$dMY1\ (kg/d) = -7.46_{(\pm 0.3476)} + 0.1526_{(\pm 3.52E\text{-}6)} * ivNDFd \quad (P<0.001)$$

$$dMY2\ (kg/d) = -11.04_{(\pm 0.5184)} + 0.2265_{(\pm 0.0010)} * ivNDFd \quad (P<0.001)$$

In Figure 2 the plots of the estimated variation of daily milk yield (dMY1, dMY2, adjusted for year effect) and the *in vitro* NDFd are shown.

From the equation slopes it can be calculated that a variation of ivNDFd by ±1.0% changes the NEl of corn silage to produce an expected variation in milk yield of ±0.15

Figure 2. Estimated variation in daily milk yield of dairy cows (adjusted for year effect) fed corn silages differing in NEl content (NRC, 2001) and intake (Oba and Allen, 2005) according to their *in vitro* measured NDFd (ivNDFd).



kg/d. If the corn silage DMI is calculated from both NDF content (Mertens, 1987) and NDF degradability (Oba and Allen, 1999, 2005) and the NEl incorporates also the measured NDF degradability value, a similar variation in ivNDFd of corn silage (±1.0 %) makes it possible to calculate an overall expected difference in MY of ±0.21 kg/d. Therefore, on the basis of our simulation and within our assumptions, the impact of NDF degradability variations on MY of cows can be mainly attributed to variations in the NEl concentrations (about 65% of total MY variation) and to a lesser extent to modifications in intake of corn silage by cows (about 35% of total MY variation).

These simulations can be easily reproduced, but the present results are strictly dependent on the specific characteristics of dataset of corn silages (in terms of chemical composition, ivNDFd range and average lab) and by specific dietary conditions in models (dietary NDF concentration, contribution of corn silage to dietary NDF, animal LW, etc.).

However, a critical point of such approach could be the direct inclusion of *in vitro* NDFd into the NRC summative equation (2001) and in available models (Schwab *et al.*, 2003; Allen, 2006; Shaver, 2006) without any modification, such as a *in vitro* to *in vivo* transformation. Therefore, the accuracy of animal performance predictions based on *in vitro* data should be further verified with dedicated *in vivo* experiments.

Conclusions

The experimental data and simulations in present study indicate that ivNDFd is highly variable in corn silage populations and differences in this nutritional parameter have an appreciable impact on the predicted milk yield from dairy cows. In addition, the poor capacity of lignin content to predict ivNDFd of corn silages has been confirmed.

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