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Short communication

Short communication: The relationship between dietary particle size and undegraded neutral detergent fibre in lactating dairy cows: A prospective cohort observational study

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A B S T R A C T

Physically effective NDF (peNDF) and undegraded aNDF at 240 h (uNDF.240) are important parameters for characterizing NDF in fibre evaluation and are associated with dietary physical form and fibre digestibility characteristics. A new concept that combines peNDF and uNDF.240, physically effective uNDF.240 (peuNDF.240 = pef × uNDF.240), was recently established. The peuNDF.240 value allows determination of dry matter intake (DMI), and the productive response of cows even in the absence of variation in DMI or when cows are fed rations with low uNDF.240 and high peNDF or rations with high uNDF.240 and more finely chopped fibre. The aim of this study was to improve our understanding of the relationships between dietary uNDF.240 content to other fibre fractions, average cow DMI, gross feed efficiency, and milk yield at the farm level. Furthermore, the relation between peuNDF.240 and the productive response of cows was also investigated at the farm level. In the Po' Valley, which is a representative area for dairy production in Italy, a cohort of 22 Holstein dairy farms was monitored over two years (2019–2020). Information regarding average cow DMI, milk yield, and ration composition was obtained through interviews with farmers, and feed samples were collected and chemically analysed. Farms were classified according to their dietary uNDF.240 (% of DM) content: low (uL) ≤ 8.29 or high (uH) > 8.29. Farms with low dietary uNDF.240 used less alfalfa forage as a fibre source compared with farms with high dietary uNDF.240 (6.27 *vs.* 15.5 % of DM) and showed higher average milk yield (35.9 *vs.* 33.6 kg/cow/day, respectively) and similar DMI (23.9 *vs.* 24.3 kg/cow/day, respectively). Dietary peuNDF.240 was negatively related to milk yield (milk yield = 47.4 – 1.87 peuNDF.240, $R^2 = 0.62$, adjusted $R^2 = 0.60$, residual standard error (RSE) = 1.87, P = 0.001) and gross feed efficiency (gross feed efficiency = 1.96 – 0.08 peuNDF.240, $R^2 = 0.65$, adjusted $R^2 = 0.64$, RSE = 0.07, P = 0.001). The results of this study have practical significance for farmers, as they suggest that the inclusion of low digestible forages in the ration (*i.e.,* late-harvested alfalfa characterized by high uNDF.240) may require more fine shredding to reduce the overall value of peuNDF.240 and increase cow production.

1. Introduction

The control of the forage-to-concentrate ratio has been critically important in dairy cow feeding in Italy. The Parmigiano-Reggiano

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(The Parmigiano Reggiano Cheese Consortium, 2018) and Grana Padano (The Consorzio Tutela Grana Padano, 2019) regulations require at least 50 % of the dry matter (DM) of the ration to come from fodder and at least 75 % from local crops. Both, the use of forages from local production and the selection of crops have economic, environmental, and social impacts (Martin et al., 2017; Tabacco et al., 2018; Zucali et al., 2018). Moreover, the provision of forages in the ration promotes chewing and saliva production in lactating cows (Beauchemin et al., 2008), raising the ruminal pH and consequently reducing the risk of metabolic diseases such as subacute ruminal acidosis (SARA), which can be prevalent even in well-managed dairy herds (Morgante et al., 2007). The onset of SARA negatively affects milk yield and quality and rumen functionality by reducing the growth of fibrolytic bacteria and fibre degradation in the rumen as well as the microbial AA supply to the small intestine (Plaizier et al., 2009; Yang and Beauchemin, 2009).

The fibre in the ration can negatively affect feed intake (Mertens, 1987; Dado and Allen, 1995). Forages with low fibre digestibility and coarse particles because of their filling effects in the rumen can limit intake, which does not permit lactating cows to achieve their maximum milk production. Since the early 1960s (Conrad et al., 1964; Grant et al., 1990a;b;c), researchers have noted that both the amount of fibre and its chemical and physical characteristics strongly affect dry matter intake (DMI), chewing activity, cow performance, and milk quality, especially fibre from forage (Grant, 1997; Fustini et al., 2017). The development of chemical analyses based on the use of detergents by Van Soest in the 1970s (Van Soest, 1967; Van Soest et al., 1991) completely transformed the basic concepts of ruminant nutrition and redefined fibre, including fibre resistant to neutral detergent (NDF) and its different components (hemicellulose, cellulose, and lignin), to better understand the quality of the fibre itself.

Physically effective NDF (peNDF) was proposed later by Mertens (1997), and it is calculated by multiplying the NDF content by its physical effectiveness factor (pef). The value of pef is related to several factors, mainly feed particle size and shape, fragility, moisture content, method of conservation, DMI, and the ratio of eating to ruminating (Mertens, 1997). The proposed pef was originally a coefficient varying from a minimum value of 0 (*i.e.*, the NDF is ineffective for chewing activity) to a maximum of 1 (*i.e.*, the NDF is amenable to chewing activity). Mertens (1997) suggested that a pef = 1 is provided by long grass hay containing 100 % NDF and was used as a reference feed. A simple laboratory technique for the estimation of pef consists of measuring the proportion of particles that are retained by a 1.18-mm sieve using dry forage or feed with a vertical sieve (Cotanch and Grant, 2006). Furthermore, several *on-farm* methods have been developed, such as ASABE (ANSI/ASABE, 2007) and the Penn State Particle Separator (PSPS), in 1996, 2002, and 2013 (Heinrichs, 2013). The PSPS 2002 includes a 1.18-mm screen (Kononoff et al., 2003), and it is designed to determine the smallest fraction of the diet that can affect digestibility. The PSPS 2013 takes into account the fibre fraction with a diameter $\geq$ 4.00 mm, and it can be used to estimate peNDF (Heinrichs, 2013). Lignin is commonly used as an indicator of the indigestible fraction of fibre (Besle et al., 1994; Jung and Allen, 1995; Palmonari et al., 2016), and several equations have been proposed to estimate the undigested fraction of amylase- and sodium sulfite-treated NDF (aNDF) using the lignin and aNDF content, such as those proposed from the Cornell Net Carbohydrate and Protein System (CNCPS = 2.4 $\times$ lignin % of DM/aNDF % of DM, and successive modifications), which define the unavailable carbohydrate fraction (Traxler et al., 1998; Fox et al., 2004). A common method for measuring the quality of fibre is the determination of undegraded aNDF (uNDF) at 240 h (uNDF.240) as the percentage of aNDF that is not degraded after 240 h of incubation with mixed ruminal microbes (Allen and Mertens, 1988; Van Soest, 1994; Raffrenato et al., 2018).

The use of a global parameter summarizing the quality and length of the fibre, the peuNDF at 240 h (peuNDF.240), has been recently proposed and is calculated by multiplying the uNDF.240 by the pef, which refers to the TMR fraction retained on a $\geq$ 1.18-mm screen (Grant et al., 2020). The novelty of this approach indicates the need to evaluate it in the context of feeding principles for dairy cows. Grant et al. (2020) noted the following: "*Research is needed to test this relationship in alfalfa-based diets, pasture systems, and other feeding scenarios that differ markedly from a typical Northeastern and upper Midwestern U.S. diet based primarily on corn silage*." The lack of knowledge of peuNDF.240 in the Italian dairy context could impede the feeding management of dairy cows. The aim of this study was to understand the relationship between dietary uNDF.240 content to other fibre fractions, average cow DMI, gross feed efficiency, and milk yield at the farm level in a cohort of 22 Italian dairy farms. Another aim was to assess whether peuNDF.240 could be related to cow feed intake and milk production at the farm level.

2. Material and methods

An observational open cohort study was conducted over two years (from 2019 to 2020) in the northern region of Italy (Pianura Padana). The studied cohort comprised 22 dairy farms breeding Holstein cows that were monitored from a minimum of one to a maximum of three times (median = 1.5 and mode = 1, two or three consecutive visits occurred in a median of 88 or 166 days, respectively). Recruitment for the open cohort was performed using a list of eligible farms provided by a feeding company operating in the study area, and participation in the study was voluntary. Each visit was conducted by trained research personnel who, supported by the farm owner or manager, completed the survey form and collected feed and ration samples. Data were recorded, and TMR samples were only taken from the lactating cow groups. The information obtained was the average days in milk, the average individual milk yield on the week of the farm visit, the number of milking cows, the amount of forage in the ration, and the TMR composition. Individual forages and other feed samples used in TMR were collected for uNDF.240 analysis. The DMI was calculated as the average daily intake in the week of the survey, corrected by the DM of the analysed TMR sample. The daily intake was calculated at farm level (only for the lactating cow group) as the total distributed ration (net of declared residuals) divided by the number of cows. To obtain a representative sample of the TMR, three subsamples of TMR of approximately 1.00 kg were collected immediately after the distribution by the mixing wagon at the two extremities and in the middle of the feeding line. The three subsamples were pooled and accurately mixed before sieving the whole sample using a PSPS 2013, made of three sieves with 19.0, 8.00, 4.00 mm hole sizes, respectively, and a bottom pan to determine the peNDF (Heinrichs, 2013). In detail, the separator was filled with 1.48 L of wet TMR and on a flat surface shaken 5 times per each side, for a total of 40 shakes. Each TMR sample was sieved twice and data averaged before statistics. The pef was calculated as the proportion of DM (Yang and Beauchemin, 2006, 2009) retained by 19.0, 8.00, and 4.00 mm sieves, assuming the same % of DM of the fractions retained on different sieves. The peNDF was calculated by multiplying the aNDF content of TMR by the pef, not accounting for the aNDF content of the particle fractions retained by each sieve, as proposed by Mertens (1997) who assumed an uniform aNDF distribution over all particle sizes. The geometric mean particle length (GMPL) of TMR samples was calculated adapting the formulas described in ASABE (ANSI/ASABE, 2007) to the PSPS hole sizes used in present trial. The TMR was analysed by a FOSS NIRSystem 5000 (Hillerød, DK). Near-infrared (NIR) calibrations were based on reference chemical analyses carried out through the following procedures: DM, #934.01 (AOAC International, 2003), aNDF with amylase and sodium sulphite used in the NDF procedure and tared F57 bags with 25 μm pore size (Ferreira and Mertens, 2007; Schlau et al., 2021), non-sequential ADF (Vogel et al., 1999) and ADL (Auger and Shipley, 2013; Thomas et al., 2013). The uNDF.240 values of the forages were evaluated by NIR analysis provided by a corporate laboratory (Cortal Extrasoy Spa, affiliate with the Dairy ONE, reporting the uNDFom as undigestible NDF on organic matter - ash free - basis, using the DaisyII Incubator (Coblentz et al., 2019) as reference method). The uNDF.240 of TMR was estimated as the weighted average of the uNDF.240 analysed in each forage used in the TMR formulation (assuming that concentrates contain 0% of dietary uNDF240). To explore the relationships between peNDF or uNDF.240 and TMR composition in terms of the use of alfalfa, DMI, and milk yield, farms were classified into two classes (Low and High) according to their dietary peNDF (% of DM) and uNDF.240 (% of DM) content, using the median of the overall values as thresholds: peL (peNDF $\leq$ 26.2) or peH (peNDF $>$ 26.2) and uL (uNDF.240 $\leq$ 8.29) or uH (uNDF.240 $>$ 8.29).

Data from repeated visits of the same farm were averaged before statistical analysis, and continuous variables were evaluated for normality by the Shapiro-Wilk test and by visual inspection of frequency distribution and Q-Q plot (quantile-quantile plot). Non-normally distributed data were reported as the median and first and third quartiles in parentheses, whereas normally distributed data were reported as the arithmetic mean and SD. Outliers were identified and removed from the dataset if their values deviated from the mean ± 3 SD. A linear model and ANOVA allowed us to test the main effects of peNDF and uNDF.240 classes and their interaction, and results were declared significant at $P < 0.05$. Linear regressions were calculated for continuous variables. All statistics were performed using R version 4.0.2 (2020−06-22).

3. Results and discussion

The monitored farms were characterized by a median number of lactating cows = 172 (first and third quartile = 91–340); ingested TMR, median =47 kg of as fed ration (first and third quartile = 45–49); days in milk, mean = 172, SD = 9.13; and lactation peak milk (kg), mean = 40.2, SD = 19.5. These findings are comparable to those reported in a previous study conducted in the Po' Valley, which is the most important region for the intensive dairy farming system in Italy (Borreani et al., 2013). As in most Italian dairy production systems (Zucali et al., 2018), the forages used in our TMR samples were mostly corn silage, grass silage, and hay. These samples were collected under ordinary farming conditions in which the GMPL of TMR varied between 5.21 and 6.97 mm (mean = 6.13, SD = 0.49).

Chemical and physical compositions of TMR, DMI, gross feed efficiency (GFE = milk yield/DMI), and milk yield according to dietary uNDF.240 class (uL *vs*. uH) are shown in Table 1.

Table 1
Chemical and physical characteristics of TMR, dry matter (DM) intake, Gross Feed Efficiency, and milk yield (least squares means ± SEM) according to dietary uNDF.240.

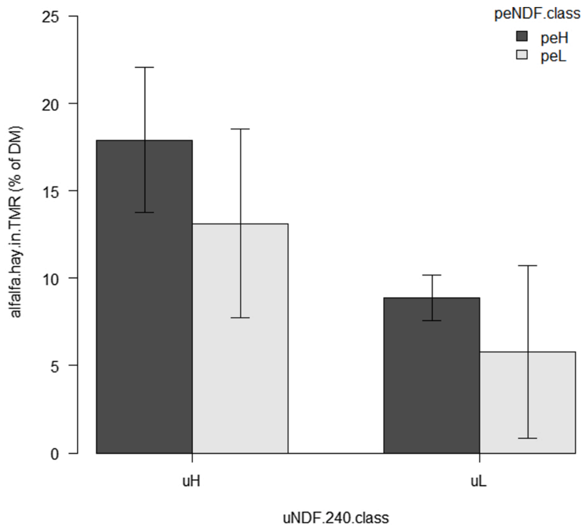
	uNDF.240[1] classes			
	uL	uH	SEM	Probability
Alfalfa hay in TMR (% of DM)	6.72	15.5	1.35	0.001
ADL (% of DM of TMR)	2.96	3.42	0.13	0.020
aNDF (% of DM of TMR)[2]	32.6	33.2	0.39	0.229
uNDF.240/ADL	2.44	2.85	0.12	0.047
aNDF/ADL	11.2	9.81	0.39	0.020
DM intake (kg)	23.9	24.3	0.31	0.390
Gross feed efficiency	1.50	1.38	0.03	0.003
Milk yield (kg)	35.9	33.6	0.79	0.041
peNDF[3] (% of DM)	26.5	26.4	0.29	0.952

[1] uNDF.240 = undegraded aNDF at 240 h of in vitro fermentation; uL (uNDF $\leq$ 8.29), uH (uNDF $>$ 8.29).
[2] aNDF = amylase-modified NDF.
[3] peNDF = physically effective aNDF.

The TMRs analysed in our trial were characterized by aNDF, mean = 32.9, SD = 1.35; uNDF.240, mean = 8.40, SD = 1.59; and peNDF, mean = 26.2, SD = 1.15. These findings are consistent with other Italian TMR samples (Mantovi et al., 2015; Tabacco et al., 2018; Zucali et al., 2018), including diverse forages in addition to alfalfa and longer chop fibre length, but differed from the results of Grant et al. (2020) (aNDF, uNDF.240 and peNDF of 34.6, 10.2 and 20.6 % of DM, respectively), who used *Phleum pratense* at different ratios (from 10.5 to 24.2 % of DM of TMR or 46.8 to 60.5 % of DM of forage) and different chop lengths. The peNDF calculated by Grant et al. (2020) accounted for pef obtained by sieving dry samples in vertical-shaker, and could be therefore lower compared with that obtained by the PSPS 2002 (Yang and Beauchemin, 2006). Our findings in Table 1 showed higher peNDF with respect to Grant et al. (2020) at similar aNDF contents. In our study, the percentage of alfalfa (% of ration DM) was higher for the uH class than for the uL class, and no differences were observed for the peNDF class and the uNDF.240 × peNDF classes interaction (Fig. 1). Palmonari et al. (2016) showed that grass hay and corn silage differed from alfalfa for the uNDF.240/ADL ratio (3.22, 3.11, and 2.37, respectively), and the ratio for alfalfa did not differ (P = 0.516) from the value = 2.4 used in the CNCPS formula. Similar patterns were described by Raffrenato et al. (2019), who reported uNDF/ADL values of 3.38, 3.53, and 2.53 for conventional corn silages, grasses, and alfalfa forages, respectively. In our study, there was no difference in the uNDF.240/ADL ratio and NDF content between the uL and uH classes, but ADL content was higher in the uH class. These findings might be explained by the use of late-harvested and lignified alfalfa in the ration, and their low digestibility.

The milk yield was higher in the uL class, and the DMI did not differ between classes (Table 1). These findings confirm that the use of forages with a high ADL content, a high percentage of uNDF.240, and a low ratio aNDF/ADL at an equal pef value and feed intake less effectively support milk production. This result partially confirms previous findings by Grant et al. (2020), who reported that the DMI was lower only for cows fed high uNDF.240 and high peNDF, and milk production was lower for cows fed high uNDF240 or both low and high peNDF. In the linear regression, ADL was positively related to uNDF.240 (uNDF.240 = 1.71 + 0.18 ADL, adjusted R^2 = 0.33, residual standard error (RSE) = 0.38, P = 0.003), and milk yield was negatively related to uNDF.240 (milk yield = 46.1 – 1.35 uNDF.240, adjusted R^2 = 0.54, RSE = 1.95, P = 0.001) but not to peNDF (milk yield = 65.7 – 1.18 peNDF, adjusted R^2 = 0.07, RSE = 2.68, P = 0.341). Milk yield was negatively related to peuNDF.240 (Fig. 2, panel A) and had a higher R^2 for the milk production estimation compared with the use of uNDF.240 or peNDF as individual predictors. Finally, the DMI was not related to peuNDF.240 (DMI = 24.5 – 0.06 peuNDF.240, adjusted R^2 = -0.05, RSE = 1.13, P = 0.78). The coefficient of determination for the latter regression is considerably lower than that reported in the literature, which probably stems from the greater uncertainty of DMI measures in our survey data. However, the coefficient of determination increased noticeably when GFE was regressed against peuNDF.240 (GFE = 1.96 – 0.08 peuNDF.240, R^2 = 0.65, adjusted R^2 = 0.64, RSE = 0.07, P = 0.001) (Fig. 2, panel B). When low digestible forages are included in the ration, such as late-harvested alfalfa characterized by high uNDF.240, they might need to be shredded more finely to reduce the overall value of peuNDF.240, decrease the pef factor, and reduce the impact of rumen fill by less digestible material.

Some limitations of this study require consideration. The cohort study did not permit us to conduct experiments under controlled conditions; furthermore, several details regarding our farm and herd sample were not considered (e.g., pluriparous and primiparous cows were not distinguished, and farm management was not assessed). Seasonal effects in milk yield, milk quality and DMI were not considered (Ray et al., 1992; Bouraoui et al., 2002). These effects would have presumably decreased the accuracy of our results, which



	uH	uL
peH	17.9%	8.89%
peL	13.1%	5.77%

Fig. 1. Bar graph for alfalfa content in the TMR (% of DM); effects of uNDF.240 (P = 0.001), peNDF (P = 0.059), and uNDF.240 × peNDF (P = 0.677) classes. peNDF classes = peL (peNDF ≤ 26.2), peH (peNDF > 26.2); uNDF.240 classes = uL (uNDF.240 ≤ 8.29) or uH (uNDF.240 > 8.29).

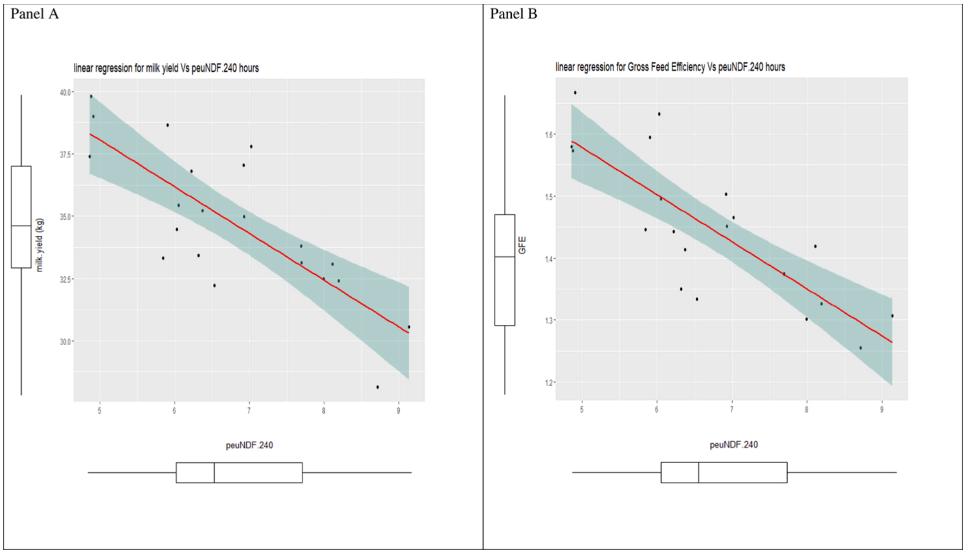


Fig. 2. Linear regression for milk yield (kg) *vs*. peuNDF.240 (panel A); milk yield = 47.4 - 1.87 peuNDF.240, $R^2 = 0.62$, adjusted $R^2 = 0.60$, RSE = 1.87, P = 0.001. Linear regression for gross feed efficiency (GFE) *vs*. peuNDF.240 (panel B); GFE = 1.96 - 0.08 peuNDF.240, $R^2 = 0.65$, adjusted $R^2 = 0.64$, RSE = 0.07, P = 0.001. The grey area represents the 95 % confidence interval for linear regression.

might explain the lower performances that were observed in our regressions compared with those in the literature. Moreover, since aNDF from byproducts does not necessarily provide effective fibre, a more accurate calculation of the peNDF should account for the actual aNDF content of each sieve. Although further investigations using a wider sample size are needed, the prospective results of this study under Italian dairy farming conditions confirmed the patterns recently documented by Grant et al. (2020). Generally, the approach used in this study has practical significance for farmers, as it could prove useful for determining the appropriate cutting length of forages based on their structural quality summarized by the peuNDF.240 value.

Author contribution for the short communication

Serva Lorenzo: Writing - original draft, Data curation; Formal analysis, Methodology
Magrin Luisa: Writing - review & editing
Andrighetto Igino: Conceptualization, Supervision
Marchesini Giorgio: Writing - review & editing

Declaration of Competing Interest

The authors report no declarations of interest.

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