Digestibility and sorting of hay-based total mixed rations employed in the Parmigiano-Reggiano area as affected by dietary particle size distribution

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SUMMARY

Introduction- Together with chemical composition, also the physical form of the Total Mixed Ration (TMR) has been demonstrated to affect dairy cattle digestive physiology and productive performance. However, few studies have been performed on the hay-based diets typically administered to dairy cattle in specific regions like the Parmigiano-Reggiano cheese production area. Moreover, almost none of these studies focused on the relationship between particle size distribution and diet digestion parameters. This relationship needs to be investigated together with the effect of particle size on sorting, in order to provide recommendation on the particle size distribution to be achieved to improve the efficiency of use of these hay-based diets.

Aim- The aim of the present trial was to investigate the relationship between TMR particle size distribution, diet digestibility and sorting of hay-based diets in lactating dairy cows.

Materials and methods- Five farms located in the Parmigiano-Reggiano production area were involved in the study. Three sampling procedures were performed in each farm with 15 day intervals at 0, 12 and 24 hours after TMR delivery. Five fecal samples were collected 12 hours after feed distribution from fresh healthy lactating cows (60 to 90 days in milk). Physical, chemical and digestibility analyses were performed on the TMR samples. Particle size distribution was determined using the Penn State Particle Separator (**PSPS**) and total tract apparent dry matter digestibility (ttaDMDe) and total tract apparent neutral detergent fibre digestibility (ttaNDFDe) were estimated using undigested NDF (**uNDF-**residual NDF after 240 h of fermentation) as a marker in both diet and feces. Dietary uNDF was calculated as weighted average of the uNDF determined on TMR samples collected at the 3 intervals assuming a 60% TMR intake in the first 12 hours after distribution. The relationship between the dietary residues retained on each sieve of the PSPS and ttaDMDe and ttaNDFDe were studied through a curve fitting procedure. The effect of particle size distribution at feed delivery on sorting was also investigated.

Results and discussion- The distribution of the TMR particles, expressed as percentage of the total mass, on the 3 screens and bottom pan was on average 12.1%, 25.2%, 35.1% and 27.4% (Upper-U-, Medium-M-; Lower-L-; and Bottom -B- respectively). The estimated digestibilities were the highest when the U sieve residues ranged between 10 -15% (DMD: 68.35% DM; NDFD: 52.76% NDF); the M sieve residue was around 25% (DMD: 68.73% DM; NDFD: 52.27% NDF), the L sieve ranged from 35 to 40% (DMD: 66.56% DM; NDFD: 50.60% NDF) and the proportion of particles retained in the bottom pan was around 40% (DMD: 68.36% DM; NDFD: 51.57% NDF). Aside from the general sorting against longer particles, the farm with the lowest geometric mean value in the delivered TMR (farm 4: 3.92 mm) shows an increase, after 12 h, in the proportion of particles held in the B. The increased proportion of the biggest particles in the diet (>19 mm) retained on the U sieves was directly related to the variation in the particle size distribution after 12 h.

Conclusions- Particle size seems to affect both digestibility and sorting parameters. A careful preparation of the hay-based TMR diet, considering the suggested values of particle size distribution, may improve the efficiency of its degradation and digestion.

KEY WORDS

Dairy cows, diet; Penn State Particles Separator, physical characteristics.

INTRODUCTION

The Parmigiano-Reggiano consortium disciplinary obliges farmers to use specific feeds providing in the same time indications about the amount of their use in the attempt to regulate diet composition and quality (https://www.parmigianoreggiano.com/it/consorzio-disciplinare-normative/) affecting the final products organoleptic and nutritional properties[1]. Thus, the diets fed in the Parmigiano-Reggiano cheese making area consisting of all dry hay, usually with a high proportion of alfalfa. No fermented forages or feeds can be fed and the majority of the forage should be harvested within the region. The TMR preparation using unchopped dry forages lead to higher variability in dietary roughage particle size and this

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in turn deeply affect rumination and passage rate, the latter being connected to the achievement of a critical particle size. Aside from the chemical parameters generally employed to evaluate diet adequacy also the physical form of the Total Mixed Ration (TMR) has been, in fact, demonstrated to deeply affect feeding behaviour (e.g. sorting), digestion process as well as milk quality and yield [2, 3]. Several parameters and tools have been developed to describe these characteristics of the diet. Among them, the most important are the physical effective NDF (peNDF) and dietary particle size distribution measurement. The former is partially dependent on the second and has been demonstrated to relate with chewing activity, digestion and passage rate [4-6]. Similarly, the particle size distribution affects chewing activity, saliva production, ruminal pH, fermentation processes, feeding behaviour and passage rate exerting a direct effect on feeds and fiber degradation[5]. In this scenario, the use of the Penn State Forage Particle Separator (PSPS) represents a good field tool in order to measure TMR particle size distribution, to study its relationship with the productive parameters and to monitor the sorting activity[2] in the attempt to improve the efficiency of the digestion process.

Several studies have been performed evaluating the effects of peNDF and particle size distribution on intake, digestibility and performance, mainly in silage based diets, but also in high forage based diets[4, 7]. In many cases, the particle size reduction demonstrated a positive correlation with the NDF digestibility[4, 8], and along with dietary NDF content and quality (expressed also by its undigestible fraction - uNDF[9]) could explain the 41% variability of the total tract NDF digestibility[8].

However, few studies have been performed in hay-based diets, which are typically administered in specific regions like the Parmigiano-Reggiano area. In this area the number of farmers feeding cows with the TMR technique has been increasing in the last 20 years, but there are few indications regarding the dietary physical characteristics. In this case, in fact, the particle size distribution was studied mainly in relationship to its effect on the peNDF[2], on the potential sorting after distribution[10] and on the productive performance[1,2].

The relationship between the particle size distribution and the digestion parameters of hay-based diets needs therefore to be investigated in order to provide indications on the optimization of diet exploitation by the animals. The effect of this factor on sorting needs however to be taken into account when recommendations have to be provided.

The aim of the present work was to investigate the relationship between Total Mixed Ration (TMR) particle size distribution, diet digestibility and sorting in lactating high producing dairy cows.

MATERIALS AND METHODS

The present study complied with Italian legislation on animal experimentation and ethics (DL 04/03/2014 n. 26).

The study was conducted on 5 dairy farms located in the Parmigiano-Reggiano cheese production area, specifically in the provinces of Modena (44°46’33” N, 10°50’59” E and 44°44’2.4” N, 10°50’45.6” E) and Reggio Emilia (44°53’45.6” N, 10°34’44.4” E; 44°43’26.4” N, 10°43’1.2” E and 44°37’1.2” N, 10°40’33.6” E). The trial was performed during spring, over a period of two months. The herds ranged from 60 to 200 lactating Holstein cows receiving TMR ration (Table 1) once a day. The farms were visited on three days with 15 days interval. During each visit the following information and samples were collected: productive parameters data (herd average milk yield and composition); number of lactating cows; amount of TMR distributed and refused. On each day, 3 sampling sessions were conducted, namely at feed delivery and after 12 and 24 hours. During each session, TMR and faecal samples were collected for physical and chemical evaluations (TMR) and for the estimation of the digestibility. In particular, faecal samples were collected only 12 hours after feed distribution since this interval could be representative of the average faeces excreted daily [11, 12]. Regarding to TMR, at delivery and after 12 hours three samples of 1000 g (as fed) were collected on the feeding line: at the beginning, in the middle and at the end of the feed bunk for a total of 3 replicates per 2 sampling time per day per farm. Every TMR sample was divided in two homogeneous subsamples: one for the physical evaluation; one for chemical and nutritional laboratory analysis. The physical evaluation of the diet was performed using the Penn State Particle Separator (PSPS) according to the procedure described by Kononoff et al.[13] and Lammers et al.[14] on a total of 90 samples (3 replicates x 2 sampling time x 3 day visits x 5 farms).

The chemical analyses were performed by Near Infrared Reflectance Spectroscopy (NIRS) including dry matter (DM), ash, crude protein (CP), soluble protein (SolP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), starch and total sugars.

During the second visit of the day (12 hours after feed distribution), 5 fecal samples, from fresh, healthy lactating cows (60 to 90 DIM) were collected for the determination of the undigested neutral detergent fiber at 240 hours ($uNDF_{240}$) as a marker to estimate the total tract apparent dry matter digestibility (ttaDMDe) and total tract apparent NDF digestibility (ttaNDFDe).

The $uNDF_{240}$ was determined both in diet and feces by 240 hours *in vitro* fermentation based on Goering and Van Soest[15] method, modified as reported by Sgoifo Rossi et al.[16]. The same methodology was employed for the determination of diet and NDFD at 24 hours of fermentation.

The total digestible nutrient at 3 times maintenance intake (TDN_{3x}) was calculated for each dietary sample according to NRC (2001).

The ttaDMDe and ttaNDFDe were calculated for each cow by using average dietary uNDF from TMR samples collected at the three daily intervals as described by Righi et al.[17]. In particular, the digestibilities were estimated by assuming a 60% of ration consumption in the first 12 hours after distribution and the remaining 40% between 12 and 24 hours following feed delivery, according to the circadian feeding behaviour described by Harvatine[18]. The dietary uNDF used for digestibility calculation was thus estimated as weighted average of the mean dietary uNDF between delivery and 12 hours after feed distribution and the mean dietary uNDF between the latter interval and the 24 hours after feed delivery.

After 24 hours from TMR distribution, the herd total intake was estimated by the difference between the amount of feed delivered and orts (Total intake = weight of distributed TMR - weight of the 24 hours residue), whereas the individual dry matter intake was calculated by dividing the total intake by the number of the lactating animals, accounting for moisture. The same calculation was repeated at each daily visit (total of 3) during the whole trial.

Table 1 - Ingredients included in the TMR diets administered to the herds involved in the study (%DM).

	Farm				
	1	2	3	4	5
Mixed hay	12.0	-	20.0	-	7.2
2nd cut alfalfa hay	9.7	14.3	20.0	50.5	38.5
3rd cut alfalfa hay	9.7	26.0	-	-	-
4th cut alfalfa hay	8.5	-	-	13.6	-
Wheat straw	-	-	4.2	-	4.3
Corn grain meal	23.2	20.6	28.6	18.9	26.9
Corn grain flacked	8.4	-	4.1	3.8	-
Barley grain meal	-	7.6	4.1	-	-
Barley grain flacked	-	-	-	3.8	-
Wheat grain meal	-	-	-	-	5.8
Soybean meal	6.2	-	-	-	7.7
Soybean flacked	-	-	2.3	1.9	-
Soybean expeller	6.2	-	-	-	-
Wheat bran	-	-	2.0	-	-
Soybean hulls	-	-	2.1	-	-
Beet pulp	4.6	-	2.1	-	-
Concentrate mix[1]	10.1	29.1	10.5	7.5	9.6
MinVit	1.5	2.2	-	-	-
Forage (%)	39.83	40.36	44.17	64.12	50.00
Concentrate (%)	60.17	59.64	55.83	35.88	50.00
Forage:Concentrate	0.66	0.68	0.79	1.79	1.00

[1] Concentrate mix of farm 1 (% DM): barley ground, 43.0; wheat bran, 21.9; corn flacked, 21.9; molasses, 13.2. Concentrate mix of farm 2 (% DM): wheat bran, 27.8; beet pulp, 25.3; corn meal, 14.9; soybean meal, 13.0; sunflower meal, 12.8; molasses, 6.1. Concentrate mix of farm 3 (% DM): soybean meal, 57.2; sunflower meal, 19.0; vitamin-mineral premix, 14.2, molasses, 9.2. Concentrate mix of farm 4 (% DM): wheat bran, 43.0; linseeds extruded, 34.8; vitamin-mineral premix, 22.2. Concentrate mix of farm 5 (% DM): beet pulp, 50.6; soybean hulls, 16.3; linseed extruded, 12.0; vitamin-mineral premix, 9.5; molasses, 7.3; wheat bran, 4.2. Three samples per farm were collected.

From milk yield and milk composition, the energy corrected milk was calculated using the equation reported in Comino et al.[3] as follows:

$ECM = 0.327 \times \text{milk lbs} + 12.97 \times \text{fat lbs} + 7.21 \times \text{protein lbs}$

and values were then converted to kg.

Sorting was measured as variation of the retained material proportion on each sieve and the bottom pan expressed as absolute value of the difference from the initial value - the latter being measured in each farm in the 3 positions of the feed bunk in the 3 days considered - at 12 hours after feed delivery. These variations (%) were then regressed over the initial value (%) of each sieve and of the bottom pan to obtain the relative curves.

Statistical analysis was performed using the SPSS for Windows software package (version 26.0; SPSS Inc., Chicago, IL). The differences between chemical composition parameters in the diets, milk yield and milk composition, as well as ECM, feed efficiency (FE), peNDF, geometric mean length (X_{gm}) and geometric standard deviation (S_{gm}) were evaluated through the ANOVA one-way procedure using the farm as fixed factor, and the different parameters measured as dependent variables. The amount of the residue retained on each sieve was expressed as a percentage of the total sample amount (as fed) and compared between farms through the univariate procedure of the General Linear Model using the farm as fixed factor, and farm visit and position in the feed bunk as random effects. The amount of the residue on each sieve were also regressed over the ttaDMDe and ttaNDFDe values in order to establish the relationship between feed particle size distribution and diet digestibility. The correlations between ttaDMDe and ttaNDFDe and compositional parameters of the diets were previously tested in order to exclude any significant interference derived from diet composition on the mentioned regressions.

RESULTS

The chemical composition of the TMRs supplied in the farms involved in the present study is reported in table 2. Differences were found in CP (ranging from 15.74 to 18.15% DM; $P \leq 0.001$), EE (ranging from 2.65 to 4.76% DM; $P \leq 0.001$), SolP (ranging from 5.23 to 6.19% DM; $P=0.010$), starch (ranging from 19.84 to 23.31% DM; $P=0.001$), NDF (ranging from 30.51 to 35.25% DM; $P \leq 0.001$), ADF (ranging from 19.58 to 24.29% DM; $P \leq 0.001$) and uNDF (ranging from 29.82 to 40.16% NDF; $P \leq 0.001$) levels. No differences were found in NSC levels (ranging from 35.06 to 38.03% DM) and in TDN and NDFD values, that ranged between 60.62 and 64.03% of DM and from 37.17 to 45.53% of NDF respectively.

Table 3 reports data on feed intake, milk productivity and FE. Farm 1 showed the highest DMI (24.02 kg), milk yield (43 kg) and ECM (41.32 kg), and one of the highest FE, all these parameters were significantly different among farms ($P \leq 0.001$). The highest FE (1.77) was found in farm n°4 where the lowest DMI (19.20 kg) and intermediate milk yield and ECM (33.94 kg and 34.00 kg, respectively) were obtained. The farm 5 showed

Table 2 - Chemical composition of the TMR diets administered to the herds involved in the study (data are expressed as least square means).

	Farm[1]						
	1	2	3	4	5	SEM	P-value
Chemical characteristics[2]							
DM (% as fed)	67.73	70.78	75.9	74.87	77.66	1.393	0.146
Ash (% DM)	10.4	9.17	9.8	10.23	10.2	0.228	0.455
CP (% DM)	18.15^{c}	15.74^{a}	17.32^{b}	17.29^{b}	17.17^{b}	0.161	<0.001
EE (% DM)	3.2^{b}	2.65^{a}	3.38^{b}	4.76^{d}	4.09^{c}	0.121	<0.001
SolP (% DM)	5.72^{ab}	5.47^{a}	5.6^{a}	6.19^{b}	5.23^{a}	0.091	0.010
Starch (% DM)	20.14^{a}	19.84^{a}	20.9^{a}	20.1^{a}	23.31^{b}	0.161	0.001
Sugars (% DM)	4.48	4.47	3.83	4.11	3.57	0.312	0.308
NSC (% DM)	35.06	37.2	36.86	36.67	38.03	0.351	0.098
NDF (% DM)	33.2^{c}	35.25^{d}	32.65^{bc}	31.06^{ab}	30.51^{a}	0.383	<0.001
ADF (% DM)	23.06^{bc}	24.29^{c}	22.37^{b}	20.16^{a}	19.58^{a}	0.366	<0.001
ADL (% DM)	5.04	5.15	4.95	5.22	4.89	0.061	0.411
TDN_{3x} (% DM)	62.03	60.62	62.13	64.03	63.26	0.452	0.152
NDFD (% NDF)	38.08	40.52	45.53	37.17	42.98	1.139	0.123
NE l (Mcal/Kg)	1.45^{ab}	1.38^{a}	1.45^{ab}	1.51^{b}	1.48^{b}	0.015	0.048
$uNDF_{240}$ (%NDF)	40.16^{c}	34.06^{ab}	30.85^{a}	37.55^{bc}	29.82^{a}	0.887	<0.001

[1]a-d: Means within a row without a common superscript letter differ for p<0.05. Total number of samples analysed = 45
[2]DM, dry matter; CP, crude protein; EE, ether extract; NSC, non-starch carbohydrate; NDF, neutral detergent fibre; ADF, acid detergent fibre; ADL, acid detergent lignin; TDN_{3x}, Total Digestible Nutrients at 3 times maintenance intake, calculated according to NRC (2001); NDFD, *in vitro* NDF digestibility at 24 hours; $uNDF_{240}$, undigestible NDF expressed as a percentage on NDF.

Table 3 - Dry matter intake (DMI), milk production and energy corrected milk (ECM), milk composition, and feed efficiency (FE) of the herds involved in the study (data are expressed as least square means).

	Farm[1]						
	1	2	3	4	5	SEM	P-value
DMI, kg/d	24.02^{e}	21.63^{c}	19.72^{b}	19.20^{a}	23.47^{d}	0.520	≤0.001
Milk Yield (kg/d)	43.00^{d}	38.04^{c}	29.00^{a}	33.94^{a}	29.50^{b}	1.426	≤0.001
Fat (%)	3.60	3.74	3.69	3.83	4.53	0.093	0.081
Protein (%)	3.43^{a}	3.31^{a}	3.33^{a}	3.59^{b}	3.20^{a}	0.025	0.010
ECM (kg/d)	41.32^{b}	36.85^{c}	27.96^{a}	34.00^{ac}	31.19^{b}	1.425	≤0.001
FE	1.72^{b}	1.70^{c}	1.42^{b}	1.77^{b}	1.33^{a}	0.047	≤0.001

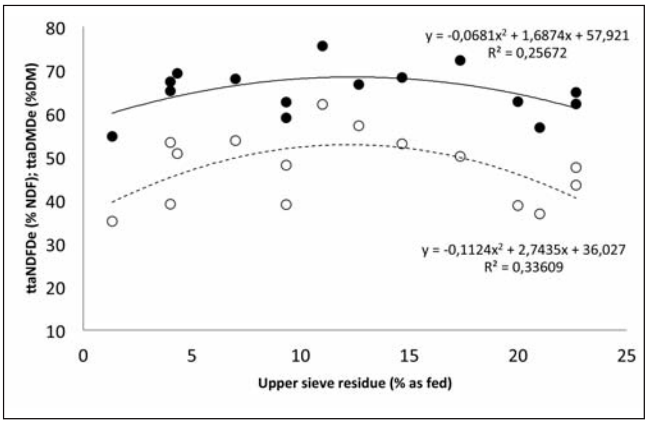
[1]a-e: Means within a row without a common superscript letter differ for p<0.05. Total number of data recorded per farm = 15

the lowest FE (1.33), medium-high level of intake and lower milk production compared to the other farms. However, the latter farm showed the numerically highest milk fat proportion (4.53%; P=0.081) with the lowest percentage of protein (3.2%; P=0.010), leading to an intermediate level of ECM (31.19 kg/d). Faecal uNDF was different in the farms considered (P≤0.001) with farm 5 showing the lowest value compared to farms 1, 3 and 4.

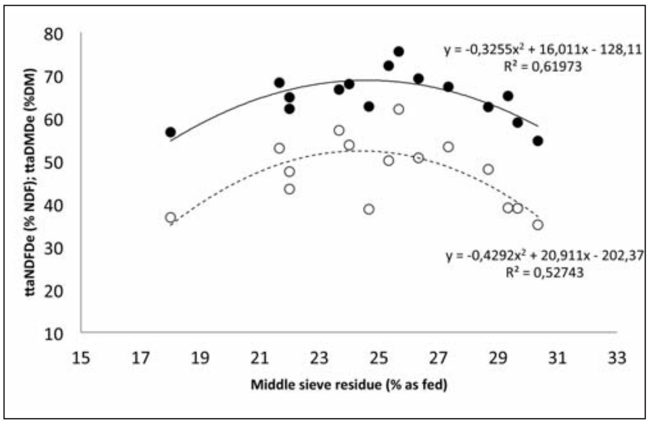
The distribution of the TMR particles in the considered diets are reported in table 4, as a percentage of the total mass. The values obtained on the 3 sieves and on the bottom pan were on average 12.1%, 25.2%, 35.1% and 27.4% (Upper-U-, Middle-M, Lower-L- and Bottom-B- respectively). The highest proportion of particles retained in the U sieve (size >19 mm) was found in the farms 3 and 5 (P≤0.001), which also showed the lowest amount of particles in the M sieve (particle size between 8 and 19 mm; P≤0.001). Farm 2 revealed the lowest value for the U and the numerically highest proportion of particles in the M sieve. Farms 2 and 4 showed the highest proportion of particles retained in the L sieve (P≤0.001). Farms 1 and 3 exhibited the highest proportion of particles smaller than 1.18 mm (B pan) compared to the farms 2 and 5 (P=0.003). The $peNDF_{1.18}$ of the farm 2 was the highest (P≤0.001), while farm 4 showed the lowest value for the $peNDF_8$ (P=0.014). Farm 5 showed longer particles compared to farms 4, 1 and 2 (P=0.011).

The highest ttaDMDe and ttaNDFDe were found in farm 4 (P≤0.001). The lowest ttaDMDe was observed in farms 2 and 3, whereas, the lowest ttaNDFDe was observed for farm 2 (P≤0.001) as reported in the table 4.

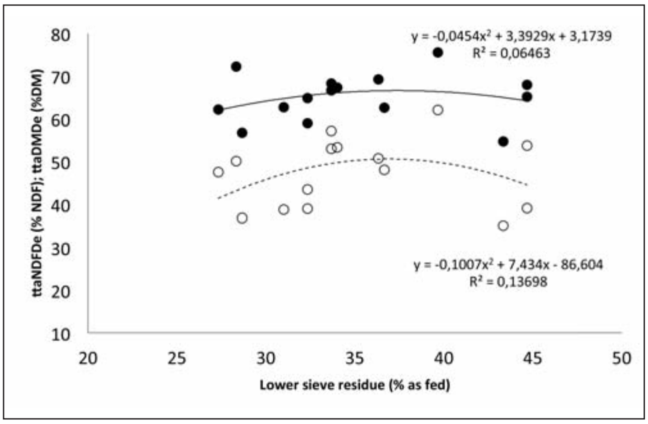
Based on the regressions between the proportions of particles retained on each sieve and the estimated digestibility parameters, it appeared that, both ttaDMDe and ttaNDFDe reached the highest levels when the U sieve residue was at 12% (Graph 1), the proportion of particles retained on the M sieve was 25% for the ttaDMDe and 24% for the ttaNDFDe (Graph 2) and



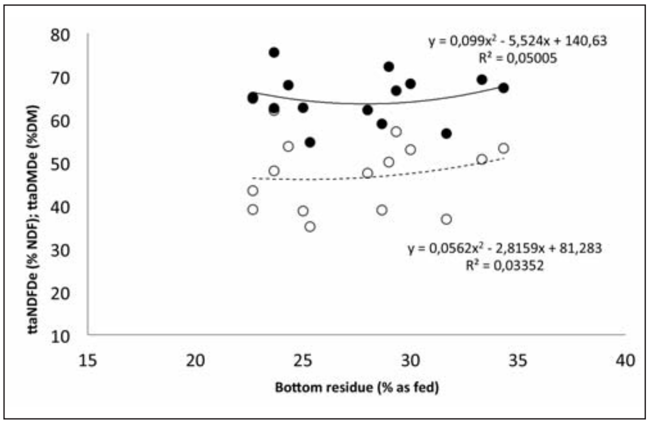
Graphic 1 - Variation of the estimated total tract apparent dry matter digestibility (ttaDMDe - continuous line) and of the estimated total tract apparent neutral detergent fibre digestibility (ttaNDFDe - dotted line) as a function of the proportion of particles retained on the upper screen of the Penn State Particle Separator (% as fed).



Graphic 2 - Variation of the estimated total tract apparent dry matter digestibility (ttaDMDe - continuous line) and of the estimated total tract apparent neutral detergent fibre digestibility (ttaNDFDe - dotted line) as a function of the proportion of particles retained on the medium screen of the Penn State Particle Separator (% as fed).



Graphic 3 - Variation of the estimated total tract apparent dry matter digestibility (ttaDMDe - continuous line) and of the total tract apparent neutral detergent fibre digestibility estimated (ttaNDFDe - dotted line) as a function of the proportion of particles retained on the lower screen of the Penn State Particle Separator (% as fed).

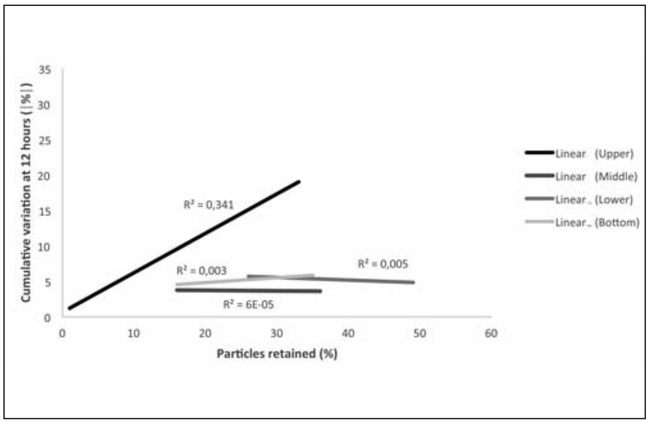


Graphic 4 - Variation of the estimated total tract apparent dry matter digestibility (ttaDMDe - continuous line) and of the total tract apparent neutral detergent fibre digestibility estimated (ttaNDFDe - dotted line) as a function of the proportion of particles collected in the bottom pan of the Penn State Particle Separator (% as fed).

the proportion of particles on the L sieve was 37%. Concerning the residues on the B, ttaDMDe and ttaNDFDe were depressed when the proportion of particles were respectively equal to 28% and 25% (Graph 3 and 4 respectively).

The variation of the proportions describing the dietary particle distribution after 12 hours from the feed delivery is reported in table 5. In three out of four farms the particles retained on the U sieve increased (P<0.05) and in farm 2 the value after 12 hours was numerically higher even if not significantly. No differences were observed in the M sieve with exception for the farm 1 which showed an increase of the particles retained on this sieve (P≤0.001). A decrease in the proportion of particles retained on the L sieve was observed in the majority of the farms after 12 hours from TMR distribution (P<0.05) and no effect was observed for this sieve in farm 1. Contrasting results, dependently to the considered farm, were observed concerning the proportion of particles retained in the bottom pan. In fact, farms 1 and 3 showed a decreased proportion of particles held in the bottom pan (P=0.091 and P≤0.001 respectively), while no effects were observed in farms 2 and 5. Moreover, an increased proportion of particles was observed in farm 4 diet (P<0.05).

In graph 5, the variation of the particle size distribution during the first 12 hours after feed administration is showed in relation with the amount of particles retained on each sieve and in the B pan at the delivery in the feed bunk. The increased pro-



Graphic 5 - Relations between the cumulative variation (Δ) of particles size distribution in the first 12 hours after feed delivery and the proportion of particles retained on each sieve and in the bottom pan of the Penn state Particle Separator at the feed delivery.

Table 4 - Estimated total tract apparent dry matter and neutral detergent fiber digestibilities (ttaDMDe; ttaNDFDe) and particle size distribution measured through the Penn State Particle Separator, of the total mixed diet fed to the cows of the studied herds (data are expressed as least square means).

	Farm[1]						
	1	2	3	4	5	SEM	p-value
Physical characteristics[2]							
19 mm	10.22[b]	4.89[a]	18.78[c]	7.44[ab]	19.11[c]	1.161	≤0.001
19.0- 8.0 mm	27.44[bc]	29.44[c]	21.22[a]	25.33[b]	22.78[a]	0.566	≤0.001
8.0- 1.18 mm	31.56[a]	41.56[b]	29.89[a]	40.22[b]	32.33[a]	0.881	≤0.001
<1.18 mm	30.67[b]	23.89[a]	29.67[b]	27.11[ab]	25.89[a]	0.659	0.003
$peNDF_{1,18}$ (% NDF)[3]	23.01[a]	26.71[b]	22.84[a]	22.72[a]	22.63[a]	0.372	≤0.001
$peNDF_8$ (% NDF)[4]	12.53[b]	12.1[b]	13.1[b]	10.17[a]	12.76[b]	0.303	0.014
X_{gm} mm[5]	4.06[a]	4.09[a]	4.57[ab]	3.92[a]	5.01[b]	0.116	0.011
S_{gm} mm[6]	3.41[b]	2.94[a]	3.74[c]	3.10[a]	3.58[bc]	0.054	≤0.001
Estimated total tract apparent digestibility[7]							
ttaDMDe (%DM)	66.11[b]	60.80[a]	61.81[a]	70.89[c]	65.23[b]	0.620	≤0.001
ttaNDFDe (%NDF)	47.41[b]	40.69[a]	47.13[b]	55.46[c]	45.02[ab]	0.578	≤0.001

[1] A-D: Means within a row without a common superscript letter differ for p<0.05. [2]Total number of samples : 45; [3]$peNDF_{1.18}$: physically effective NDF, ration NDF multiplied by amount of DM > 1.18 mm; [4]$peNDF_8$: physically effective NDF, ration NDF multiplied by amount of DM > 8.0 mm; [5]X_{gm}: geometric mean length; [6]S_{gm}: geometric standard deviation; [7] Total number of samples: 75.

portion of the bigger particles (retained on the U sieve) is directly related to the variation in the particle size distribution after 12 hours, even if this relationship does not appear to be strong (R^2=0.341). Any relationship was found with the amount of particles retained on the other sieves and in the B pan.

DISCUSSION

The diets were typical of the Parmigiano-Reggiano cheese making area, and were similar to the hay-based diets described by Comino et al.[3]. Indeed, as reported in table 1, the diets considered in the present study were characterized by a forage to concentrate ratio (F:C) ranging from 0.66 to 0.79 with the only exception of farm 4 which included higher levels of forage (F:C= 1.79). In general, the forage portion comprised a big amount of alfalfa hay from different cuts, providing both fiber and proteins. In addition, diets 1, 3 and 5 were comprised of mixed hay, and rations of farms 3 and 5 included also wheat straw as roughage. The diets considered included on average 22.78% of corn meal, as a main source of starch, whose level ranged from 18.9 to 28.6% of the diet DM. Additionally, a customized concentrate mix was supplied in all cases to fulfil the lactating cattle requirements.
Generally, the composition of the diets was similar among the farms considered. The highest dietary CP content observed in the farm 1 might be related to the highest milk production yielded by the relative herd, in agreement with results from Comino et al.[3]. The EE content was the highest in farm 4 and the lowest in farm 2. The high level of EE in farm 4 was probably one of the reasons for the high net energy of lactation (NEl) observed, despite the higher F:C in this diet. In fact, farm 4 ration contained the highest amount of forages that could have led to the rumen filling which justify the lowest DMI. Moreover, the high forage proportion in the same diet could have improved the acetic acid and butyric acid production in the rumen, along with a higher microbial protein synthesis, both reflected in the milk composition as high milk fat and protein levels. However, dietary EE and CP content are not considered as major variables affecting the total tract digestibility, which is investigated in the present trial. The starch content was the highest in farm 5 but from a practical point of view, the level observed can be considered comparable to the values of the other farms. Farm 2 showed the highest level of NDF content in the diet, while the other farms were similar. Despite the slight differences in terms of chemical composition between the studied rations, TDN_{3x} and NDF digestibility of the complete diets were unaffected, indicating that the differences found in the total tract digestibility are mainly due to the particle size distribution.
The proportion of the TMR particle size distribution was comprised in the same ranges identified by other authors in the Parmigiano-Reggiano area[1,2,8]. In particular, they were similar to the ranges reported by Fustini et al.[10] which were 0.0 to 19.6% for U, from 14.0 to 50.0% for M, from 22.9-46.0 for L and from 13.6 to 53.2 for the B.
In the present study the diets were comparable for their peNDF, and close to the value of $peNDF_{1.18}$ of 20-22% recommended by Mertens[6] in almost all cases. An exception was observed in farm 2 which showed the highest value having 75.89% of the particles longer than 1.18 mm and the highest NDF content in the diet, resulting in a $peNDF_{1.18}$ of about 27% of NDF. Moreover, $peNDF_8$ was the lowest in farm 4 diet which, in fact, showed the highest content of particles retained in the lower screen. The same diet showed the numerically lowest geometric mean length of the particles. Despite the possible effect of this parameter on the DMI[7], that was not investigated in the present study, some authors demonstrated that a decrease in TMR particle length, increased nutrients digestibility[4, 19] and milk production[8] though reducing the milk protein content[4]. Our results partially agree with those of Stojanovic et al.[4] and Haselmann et al.[19] since the farm with the lowest dietary value of X_{gm} of particles size showed the highest ttaDMDe, ttaNDFDe and milk protein lev-

Table 5 - Variation of the particle size distribution at 12 hours after feed delivery (values are expressed as %).

Diet	Sieve[1]	T0 (%)[2]	T12 (%)[3]	SEM	p-value
1	U	10.22	6.67	1.144	0.130
	M	27.44	33.33	1.147	≤0.001
	L	31.56	32.78	0.573	0.414
	B	30.67	27.33	1.163	0.091
2	U	4.89	8.00	1.467	0.183
	M	29.44	28.44	0.664	0.507
	L	41.56	38.44	1.293	0.042
	B	23.89	25.33	0.642	0.458
3	U	18.78	32.89	3.067	≤0.001
	M	21.22	18.78	1.120	0.110
	L	29.89	25.00	1.064	0.002
	B	29.67	23.00	1.146	≤0.001
4	U	7.44	12.44	0.962	0.036
	M	25.33	23.33	0.642	0.188
	L	40.22	33.11	1.645	≤0.001
	B	27.11	31.11	1.204	0.044
5	U	19.11	28.33	1.918	≤0.001
	M	22.78	24.89	0.538	0.165
	L	32.33	24.89	1.253	≤0.001
	B	25.89	23.00	1.189	0.142

[1]U: upper (19.0 mm), M: middle (8.0 mm), L: lower (1.18 mm) and B: bottom pan; [2] Proportion of particles at feed delivery; [3] Proportion of particles 12 hours after feed delivery. Total number of samples: 45 at each time point.

els. This could be related to a higher content of particles retained on the lower sieve: these are represented, usually, by the finest forage particles -leaves and small stems scraps- and concentrate fragments -particles of pellets, flakes and shreds- generated by a more intense mixing and chopping procedure and characterized by high digestibility. The presence of these residues, given by particles over the critical size, associated to the low intake observed is expected to increase the ruminal retention time with an improvement in the extent of digestion. The latter could be also enhanced by the relatively high ratio between the surface area for the microbial attack and the mass of the particles, thus leading to a feed efficiency raise.

The study of the relationship between the proportion of residual particles retained on each sieve and the ttaDMDe and ttaNDFDe showed a relationship between the residues collected on the M screen and the digestion parameters. The latter showed very weak relationships with particles retained on the U screen. Whereas, the amount of residues reaching L screen and the bottom pan did not show specific relationship with the digestibility parameters; particles smaller than 1.18 mm are, in fact, poorly retained in the rumen but exploited in the fermentation process[20]. Some indications can be, however, obtained by the examination of the graphic representation of the mentioned regressions.

Apart from the specific optimal value for each sieve residue stated in the results section, it appears that the ttaDMDe and ttaNDFDe were generally higher when the U sieve residues ranged between 10 and 15%, the amount of particles on the M sieve were around 25%, the proportion of particles on the L sieve ranged from 35 to 40% and the percentages of residues on the B were around 40%. The number of observations was however limited and these values should be considered as preliminary indications. Based on these results, it could be speculated that these proportions of particles in the diets can improve the ruminal mat formation optimizing, accordingly to Zebeli et al.[5], ruminal environment stimulating chewing and ruminal motility and promoting particle retention, modulating in the same time the passage rate.

Concerning sorting, an increase in the proportion of the particles bigger than 19 mm (retained on the U screen) was observed in the majority of the farms after 12 hours from the TMR distribution. Four farms showed a significant reduction in the percentage of particles retained on the L sieve within a general decline in the proportion of the smallest particles retained (<8 mm). The highest variation in the smallest particles was observed in the farms 3 and 5 whose percentage of particles longer than 8 mm were higher in the initial TMR. These results apparently indicated a sorting effect against longer particles in favour of small particles according to several studies[21,22]. However, the farm with the lowest X_{gm} in the TMR at the delivery was the only one showing an increase in the proportion of particles held in the bottom pan after 12 hours, highlighting the importance of the dietary particles size also in the feeding behaviour.

In general, the proportion of particles bigger than 19 mm in the TMR was positively related with the relative variation of particle size distribution during the first 12 hours after feed delivery. This is particularly evident in farms 3, 4 and 5, indicating that the higher initial presence of bigger particles was responsible for the feed sorting of the diet. Consistently to the results from Leonardi and Armentano[22], that found a strong correlation between the proportion of particles retained on the 26 mm screen and the feed sorting, in the present study cows generally sorted in favour of smaller particles than the longer ones.

CONCLUSIONS

Total mixed ration particle size seems to affect both digestibility and sorting parameters.
The estimated total tract digestibilities of both DM and NDF, in alfalfa hay-based TMR diets typical of the Parmigiano-Reggiano cheese production area, appear higher when the proportion of particles retained in the U sieve ranges between 10 and 15%, is around 25% in the M residues, ranges between 35 and 40% in the L and is around 40% in the bottom pan.
The U sieve residues particle are the main responsible for the feed sorting after meal distribution.
A careful preparation of the hay-based TMR diet, considering the suggested values of particle size distribution, may improve the efficiency of its degradation and digestion.

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