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# Changes of particle size distribution and chemical composition of a hay-based ration offered once or twice daily to dairy cows

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## ABSTRACT

The objective of this experiment is to evaluate the changes of particle size distribution and chemical composition of the total mixed ration (TMR) based on hay as the main forage component ("dry" TMR) and distributed once (7.00 am) or twice (7.00 am and 1.00 pm) daily to 32 lactating cows. The trial was divided in two periods of 14 days each. Diet (DM=53.7%) was formulated in order to assure the nutritional requirements of cows producing 24 kg/d of milk (crude protein=14.4% DM; NDF=40.9% DM; milk FU=0.88/kg DM) and additional amounts of concentrates were distributed using automatic feeders. Four TMR samples were collected daily (7.00 am, 10.00 am, 1.00 pm, 4.00 pm) for six days during each experiment period for a total number of 48 feed samples. Each feed sample was subjected to the estimation of the particle size distribution using the separator of Pennsylvania State University composed of two sieves (diameters of 19 and 8 mm) and a collector on the bottom, and to the determination of the chemical composition. Changes of all three particle size fractions for TMRs were observed during the day with distributions of the TMR both once and twice daily. With the once daily distribution, the large particles fraction increased linearly ( $P<0.001$ ) from 19.7 to 23.4, 32.2, and 35.1%, while the finest particle fraction decreased (from 60.1 to 58.3, 50.0, 47.8%). According to particle size changes, the chemical composition varied significantly at the different times of sampling when TMR was distributed once daily. Significant variations of DM were detected for TMR with a linear ( $P<0.001$ ) increase (from 54.4 to 57.9, 60.7, 61.5%). Considering once TMR distribution, the values of NDF and starch showed an opposite trend with an increase of 6.5 and a decrease of 8.3 points from 7.00 am to 4.00 pm (i.e., 9 hrs after distribution). Correlations were estimated between chemical and physical characteristics of TMRs. NDF content was positively and significantly correlated to the fraction of particles retained by a 19 mm sieve ( $r=0.42$ ;  $P<0.001$ ) and negatively correlated with the smaller particles ( $r=-0.51$ ;  $P<0.001$ ). In conclusion, when the TMRs are prepared excluding corn/hay silages, twice daily distributions of diet can avoid the selection of large feed by the cows, thereby preserving both a uniform particle size distribution and a steady chemical composition of the diet during the day. However, the cost for the extra time needed for twice daily distribution should be carefully considered.

*Key Words:* Total mixed ration, Hay based diet, Dairy cow, Particle size.

## RIASSUNTO

VARIAZIONI DELLE DIMENSIONI DELLE PARTICELLE E DELLA COMPOSIZIONE CHIMICA DI UNA DIETA UNIFEED A SECCO DISTRIBUITA UNA O DUE VOLTE AL GIORNO A VACCHE IN LATTAZIONE

*Obiettivo della sperimentazione è valutare le variazioni delle dimensioni delle particelle e della composizione chimica di una dieta unifeed basata sull'impiego di fieno (unifeed "a secco") e distribuita una o due volte al giorno a vacche in lattazione. La prova è stata effettuata in due periodi di 14 giorni ciascuno durante i quali 32 vacche in lattazione hanno rice-*

vuto rispettivamente una volta al giorno (alle ore 7.00) o due volte al giorno (alle ore 7.00 e alle 13.00) una dieta unifeed (SS=53,7%) in grado di soddisfare i fabbisogni nutritivi di bovine con una produzione media di latte di 24 kg/d (proteina grezza=14,4% ss; NDF=40,9% ss; UFL=0,88/kg ss). Le bovine con fabbisogni superiori hanno ricevuto quote aggiuntive di concentrati attraverso gli autoalimentatori. In 6 giorni, durante ciascun periodo sperimentale, sono stati raccolti 4 campioni giornalieri di unifeed (prelevato a metà della corsia di alimentazione) alle ore 7.00, 10.00, 13.00 e 16.00. I 48 campioni sono stati sottoposti al frazionamento delle particelle in classi di dimensioni diverse utilizzando il separatore della Pennsylvania State University dotato di due crivelli (19 e 8 mm di diametro dei fori) e di un contenitore sul fondo. I campioni sono stati sottoposti ad analisi della composizione chimica (sostanza secca, proteina grezza, NDF, amido, ceneri) secondo le metodiche ufficiali. I risultati della prova hanno evidenziato che, nel corso della giornata, la distribuzione delle particelle subisce variazioni significative a carico di tutte le tre frazioni, particolarmente evidenti quando la distribuzione dell'unifeed avviene una volta al giorno. In questo caso infatti le particelle con asse maggiore superiore a 19 mm aumentano linearmente ( $P<0,001$ ) passando da 19,7 a 23,4, a 32,2 e a 35,1%, mentre quelle raccolte sul fondo diminuiscono progressivamente (da 60,1 a 58,3, a 50,0 e a 47,8%). In relazione a queste variazioni anche la composizione chimica della dieta si modifica sostanzialmente ai diversi tempi di prelievo. Variazioni significative sono state osservate a carico della sostanza secca con un lineare ( $P<0,001$ ) incremento di questo parametro nel caso di distribuzione singola dell'unifeed (da 54,4 a 57,9, 60,7, 61,5%). Considerando sempre un'unica distribuzione giornaliera, i valori di NDF e di amido sono variati con un andamento opposto manifestando rispettivamente un incremento di 6,5 e un decremento di 8,3 punti percentuali nell'intervallo di tempo tra i prelievi effettuati alle 7.00 e alle 16.00. Dall'analisi delle relazioni fra caratteristiche chimiche e fisiche della diete unifeed sono emerse correlazioni positive tra tenore di NDF della dieta e la frazione delle particelle con asse maggiore di 19 mm ( $r=0,42$ ;  $P<0,001$ ) e correlazioni negative tra NDF e particelle più piccole ( $<8$  mm) ( $r=-0,51$ ;  $P<0,001$ ). In conclusione, si può affermare che nel caso di diete unifeed che non prevedono l'inclusione di insilati la distribuzione della dieta in due frazioni giornaliere consente di mantenere una più omogenea distribuzione delle particelle e una composizione chimica più costante nell'arco della giornata, anche a seguito della più rapida utilizzazione da parte degli animali. La maggior richiesta di tempo per la preparazione e distribuzione dell'unifeed richiede tuttavia un'attenta valutazione tecnica dei rapporti costi/benefici.

Parole chiave: Unifeed, Dieta a base di fieno, Vacche da latte, Dimensioni delle particelle.

## Introduction

The use of total mixed rations (TMR) in the feeding of lactating cows is widespread in large and highly mechanized dairy farms and it is mostly based on corn silage as the main component. Indeed the inclusion of corn silage in TMR reduces the cost of feeding and improves the final characteristics of TMR (better mixing, high moisture and low dust) (Bonsembiante, 1983; Berzaghi *et al.*, 2001). However, the use of corn silages and hay silages or other feeds subjected to microbial fermentation is limited by environmental conditions (i.e., mountain areas) or by cheese production regulations (i.e., forbidden for Parmigiano Reggiano cheese production; Pecorari *et al.*, 2001). The production and distribution of long hays-based TMR, have shown mechanical problems of the chopper mixer wagon and problems related to the selection of the different ingredients by cows (Weinberg and Ashbell, 2003).

Monitoring the TMR quality is essential in order to continuously evaluate the balancing of the diet with respect to the specific nutritional requirements of dairy cows. The chemical param-

eters for a balanced diet (i.e. rumen degradable protein, fermentable organic matter, NDF, starch, non structural carbohydrates, net energy) have been stated by INRA (1988) and, more recently, by NRC (2001). In addition to the other nutrients, a specific requirement for ruminants and, particularly, for dairy cows, is related to the amount and quality of fibre (NDF) (NRC, 2001). When the minimum fibre requirement is not met, cows show metabolic disorders such as reduced total DM digestibility, reduced milk fat percentage, displaced abomasum, and increased incidence of ruminal parakeratosis, laminitis, acidosis, and fat cow syndrome (Sudweeks *et al.*, 1981; Weston and Kennedy, 1984). However, cows assuming sufficient amounts of NDF with finely chopped forage or feeding diets subject to large variations in the chemical composition over the day can also exhibit the same metabolic disorders as cow fed a diet deficient in fibre. Therefore, an adequate particle length/size of forages and a limited process of diet demixing over time are necessary for a proper ruminal function (Lammers *et al.*, 1996) and, therefore, for animal welfare.

A simple approach to determine the particle

size distribution of TMR was developed through studies conducted by Pennsylvania State University (Heinrichs *et al.*, 1999) and was based on the fractionation of complete diets by a separator made up of two sieves (diameters of 19 and 8 mm) with a collector at the bottom. An additional sieve (1.18 mm) can be used in order to evaluate the amount of particles escaping the rumen, i.e. without any effects on the chewing (Mertens, 1997).

Recently several experiments were carried out in Italy in order to verify the effect of diet composition and mixing time on the particle size distribution of TMR and the effect of different particle distribution of TMR on dairy cow production (Berzaghi *et al.*, 2001; Spanghero, 2002; Mancin *et al.*, 2004). However, all studies have been conducted considering TMRs based on a high inclusion of corn silage. On the contrary, no information is available on particle size distribution of TMR based exclusively on hay as forage components. The aim of this experiment is to evaluate the changes of particle size distribution and chemical composition of a hay-based TMR collected at different times after distribution. The effect of two different frequencies of TMR distribution (once vs. twice daily) is also evaluated in the trial.

## Material and methods

The experiment was performed during the months of June and July. A daily total mixed ration was prepared for lactating cows using a horizontal chopper mixer wagon (Faresin TMR 500, Breganze - VI, Italy) with 5m<sup>3</sup> capacity. Two subsequent experimental periods of 14 days were considered. In the first period, 32 cows were fed a TMR once daily (7.00 am) and, in the second, the same TMR was prepared and distributed twice daily to cows (7.00 am and 1.00 pm). In the latter case the first TMR accounted for 60% of the total amount distributed daily. The schedules of diet distribution were chosen both to respect the practical farm conditions and to meet the requirements of cows that assume more than 2/3 of daily rations during the diurnal hours of the day (Berzaghi *et al.*, 2001). The mixer was filled to approximately 70% of wagon capacity with the once daily distribution while with twice daily distribution 39 and 26% of wagon capacity were

reached. The work program of the feeder mixer wagon was for each TMR: 1) addition of the hays, 2) hay chopping (10 minutes at 24 rpm of the auger), 3) addition of concentrates 4) addition of water and 5) mixing time (9 minutes at 12 rpm of the auger).

Ingredients and chemical composition of TMR are given in Table 1. The total mixed ration was balanced to meet the nutritional requirements of cows producing 24 kg/d of milk (INRA, 1988; NRC, 2001). The additional requirements of higher producing cows were assured by separate addition of concentrates using automatic feeders.

For once daily TMR, four feed samples were collected at distribution and after 3, 6 and 9 hrs from distribution, while for twice daily TMR administration, samples were collected at each distribution, and after 3 hrs. Indeed, according to previous works (Berzaghi *et al.*, 2001), during the early 3 hrs after TMR distribution, the DM intake of the cows is higher than 1/3 of the total daily intake. In addition, it is expected that in this period the processes of feed demixing and selection have the major effect on the chemical composition of the feed intake. Therefore, for each frequency of

Table 1. Ingredients and chemical composition of total mixed ratio used.

|                                     |        |  |      |
|-------------------------------------|--------|--|------|
| Ingredients:                        |        |  |      |
| Permanent meadow                    | % DM   |  | 27.3 |
| Alfalfa hay                         | "      |  | 17.6 |
| Dehydrated alfalfa hay              | "      |  | 6.7  |
| Corn meal                           | "      |  | 8.8  |
| Cracked corn seeds                  | "      |  | 14.9 |
| Protein-mineral premix <sup>1</sup> | "      |  | 10.6 |
| Dry beet pulp                       | "      |  | 5.2  |
| Soybean meal                        | "      |  | 8.8  |
| Chemical composition:               |        |  |      |
| Dry Matter                          | %      |  | 53.7 |
| Crude Protein                       | % DM   |  | 14.4 |
| NDF                                 | "      |  | 40.9 |
| Milk Feed Units                     | /kg DM |  | 0.88 |

<sup>1</sup>Contained in kg of feed compound: vit. A 75,000 U, vit. D<sub>3</sub> 4500 U, vit. E 45 mg, vit. B<sub>1</sub> 7.5 mg, vit. B<sub>12</sub> 0.0015 mg, vit. PP 450 mg, Mn 150 mg, Fe 150 mg, Cu 3 mg, Co 1.5 mg, I 3 mg, Zn 150 mg, Se 0.45 mg.

TMR distribution (i.e. experimental period) four samples were collected for six days at 7.00 am, 10.00 am, 1.00 pm, and 4.00 pm, always at the same position of the manger. A total of 48 samples of about 2.5 kg of TMR were collected. Then each one was sub-sampled to obtain a final representative sample of 500 g that was separated in two fractions of 250 g and pre-dried in a forced ventilated oven at 60°C for 48 hrs.

A first sub-sample was ground through a mill with a 1 mm screen (Retsch ZNC) and analysed for dry matter, ash, crude protein, NDF, and starch (Van Soest *et al.*, 1991; AOAC, 2000). A second one was subjected to evaluation of the particle size distribution using the Pennsylvania State Particle Separator (PSPS) composed of two sieves (diameters of 19 mm and 8 mm, respectively) and a collector at the bottom according to the procedure described by Heinrichs and Kononoff (2002). This method suggests sieving fresh samples of both feed and of TMRs because it is not practical to recommend analysis at standard moisture content during field measurements. Nevertheless, the same authors confirm that the moisture content may affect the particle size measurements of both feeds and TMRs (Kononoff *et al.*, 2003). Therefore, in this trial the sieving procedures were performed on pre-dried samples to avoid both underestimation of the small particle fractions and confused effects between sampling time and DM percentage of the sample. Each TMR sample was inserted in the upper sieve and then the apparatus was shaken horizontally 5 times for each side of sieve and then the process was repeated for a second time.

A total of 40 shakes per sample with a frequency of at list 1.1 Hz and a stroke length of 17 cm

were performed. The amount of the material collected in each sieve and in the bottom pan was weighed and expressed as percentage of the total sample weight. All physical separations were performed by the same operator. Data obtained from each feed sample were analysed by a General Linear Models procedure of the SAS-STAT (1990) according to the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where  $\mu$  = overall mean;  $\alpha_i$  = effect of frequency of daily TMR distribution ( $i=1,2$ );  $\beta_j$  effect of sampling time ( $j=1,4$ );  $(\alpha\beta)_{ij}$  = effect of the first order interaction;  $\varepsilon_{ijk}$  = residual error. Linear and quadratic components were also tested using the mean squares of interaction between frequency of TMR distribution and sampling time.

## Results and discussion

### *Particle size distribution of TMR*

The results of ANOVA for particle size distribution are reported in Table 2. On average the fractions of TMR particles in the different sieves was 23.0, 20.8 and 56.2% for upper sieve, middle sieve and bottom pan, respectively. The average percentage of TMR fraction having particles > 19 mm was substantially higher than that reported by Heinrichs and Kononoff (2002), that suggested a mean incidence ranging from 2 to 8%. On the contrary, the middle fraction (8-19 mm) was lower than that recommended (30-50%), while the percentage of the fraction collected in the bottom pan (< 8 mm) was in general within the suggested range (30-50%). In this experiment the percentages of upper and middle fractions of TMR were respectively higher and lower than those reported

Table 2. ANOVA for percentage of particle size fractions obtained by Pennsylvania State University separator (mean square and *P*).

| Particle size of TMR | Frequency of distribution (F) | Sampling time <sup>1</sup> (S) | F x S interaction | Mean square error |
|----------------------|-------------------------------|--------------------------------|-------------------|-------------------|
| > 19 mm              | 1200.51***                    | 274.10***                      | 135.54*           | 37.25             |
| 8-19 mm              | 333.41***                     | 29.16*                         | 6.66              | 10.27             |
| < 8 mm               | 253.85**                      | 131.59**                       | 131.58**          | 30.24             |

<sup>1</sup> Time of TMR distribution.

\*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ ; \*:  $P < 0.05$

by other Authors that sieved both fresh (Spanghero, 2002; Mancin *et al.*, 2004) and pre-dried (Berzaghi *et al.*, 2001) TMR samples collected in Italian farms. However, all these authors dealt with TMRs that included corn silage as mean component, while the diet used in our experiment is based largely (51.6% on DM) on hays (permanent meadow, alfalfa hay, dehydrated alfalfa hay; Table 1). Calberry *et al.* (2003) found that, replacing alfalfa silage with chopped alfalfa hay (9.8% on DM), the proportion of particles of fresh samples passing through the 8 mm sieve increased from 55.2 to 61.9%.

The effect of frequency of TMR distribution (once or twice daily) was statistically significant ( $P < 0.01$ ) for all fractions of different particle size (Table 2). On average, the percentage of large particles (>19 mm) of single distribution was significantly higher ( $P < 0.001$ ) than that of twice daily distribution (27.6 vs 18.3%, respectively). On the contrary, the middle and lower fractions of single distribution were lower than that of twice daily distribution (18.3 vs 23.2% and 54.1 vs 58.4% respectively;  $P < 0.01$ ). This result could be related to the higher chopping efficiency of feeder mixer wagon when a lower amount of TMR was prepared twice daily, although usually mixer wagons are designed to achieve the higher mixing efficiency when are filled at about 80% of their maximum capacity.

The interaction between frequency of TMR distribution and sampling time was significant ( $P < 0.05$ ) both for largest (>19 mm) and smallest (<8 mm) particle percentages (Table 3).

Considering the once daily distribution, the percentages of the fraction collected in the upper sieve increased linearly ( $P < 0.01$ ) during the day: from 19.7 to 23.4, 32.2, and 35.1% after 0, 3, 6 and 9 hrs after distribution of feeding, respectively). The highest change in the upper fraction percentage was observed between 3 and 6 hrs after the TMR distribution, corresponding to the period between 10.00 am and 1.00 pm. In contrast, the lower fraction (<8 mm) showed the linear tendency ( $P < 0.002$ ) to decrease over time (from 60.1 to 47.8%). More limited and non significant variations of large particle fraction for silage-based TMRs were observed by Masoero *et al.* (2001) after 8 hrs from feeding (from 24.9 to 27.6%). Using diets based on chopped alfalfa hay, Calberry *et al.* (2003) found on fresh samples that the fraction of particles retained by the 19 mm sieve of orts was greater than that of TMR.

When the TMR was distributed twice daily, the changes of the different particle size fractions during the day were limited. The variation in the upper fraction percentage was not significant: from 15.3 to 19.8% after the first TMR distribution and from 16.3 to 21.9% after the second TMR dis-

Table 3. Particle size distribution (% DM) of TMR. Interaction of frequency of TMR distribution<sup>1</sup> and sampling time

| Frequency of distribution <sup>1</sup> | Particle size | Sampling time     |                    |                    |                   | P   |    |
|--|---------------|-------------------|--------------------|--------------------|-------------------|-----|----|
|  |               | 7.00 am           | 10.00 am           | 1.00 pm            | 4.00 pm           | L   | Q  |
| - once daily:                          |               |                   |                    |                    |                   |     |    |
|  | > 19 mm       | 19.7 <sup>A</sup> | 23.4 <sup>A</sup>  | 32.2 <sup>B</sup>  | 35.1 <sup>B</sup> | *** | ns |
|  | 8-19 mm       | 20.2              | 18.3               | 17.8               | 17.1              | ns  | ns |
|  | < 8 mm        | 60.1 <sup>A</sup> | 58.3 <sup>A</sup>  | 50.0 <sup>B</sup>  | 47.8 <sup>B</sup> | *** | ns |
| - twice daily:                         |               |                   |                    |                    |                   |     |    |
|  | > 19 mm       | 15.3 <sup>a</sup> | 19.8 <sup>ab</sup> | 16.3 <sup>ab</sup> | 21.9 <sup>b</sup> | ns  | ns |
|  | 8-19 mm       | 24.8 <sup>b</sup> | 23.5 <sup>b</sup>  | 24.4 <sup>b</sup>  | 20.3 <sup>a</sup> | *   | ns |
|  | < 8 mm        | 59.9              | 56.7               | 59.3               | 57.8              | ns  | ns |

<sup>1</sup> once daily distribution of TMR was supplied at 7.00 am, twice daily distribution at 7.00 am and 1.00 pm.

L= Linear component; Q= Quadratic component.

\*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ ; \*:  $P < 0.05$ ; ns: not significant.

Within row A,B =  $P < 0.01$ ; a,b =  $P < 0.05$ .

tribution. Both these changes were always of the same magnitude as those observed after three hours from distribution when TMR was distributed once daily. However, due to the reduced amount distributed and to the feed consumption, the overall changes in the distribution of the particle size were lower than those observed within the once daily distribution. The percentages of the middle fraction were similar after the first distribution and tended to decrease after the second distribution (from 24.4 to 20.3%;  $P < 0.05$ ). No changes were observed for the lower fraction.

#### *Chemical composition of TMR*

The average chemical composition of TMR was similar to that calculated on the basis of the chemical composition of the single ingredients included in the diet (Table 1). In particular the levels of dry matter, crude protein, and NDF were on average 55.0%, 14.8% DM and 40.3% DM, respectively.

Chemical composition of TMRs was affected by the frequency of distribution ( $P < 0.05$ ) (Table 4). Dry matter and crude protein contents of TMRs distributed once daily were lower than those observed with twice daily distribution (58.6 vs 61.4% and 14.5 vs 15.0% DM, respectively;  $P < 0.05$ ). As reported above, once daily TMR distribution produced a higher large particles fraction than that of twice daily distribution. Generally, the fraction of large particles (> 19 mm) are represented by forage components of TMR. Therefore, the higher amount of forages linked to the once daily distribution could be related to the lower DM and CP contents in the diet.

The interaction between frequency of TMR distribution and sampling time was significant ( $P < 0.001$ ) for dry matter content of TMR (Table 5). Considering the single distribution, dry matter level increased linearly ( $P < 0.001$ ) increasing of the sampling time after the TMR distribution (from 54.4 to 57.9, 60.7, and 61.5%). This increase of DM level is probably due to the experimental period (June and July) with high environmental temperature during the day. An opposite trend of changes in NDF and starch contents after distribution was detected. Instead, NDF and starch values tended, respectively, to increase and to decrease linearly ( $P < 0.001$ ) during the day. Because of the rapid loss of moisture in the TMR, it is possible that cows tended to select the mixing diet and chose the concentrate as respects the forage component. This propensity could also be related to the linear increase in the larger particle size of TMR distributed once daily as previously reported.

On the other hand, no significant variations among sampling times were observed on chemical composition when TMR was distributed twice daily, with the exception of the NDF % which increased from the first to the last sampling time ( $P < 0.001$ ).

In general, the crude protein content showed small changes during the day considering both once or twice daily distribution and this could be related to the high amount of protein supplied with forages, i.e. about 45% of the total protein of the diet.

#### *Correlation between chemical and physical characteristics of TMR*

The coefficients of correlation between the chemical parameters and the percentages of parti-

Table 4. ANOVA for chemical composition (on DM basis) of TMR samples.

|        | Frequency of distribution (F) | Sampling time <sup>1</sup> (S) | F x S interaction | Mean square error |
|--------|-------------------------------|--------------------------------|-------------------|-------------------|
| DM     | 23.63*                        | 36.52***                       | 34.50***          | 4.47              |
| CP     | 4.27*                         | 2.08*                          | 1.65              | 0.95              |
| NDF    | 9.57                          | 107.70***                      | 6.84              | 8.42              |
| Starch | 0.04                          | 73.91**                        | 25.40             | 12.30             |
| Ash    | 0.82                          | 0.37                           | 0.30              | 0.99              |

<sup>1</sup> Time of TMR distribution.

\*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ ; \*:  $P < 0.05$ .

Table 5. Chemical composition for TMR. Interaction of frequency of TMR distribution<sup>1</sup> and sampling time.

| Frequency of distribution <sup>1</sup> | Proximal composition |      | Sampling time      |                     |                     |                    | P   |    |
|--|----------------------|------|--------------------|---------------------|---------------------|--------------------|-----|----|
|  |                      |      | 7.00 am            | 10.00 am            | 1.00 pm             | 4.00 pm            | L   | Q  |
| - once daily:                          |                      |      |                    |                     |                     |                    |     |    |
|  | DM                   | %    | 54.4 <sup>A</sup>  | 57.9 <sup>B</sup>   | 60.7 <sup>C</sup>   | 61.5 <sup>C</sup>  | *** | ns |
|  | CP                   | % DM | 14.52              | 14.88               | 14.44               | 14.23              | ns  | ns |
|  | NDF                  | "    | 36.67 <sup>a</sup> | 39.09 <sup>ab</sup> | 40.83 <sup>bc</sup> | 43.14 <sup>c</sup> | *** | ns |
|  | starch               | "    | 21.63 <sup>c</sup> | 17.98 <sup>b</sup>  | 16.04 <sup>ab</sup> | 13.31 <sup>a</sup> | *** | ns |
|  | ash                  | "    | 8.52               | 8.65                | 8.61                | 8.63               | ns  | ns |
| - twice daily:                         |                      |      |                    |                     |                     |                    |     |    |
|  | DM                   | %    | 61.2               | 61.9                | 60.1                | 62.4               | ns  | ns |
|  | CP                   | % DM | 15.98              | 14.79               | 15.05               | 14.42              | ns  | ns |
|  | NDF                  | "    | 37.14 <sup>a</sup> | 41.84 <sup>bc</sup> | 40.29 <sup>b</sup>  | 43.72 <sup>c</sup> | *** | ns |
|  | starch               | "    | 18.94              | 16.37               | 17.55               | 16.32              | ns  | ns |
|  | ash                  | "    | 8.36               | 8.73                | 8.00                | 8.39               | ns  | ns |

<sup>1</sup> once daily distribution of TMR was supplied at 7.00 am, twice daily distribution at 7.00 am and 1.00 pm.

L= Linear component; Q= Quadratic component.

\*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ ; \*:  $P < 0.05$ ; ns: not significant.

Within row A,B =  $P < 0.01$ ; a,b =  $P < 0.05$ .

Table 6. Correlation coefficients between chemical composition and particle size.

|        | Percentage of TMR with particle size |         |            |
|--------|--------------------------------------|---------|------------|
|        | > 19 mm                              | 8-19 mm | < 8 mm     |
| DM     | +0.0885                              | -0.1180 | -0.0398    |
| CP     | -0.3625                              | +0.1486 | -0.3950    |
| NDF    | +0.4242***                           | -0.0814 | -0.5145*** |
| Starch | -0.3739**                            | +0.0722 | +0.4562*** |
| Ash    | +0.0271                              | +0.0012 | -0.0423    |

\*\*\*:  $P < 0.001$ ; \*\*:  $P < 0.01$ .

cle size of the three fractions of TMR samples are reported in Table 6. The percentages of the three particle size fractions were low and not significantly correlated to the dry matter, crude protein and ash content of the TMRs. Contrarily, significant correlations between NDF and starch content of the TMRs with particle distribution were observed. In particular, the NDF content of the TMRs was positively correlated with large particles ( $r = +0.42$ ;  $P < 0.001$ ) and negatively correlated with the percentage of particles of small size ( $r =$

0.51;  $P < 0.01$ ). On the contrary, the correlation coefficients between starch content of the TMRs and large and fine particles were respectively negative ( $r = -0.37$ ;  $P < 0.01$ ) and positive ( $r = +0.46$ ;  $P < 0.01$ ). Heinrichs *et al.* (1999), using a large dataset of TMRs samples ( $n = 831$ ) collected in dairy farms within the USA, observed a lower but significant correlation between the content of NDF and the fraction of particles on the top screen ( $r = 0.23$ ;  $P < 0.001$ ). Similarly, from data collected in 5 dairy farms in Italy, Mancin *et al.* (2004) observed a sig-



nificant correlation between the NDF level and the percentage of large particles ( $r=+0.19$ ;  $P<0.01$ ). Both these studies were carried out on corn silage-based TMR.

The results of the present study suggest the suitability of estimating the daily change of the chemical composition of the hay-based TMR by measuring the changes of the particle size distribution. However, further study will be required to adapt this simple tool for the estimation of dietary variations in farm practice, where it is not practical to recommend the analysis of the TMR particle size on pre-dried samples. In agreement with other studies, the evaluation of the TMR particle size distribution could be an easy and useful monitoring system to prevent digestive and metabolic disease due to errors in TMR preparation.

## Conclusions

This experiment has confirmed that total mixed rations for lactating cows, prepared without the inclusion of corn/hay silages, could result in problems related to the demixing of the different ingredients. Indeed, the fibrous and coarse particles tended to separate from the whole mix and, due to low specific gravity, to migrate to the upper layers of the mixture. Consequently, the cows can more easily select against large and less appetizing feed particles in favour of finer ones. As a result, the proportion of the diet ingested by cows is less coarse and different from the chemical point of view than that of corn silage-based TMR.

The results of this experiment also suggests that the twice daily distributions of TMR can eliminate large feed selection by cows due to better particle size distribution and a steady chemical composition of the diet during the day. With twice daily feeding, particle size distribution and chemical composition of TMR can vary slightly after three hours from the distribution. However, from the practical point of view, the increase in daily work spent to prepare and distribute the feed, only partially balanced by the reduction of the single mixer preparation time, should be carefully considered. Moreover, twice daily distribution can force the lactating cows to consume all the available amount of the TMR, thereby allowing a limited feed selection by animals. In farm practice, the changes of the

chemical composition of TMR during the day can be easily and rapidly approximated from the variations of the particle size distribution.

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