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SHORT COMMUNICATION

Precision in the measurement of dairy feed fractions based on particle size.

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ABSTRACT

The aim of this research note is to evaluate the variability of the physical measurements obtained by a separator of feeds for dairy cows based on particle size. Fresh samples of total mixed ration (TMR) and corn silage were collected from four dairy units and were immediately fractionated using a particle separator (NASCO®, Pennsylvania State University) composed of two sieves (diameters of 19 and 8 mm) and a collector on the bottom. Repeatability expressed as standard deviation was similar between fractions (>19, 8-19 and <8 mm fractions: ± 1.9 , ± 1.4 and $\pm 2.2\%$ for TMR samples; ± 2.0 , ± 1.7 and $\pm 1.5\%$ for corn silages), but when expressed as coefficient of variation there were, for both samples, large differences between the values for fraction exceeding 19 mm (44.7 and 35.1 %, respectively for TMR and corn silages) and the middle (3.9 and 2.5 %, respectively for TMR and corn silages) and bottom fractions (3.7 and 5.6 %, respectively for TMR and corn silages). The between operator reproducibility was very close to repeatability and this indicates that the operator's contribution to the overall variability is marginal in comparison with residual variability. In conclusion, different operators properly trained in the use of the NASCO® separator can produce satisfactory repeatable and reproducible values for the middle and the bottom fractions.

Key words: Dietary particle size, Dairy cow, Repeatability, Reproducibility.

RIASSUNTO

PRECISIONE STATISTICA DELLE MISURE DI FRAZIONAMENTO DI CAMPIONI ALIMENTARI PER BOVINE DA LATTE SULLA BASE DELLA DIMENSIONE DELLE PARTICELLE

Nel presente lavoro sono stati stimati i parametri di precisione statistica (ripetibilità e riproducibilità tra operatori) delle misure di frazionamento di campioni alimentari per bovine da latte sulla base della dimensione delle particelle. È stata usata una attrezzatura composta da due setacci e un raccoglitore di base messa a punto dalla Pennsylvania State University (NASCO®) che consente la separazione dell'alimento in 3 frazioni (lunghezza delle particelle > 19, 8-19 e <8 mm).

Campioni di insilato ceroso di mais e di diete complete sono stati raccolti in quattro allevamenti di bovine da latte, scelti sulla base di uniformi caratteristiche dimensionali, gestionali e produttive (80-100 bovine in lattazione; stabulazione libera a gruppo unico alimentato con tecnica unifeed; circa 9000 kg di latte per lattazione). I campioni sono stati sottoposti a setacciatura in doppio da 8 operatori appositamente addestrati all'operazione. Pur riscontrando una discreta variabilità tra gli allevamenti, in generale, le diete complete hanno presentato dimensioni medie delle particelle (4.2, 35.1 e 60.7%, rispettivamente per le frazioni >19, 8-19 e <8 mm) inferiori a quelle suggerite come ottimali nelle condizioni nord-americane; al contrario, la dimensione media delle particelle degli insilati (5.7, 68.3 e 26.0%, rispettivamente per le frazioni >19, 8-19 e <8 mm) è risultata superiore a quanto mediamente rilevabile nel nord-america. La ripetibilità,

espressa come coefficiente di variabilità, è risultata, per entrambi i campioni, molto elevata per la frazione a particelle di dimensione maggiore (44.7 e 35.1 %, rispettivamente per le diete complete e i silomais) in confronto alla frazione intermedia (3.9 e 2.5 %, rispettivamente per le diete complete e i silomais) e a quella più fine (3.7 and 5.6 %, rispettivamente per le diete complete e i silomais). La riproducibilità tra operatori ha fatto registrare valori molto simili alla ripetibilità e questo indica, nelle condizioni adottate, un marginale contributo dell'effetto dell'operatore alla variabilità complessiva delle misure. In conclusione, la frazione di setacciatura più grossolana presenta valori molto variabili, mentre per le altre due i parametri di ripetibilità e riproducibilità tra operatori sono da ritenersi molto soddisfacenti. Pertanto, operatori addestrati all'impiego della attrezzatura possono fornire valori di frazionamento di unifeed per bovine da latte e silomais sufficientemente ripetibili e riproducibili per le frazioni a granulometria intermedia e fine.

Parole chiave: Dimensione particelle alimentari, Bovine da latte, Ripetibilità, Riproducibilità.

Introduction

A balanced ratio between fast and slow fermentable substrates (i.e., starch and fiber) is an essential dietetic requisite for dairy diets to allow suitable rumen ecological conditions. In events of fiber shortage, the time spent ruminating and the saliva production are reduced and, therefore, the amount of buffers which reach the rumen is also depressed. There are several chemical parameters to monitor the diet composition (e.g., NDF, ADF, starch, non structural carbohydrates) and the respective recommendations have been recently updated for dairy cows (NRC, 2001). However, these analytical data and parameters are insufficient for an overall dietetic evaluation, as the rumination stimuli and the intensity of rumen fermentation are also related to the dietary particle dimension and to the physical effectiveness of the diet (Mertens, 1997). Therefore, additional recommendation have been proposed, such as the dietary NDF content of forages in long chop (NRC, 2001) and/or the effective NDF which is a parameter based on chewing time (Mertens, 1997).

A complementary approach, recently proposed by researchers at the Pennsylvania State University (Heinrichs, 1996; Lammers *et al.*, 1996) is to fractionate complete diets by a separator (NASCO's® particle separator) made up of two sieves and a bottom pan. This simple apparatus is suitable for use by the extension services as an on-farm tool to assist in assessing dietary particle size (Heinrichs *et al.*, 1999) and it has also been utilised in experimental works to characterize dif-

ferent dietary treatments and feeds (Andrighetto *et al.*, 1998; Berzaghi *et al.*, 2001; Masoero *et al.*, 2001; Johnson *et al.*, 2002).

However, to date there is no information about the precision (e.g., repeatability and reproducibility) of this measure, an essential and preliminary prerequisite to any further utilization. The aim of this research note is to evaluate the statistical parameters of precision of this physical measurement.

Material and methods

Wet density measurement

A preliminary survey was conducted to measure the wet density of corn silage (unpacked, as feed) and total mixed rations (TMR) and to assess the most appropriate amount of sample to be sieved: 16 corn silage and 19 TMR samples (about 2-4 kg of fresh material) were collected in dairy farms with similar characteristics (situated in the East Po Valley (Italy); average lactation milk yield of about 9000 kg; feeding system based on TMR having about 50% DM and containing about 20 kg/d/head of corn silage). The TMR samples were collected within one hour after the mixer-wagon distribution in stalls, while the corn silages were manually sampled in the silo immediately after the mixer-wagon preparation. The wet density was calculated by weighting a graduated cylinder (2 liters of capacity) filled with the sample to reach a volume of 1.7 liters. This measure was repeated using all the available amount of sample and this resulted in a number of repetitions ranging from 4 to 7 per each dairy farm sample.

Farms, chemical analysis and physical separation

Four dairy units were chosen on the basis of some homogeneous characteristics (herd size of about 80-100 lactating cows, feeding system based on TMR, average lactation milk yield of about 9000 kg). From each farm a sample of about 6-7 kg of corn silage and TMR were collected using the procedure previously described and immediately stored at 4°C. A portion of each feed sample was pre-dried at 60°C, milled and then analysed for dry matter, ash, crude protein, ether extract (Association of Official Analytical Chemists, 2000) and for the NDF content (Van Soest *et al.*, 1991) using the Ankom apparatus (Ankom ®, Tech. Co., Fairport, NY, USA). For the NDF analysis the samples were pre-treated with α -amylase, ND solution contained Na sulfite and data were corrected for residual ash.

Fresh samples were fractionated within 24 h from the collection using a particle separator (NASCO's®) composed of two sieves (diameters of 19 and 8 mm, respectively) and a collector on the bottom. Five hundred g of sample were inserted in the upper sieve and then the apparatus, placed on a flat desk, was shaken horizontally five times in one direction, then rotated one-fourth turn, and again shaken five

times. This procedure was continued to obtain a complete rotation of the apparatus (4 sets of five shakes each) and was then repeated (for a total of 40 shakes, Heinrichs *et al.*, 1996). The amount of material collected in each sieve and in the bottom pan was weighed and expressed as percentage of the total sample weight. Following this procedure, eight operators (average age 21-24 years; males and females in equal proportion; properly trained in the utilisation of the separator) fractionated each sample (TMR and corn silage) in duplicate.

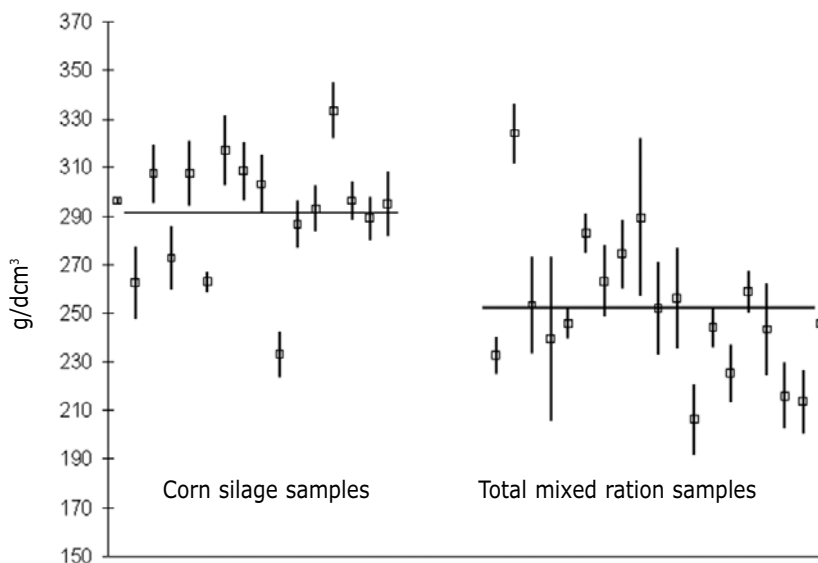
Statistical analysis

The experiment was designed as a two factorial and data were analysed according to the linear model:

$$y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon$$

where: μ = overall mean; α = effect of farm ($i=1,4$); β = effect of operator ($j=1,8$); ε residual error (SAS, 1999; PROC GLM). The standard deviation of repeatability was estimated (Youden and Steiner, 1975) as $\sqrt{(\sigma_e^2)}$, while the variance components of β and $(\alpha\beta)$ effects and the error variance (σ_β^2 , $\sigma_{\alpha\beta}^2$ and σ_e^2 , respectively) were used to estimate the between operators standard deviation of reproducibility as $\sqrt{(\sigma_\beta^2 + \sigma_{\alpha\beta}^2 + \sigma_e^2)}$.

Figure 1. Wet density of corn silage (unpacked, as feed) and total mixed ration samples (standard deviations are represented by vertical bars)



Results and discussion

The corn silage (unpacked, as feed) and TMR samples had an average DM content of 342 (+41) and 507 (+35) g/kg and a wet density of 291.4 (+24.3) and 250.7 (+28.3) g/dcm³, respectively (figure 1).

These latter data make it possible to calculate that an amount of sample equal to about 3 pints (about 1.7 liters), as suggested by Heinrichs (1996), is approximately equivalent to 500 g for corn silage and to a lower weight (420 g) for the present type of TMR samples (based on corn silage and with an average DM content of about 50%). However, in the present work a fixed weight of

sample (500 g) was used for both types of feeds (close to the volume suggested for corn silages; about one fifth higher for TMRs) to estimate the precision parameters of NASCO separator fractions at the same amount of sample.

In Table 1 the TMR formulation and the chemical composition of TMR and corn silage samples used for the measurements of the particle size distribution are presented.

The TMRs considered were based mainly on corn silage and alfalfa hay as forages with limited amounts of by-products. Substantial differentiation between farms in terms of amount and type of concentrates used (wheat bran, corn cobs silage, cereal and extracted oil seed meals) were noted.

Table 1. Total mixed ration formulation and chemical composition of TMR and corn silage samples used for the measurements of the particle size distribution.

		Dairy farms			
		A	B	C	D
TMR formulation:					
Corn silage	Kg/d	24.0	15.0	20.0	25.0
Corn cob silage	"	=	4.5	=	5.0
Alfalfa hay	"	4.8	4.0	3.0	6.0
Extracted oil seed meals	"	2.7	6.6	3.7	2.0
Cereal meals	"	1.6	2.7	6.7	=
Wheat bran	"	4.8	0.8	1.5	=
Fullfat soybean meal	"	=	=	=	2.0
Dehydrated beet pulp	"	=	1.2	=	=
Cotton seeds	"	=	2.0	=	=
Molasses	"	1.1	=	=	=
Chemical composition					
TMR:					
Dry matter	%	52.1	58.4	62.8	47.9
Crude protein	% DM	15.2	18.8	15.5	16.1
NDF	"	33.5	32.5	30.4	31.2
Ether extract	"	2.8	4.0	2.9	3.5
Ash	"	6.9	7.0	6.2	5.8
Corn silage:					
Dry matter	%	38.8	34.7	38.0	28.7
Crude protein	% DM	8.5	8.1	7.9	8.3
NDF	"	40.7	40.7	37.2	43.5
Ether extract	"	3.2	3.8	3.3	2.4
Ash	"	3.6	3.3	2.8	4.1

Crude protein content ranged from 15.2 to 18.8 % DM, while the NDF contents (between 30.4 and 33.5% DM) were within the recommended ranges suggested for high yielding dairy cows (NRC, 2001). The corn silages had similar chemical composition, apart from the low DM content of one sample (29%) with respect to the others (35-39%).

Values of physical fractions of both TMR and corn silage samples (Table 2) differed significantly between farms ($P < 0.01$).

In general, the TMRs had a lower average size of particles than suggested ranges (Heinrichs, 1996): the percentage of fractions having particles > 19 mm were generally lower than that recommended (6-10%), while the fractions collected in the bottom pan (< 8 mm) were higher than 30-50%. Suggested ranges for both the middle and the bottom fractions of corn silage are 40-50% (Heinrichs, 1996) while data from Northern American States (Heinrichs *et al.*, 1999) show the tendency to have

Table 2. Pennsylvania State University separator distribution data for TMR and corn silage samples and statistical parameters of precision.

	Percentage of total mixed ration with particle size			Percentage of corn silage with particle size		
	> 19 mm	8-19 mm	< 8 mm	> 19 mm	8-19 mm	< 8 mm
Overall mean	4.2	35.1	60.6	5.7	68.3	26.0
Farm						
A	8.5	32.7	58.8	8.8	61.1	30.1
B	3.7	41.1	55.2	5.6	74.7	19.7
C	2.5	22.2	75.3	2.9	61.6	35.5
D	2.2	44.5	53.3	5.5	75.6	18.9
Operator						
1	4.5	35.0	60.5	4.9	68.0	27.1
2	4.1	36.2	59.7	5.3	68.5	26.2
3	4.4	35.5	60.1	6.6	67.8	25.6
4	4.5	34.4	61.2	5.2	68.3	26.4
5	4.2	35.3	60.5	4.7	69.0	26.2
6	4.1	35.0	60.8	5.8	67.4	26.8
7	3.4	34.1	62.5	5.2	68.1	26.8
8	4.7	35.5	59.8	7.9	69.0	23.1
Effects ¹						
-farm	**	**	**	**	**	**
-operator	ns	ns	ns	*	ns	**
-farm x operator	ns	ns	ns	ns	ns	ns
Repeatability						
-standard deviation (\pm %)	1.9	1.4	2.2	2.0	1.7	1.5
-variability coefficient (%)	44.7	3.9	3.7	35.1	2.5	5.6
Between operators reproducibility						
-standard deviation (\pm %)	1.6	1.5	2.2	2.1	1.8	1.9
-variability coefficient (%)	36.6	4.3	3.6	36.5	2.6	7.4

¹ ** ($P < 0.01$); * ($P < 0.05$); ns: not significant.

a prevalence for the middle fraction ($50.8 \pm 12.3\%$) with respect to the fraction recovered on the lower screen ($41.1 \pm 14.1\%$). The corn silage samples considered in the present study had the intermediate fraction ranging from 60 to 75% and the bottom fraction between 20 and 35%. Other measurements performed on Italian corn silages from different cutting lengths or post harvesting processing gave similar values (63 to 72%, Andrighetto *et al.*, 1998) or higher values (74 to 84%, Masoero *et al.*, 2001) for the middle fraction.

The interaction between farm and operator was not significant for either sample. For corn silage samples there was a significant effect of the operator for the top screen ($P < 0.05$) and the bottom pan ($P < 0.01$) fractions.

The variance components for farm and 'farm x operator' effects, together with the residual error variance allowed the calculation of repeatability and between operators reproducibility of measurements of physical separation.

Repeatability expressed as standard deviation was similar between fractions (>19 , $8-19$ and <8 mm fractions: ± 1.9 , ± 1.4 and $\pm 2.2\%$ for TMR samples; ± 2.0 , ± 1.7 and $\pm 1.5\%$ for corn silages), but when expressed as coefficient of variation there were for both samples, large differences between the values for fraction exceeding 19 mm (44.7 and 35.1 %, respectively for TMR and corn silages) and the middle (3.9 and 2.5 %, respectively for TMR and corn silages) and bottom fractions (3.7 and 5.6 %, respectively for TMR and corn silages).

These data confirm the variability reported in the survey done by Heinrichs *et al.* (1999) where the coefficient of variability for the percentage of particles longer than 19 mm (76-80 %, TMR and corn silage samples) was two-four times that of the other two fractions (values between 21 to 34%). The between-operators reproducibility was very close to the repeatability and this indicates that contribution to overall variability of operators was marginal in comparison with residual variability. In collaborative tests for analytical determination, the reproducibility have generally higher values than repeatability because the methodology, apparatus and operators of different laboratories are important sources of variability (Lanari *et al.*, 1991). On the contrary, in the present conditions,

where the same apparatus and methodology were utilised and all operators followed a training course, all these possible variability effects were almost completely removed.

Repeatability data obtained for intermediate and bottom fraction (between 2.5 to 5.6 %) were slightly higher than those typically obtained for several analytical procedures (Lanari *et al.*, 1991). On the contrary, the fraction of particles longer than 19 mm in complete diets and corn silage samples showed very high variability (repeatability between 35.1 and 44.7 %).

Conclusions

In feeds considered in the present study (TMR and corn silages), the repeatability of measurements of fractions having middle (8 to 19 mm) and short (<8 mm) particle length is satisfactory, and this implies the need for a limited number of replicates to obtain sound average values. On the contrary, there is scarce precision in the measurement of the fraction having particles >19 mm, and, therefore, caution is needed in the utilisation of these data. Moreover, the contribution of different operators on the overall variability of these measurements is very limited.

In conclusion, different operators (e.g. consultants of extension services or feed companies, technicians of experimental stations, feed sales persons, etc.) properly trained on the use of the separator can produce satisfactory repeatable and reproducible values for the middle and the bottom fractions

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