Italian Journal of Animal Science

ISSN: (Print) 1828-051X (Online) Journal homepage: https://www.tandfonline.com/loi/tjas20

Problems concerning ovine milk clotting aptitude

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To cite this article: E Duranti, P Bolla, A Caroli, L Chiofalo, L. Di Stasio, R Fortina, M Martini, V Piccolo & A Zullo (2003) Problems concerning ovine milk clotting aptitude, Italian Journal of Animal Science, 2:1, 89-95, DOI: 10.4081/ijas.2003.89

To link to this article: https://doi.org/10.4081/ijas.2003.89

ASPA Commissions' activity

Evaluation of ovine milk clotting aptitude

ASPA Commission "Problems concerning ovine milk clotting aptitude"[1]

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ABSTRACT

A comparative study of the lactodynamographic parameters was carried out on ovine milk. Besides evaluating the repeatability and reproducibility of the analytical method, the influence of some variables such as the genetic type (three breeds), the kind of milk (whole or skimmed), and its concentration after reconstitution (12g or 20g /100 ml) was evaluated. The working plan involved 6 laboratories for the final statistic analyses, by the use of freeze-dried milk samples (adequately reconstituted on the basis of established methods) from Sardinia, Comisana, and Massese ewes. All the considered variability factors showed a highly significant effect ($P<0.001$) on the lactodynamographic parameters considered. In particular, Massese ewe milk showed the shortest curd speed (k_{20}) and the best coagulum strength (a_{30} and a_{45}), although clotting time (CT) was the highest one. The same trend was registered for skimmed milk and for the most concentrated one (20g). Repeatability values within laboratories were 96% and 97% for CT and k_{20}, lowering for a_{30} e a_{45}, (respectively 87% and 85%). Much lower coefficients were found for the among laboratories reproducibility, ranging from a maximum of 58% for CT to a minimum of 18% for k_{20}. The wide variability observed indicates that lactodynamographic parameters are comparable only within the same lab. Further investigation is needed to compare different labs in order to obtain more homogeneous results.

Key words: Ovine milk, Lactodynamographic analysis, Repeatability, Reproducibility.

RIASSUNTO

VALUTAZIONE DELL'ATTITUDINE ALLA COAGULAZIONE PRESAMICA DEL LATTE OVINO

È stato effettuato uno studio comparativo sui parametri lattodinamografici del latte ovino. Oltre a valutare la ripetibilità e la riproducibilità del metodo di analisi si è voluto valutare anche l'influenza di alcune variabili quali il tipo genetico (tre razze), il tipo di latte (intero e scremato) e la sua concentrazione in seguito a ricostituzione (12g o 20g/100 ml). Il piano operativo ha coinvolto, per le elaborazioni statistiche finali, 6 laboratori ed ha previsto l'utilizzazione di campioni di latte liofilizzati (ricostituiti secondo metodiche stabilite) di pecora Sarda, Comisana e Massese. Tutti i fattori di variabilità considerati hanno presentato un effetto altamente significativo ($P<0,001$) sui parametri lattodinamografici considerati. In particolare, il latte della pecora Massese, pur mostrando un tempo di reazione all'enzima più elevato (TC), ha presentato un tempo di rassodamento (k20) minore ed una migliore consistenza del coagulo (a30 e a45). Stesso andamento è stato registrato per il latte scremato e per quello a maggiore concentrazione (20g). I valori di ripetibilità all'interno dei laboratori sono risultati pari a 96% e 97% per le misure di TC e k20 e minori per a30 e a45, (rispettivamente 87% ed 85%). Molto inferiori risultano i coefficienti di riproducibilità tra laboratori, con un valore massimo del 58% per TC e mini-

mo per k20 (18%). La notevole variabilità osservata indica che i confronti tra le valutazioni lattodinamografiche sono possibili nell'ambito dello stesso laboratorio. Per rendere accettabili i confronti tra i diversi laboratori sono necessari ulteriori approfondimenti che consentano di ottenere risultati più omogenei.

Parole chiave: *Latte ovino, Analisi lattodinamografica, Ripetibilità, Riproducibilità.*

Introduction

In the dairy sector, food products need to reach a higher and higher quality level, not only from the nutritional and organoleptic point of view, but also concerning technological properties. The aim is that market can dispose of raw materials with different prices on the basis of specific technological properties. With regard to this point, in the future attention should be paid to milk rennet clotting aptitude, particularly important for the ovine production where milk is entirely devoted to cheese making.

Different authors (Chiofalo *et al.*, 1986, 1989; Ubertalle *et al.*, 1989, 1990; Casoli *et al.*, 1990; Manfredini *et al.*, 1992, 1993; Cecchi *et al.*, 1997; Zullo *et al.*, 1997) tried to classify the ovine milk on the basis of the lactodynamographic properties. Such an attempt runs the risk of penalising milks that require a longer clotting time, as a consequence of different genetic, environmental, and technological factors, although they can give a high cheese yield, in addition to dairy products particularly required by consumers (Martini *et al.*, 2000).

Thus, attention has often been paid by researchers to the evaluation of milk clotting aptitude, and the Scientific Association of Animal Production (A.S.P.A.) has interpreted the need of standardised lactodynamographic methodologies, in the attempt of reducing variability sources and comparing analytical results obtained in different operative conditions.

First, the Study Commission "Methodologies of evaluation of the quantitative and qualitative milk production in the main species of zootechnical interest" worked to a standardised lactodynamographic method, published in the handbook "Methods for the analysis of milk in the main livestock species" (1995).

Later (spring 1997), a ring-test was performed among the laboratories involved in the evaluation of bovine milk clotting aptitude (A.S.P.A. 1999). In November 1999, a national network was constituted in order to evaluate the same problems in ovine milk showing a very high genetic and environmental variability.

The organisation of a ring-test for the lactodynamographic analysis in ovine species presented a main problem due to the lack of freeze-dried commercial milk as a reference sample, which is on the contrary easily available for bovine species. The first step was to adequately freeze-dry and reconstitute bulk milk samples selected within the three main Italian ovine breeds, Sarda, Comisana, and Massese.

The aim of this work was to evaluate the results obtained in a comparative study of the ovine milk lactodynamographic parameters. The influence of some variability factors (genetic type, milk skimming, milk concentration) on the lactodynamographic parameters CT (clotting time), k20 (syneresis time), a30 and a45 (coagulum strength) was evaluated in addition to the reliability of the analytical method.

Material and methods

Sampling

Three Operative Units (Veterinary Medicine, Messina University, for Comisana breed; Veterinary Medicine, Pisa University, for Massese breed; *Istituto Lattiero-Caseario per la Sardegna*, Bonassai, Sassari, for Sardinia breed) carried out milk collection. Milk sampling was organised aiming to minimise variability factors. For each breed, a typical permanent flock was chosen in a plain area. Bulk milk from morning milking was collected in spring from about 40 ewes (10 animals per different lambing order: 1st, 2nd, 3rd, 4th and more), lactation length ranging from 60 to 90 days.

For each breed, a bulk milk sample (about 23 kg) was collected and freeze-dried partly as whole milk (type A), and partly after skimming (type B).

The freeze-dried samples, in addition to rennet and calcium chloride kindly provided by Hansen (Corsico, Milano, Italy), were sent to the 12 national laboratories participating to the trial. Material was sent in a refrigerated bag by express delivery, recommending to keep it at 4°C until the analysis, and to develop the lactodynamographic test as soon as possible. Instructions for the analysis were provided, together with a registration form for lactodynamographic parameters (CT, k_{20}, a_{30} and a_{45}).

The reconstitution of the freeze-dried milk, both whole and skimmed, was made starting from 12 g and 20 g ± 0.02 of powder, by adding 100 ml of a $CaCl_2$ 0.02M solution, in order to simulate fresh milk properties in the best way.

Statistical analysis

A statistical analysis was performed eliminating anomalous values, as well as data from laboratories not providing results for all the three analysed breeds. Therefore, only six laboratories were considered in the analysis.

The following linear model was fitted by the statistical package Jmp (1996):

$$y_{ijklm} = \mu + \alpha_i + \beta_j + \delta_k + \chi_l + (\alpha\beta)_{ij} + (\alpha\chi)_{il} + (\alpha\delta)_{ik} + (\beta\chi)_{jl} + (\beta\delta)_{jk} + (\alpha\beta\chi)_{ijl} + (\alpha\beta\delta)_{ijk} + \varepsilon_{ijklm}$$

where:

y_{ijklm} = considered parameter (time by time pH, CT, k_{20}, a_{30} e a_{45});
μ = overall mean;
α_i = fixed effect of the i^{th} breed (i = 1,2,3);
β_j = fixed effect of the j^{th} laboratory (j = 1,…,6);
δ_k = fixed effect of the k^{th} reconstitution type (k = 12g or 20g);
χ_l = fixed effect of the l^{th} milk type (l = whole or skimmed);
$(\alpha\beta)_{ij}$ = effect of the interaction (breed, laboratory);
$(\alpha\chi)_{il}$ = effect of the interaction (breed, milk type);
$(\alpha\delta)_{ik}$ = effect of the interaction (breed, reconstitution type);
$(\beta\chi)_{jl}$ = effect of the interaction (laboratory, milk type);
$(\beta\delta)_{jk}$ = effect of the interaction (laboratory, reconstitution type);
$(\alpha\beta\chi)_{ijl}$ = effect of the interaction (breed, laboratory, milk type);
$(\alpha\beta\delta)_{ijk}$ = effect of the interaction (breed, laboratory, reconstitution type);
ε_{ijklm} = random error.

A further statistical model was fitted aiming to evaluate a repeatability and reproducibility coefficient that could give a percent probability for an analytical parameter to be repeated in the same lab, or reproduced in different labs. The statistical method suggested by Caroli (1998) was fitted to the analytical model used in the present work.

For the repeatability coefficient evaluation within laboratories, the following random model was fitted:

$$y_{ijk} = \mu + milk_{ij} + \varepsilon_{ijk}$$

where:

y_{ijk} is the k^{-th} lactodynamographic parameter (time by time CT, k_{20}, a_{30}, a_{45}); $milk_{ij}$ is the random effect of the i^{-th} kind of milk (12 levels, from the combinations breed by milk type * by reconstitution type) within the j^{-th} laboratory.

The repeatability coefficient within labs was calculated by the ratio between the following variance components:

$$r = \frac{\sigma^2_{milk_{ij}}}{\sigma^2_{milk_{ij}} + \sigma^2_{error_{ijk}}}$$

For the evaluation of the reproducibility coefficients among laboratories the following random model was fitted:

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

where:

y_{ij} is the j^{-th} lactodynamographic parameter (time by time CT, k_{20}, a_{30}, a_{45}); $milk_i$ is the random effect of the i^{-th} kind of milk (12 levels).

The reproducibility coefficient among labs was calculated by the ratio between the following variance components:

$$R = \frac{\sigma^2_{milk_i}}{\sigma^2_{milk_i} + \sigma^2_{error_{ij}}}$$

Results and discussion

The variability factors considered and the interactions between and among them mainly showed a significant effect (P<0.001) on the lactodynamographic parameters and milk pH (table 1). The only exceptions were the interactions milk type by breed (not significant on pH), and milk type by reconstitution type (not significant for all the considered parameters, and therefore excluded from the statistical model).

The general trend reflects the results previously obtained on bovine milk (A.S.P.A., 1999). The determination coefficients of the fitted model were high, ranging from 0,93 (pH, k_{20}), 0.90 (TC), 0.87 (a_{30},), to 0.84 (a_{45}).

Breed effect

Genetic type greatly influences milk pH and lactodynamographic parameters, showing that the milk chemical and physical properties of each ovine breed significantly affect (P<0.01) the rennet clotting aptitude (table 2).

Accordingly to recent studies (Cecchi e coll. 1997, Martini e coll. 1999, Martini e coll. 2000), a higher length of the enzymatic phase was shown for Massese milk, also characterised by a pH value significantly higher than Sarda and Comisana breed (6.23 vs 6.16 and 6.11). On the contrary, syneresis is faster for Massese milk, and higher curd strength is reached after 30 and 45 minutes from the rennet addition in comparison with the other two breeds. The different physical and chemical properties of the three breeds give rise to this effect. In particular, some authors (Delacroix-Bouchet *et al.* 1994, Mariani P. *et al.* 1997, Pellegrini O. *et al.* 1997) attribute clotting trends like the one observed in Massese breed to a higher milk total protein or casein content.

The curd strength 45 minutes after the rennet addition (a_{45}) shows the same behaviour as a_{30} parameter, with slightly lower values.

Laboratory effect

Least-square means of pH and lactodynamographic parameters for the different laboratories are reported in table 3. The high variability is a clear proof of the low reproducibility of the analytical method among labs. The already mentioned

Table 1. Level of significance.

	pH	CT (min)	k_{20} (min)	a_{30} (mm)	a_{45} (mm)
Breed	***	***	***	***	***
Lab	***	***	***	***	***
Milk type (A or B)	*	***	***	***	***
Milk reconstituted type (12 g or 20 g)	***	***	***	***	***
Lab x A or B	***	***	***	***	***
Lab x 12 g or 20 g	***	***	***	***	*
Breed x Lab	***	***	***	***	***
Breed x Lab x A or B	***	***	***	***	**
Breed x Lab x 12 g or 20 g	***	***	***	***	***
Breed x A or B	ns	***	***	***	**
Breed x 12 g or 20 g	***	***	***	***	*

** : P<0.05.*
*** : P<0.01.*
**** : P<0.001.*
ns: not significant.

significant effect of most interactions between and among the variability factors (table 1) is a further evidence of the difficulty in standardising the lactodynamographic analysis.

Milk type effect

Milk fat content significantly affects both pH ($P<0.05$) and lactodynamographic parameters ($P<0.01$) (table 4). Thus, skimmed milk shows lower pH, value higher rennet clotting time and stronger curd strength at 30 minutes, according to Zumbo *et al.* (1994), whereas curd formation is faster.

Reconstitution type

The freeze-dried quantity resulting in the different concentration of the reconstituted milk significantly affects ($P<0.01$) all the analysed parameters (table 5). Reconstitution starting from 20 g of powder gives raise to more concentrated milk, resulting on one hand in lower pH and longer enzymatic phase, on the other hand, in faster clot formation and higher curd strength.

Repeatability and reproducibility

Finally, table 6 shows the repeatability and reproducibility coefficients that account in percentage terms for the variability of the lactodynamographic parameters within (repeatability) and among (reproducibility) laboratories, the theoretical limits ranging from 0% to 100%. The more repeatable or reproducible the method was, the higher the values were for the two coefficients. The probability for two measures of CT and k_{20} to be repeated in the same conditions (both same laboratory and operator) amounted respectively to 96 % and 97%. The same probability went down to 87% and 85% for a_{30} and a_{45}. The reproducibility coefficients among different labs were much lower, with a maximum value of 58% for CT, and a minimum for k_{20}.

First of all, the observed repeatability values suggest the necessity to perform a more accurate calibration of the lactodynmographic apparatus within the same lab, with particular attention to

Table 2. Least square means of pH and coagulation properties by breed.

	pH		CT (min)		K_{20} (min)		a_{30} (mm)		a_{45} (mm)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Comisana	6.11 C	0.004	4.78 B	0.069	3:40 A	0.062	35.67 B	0,47917	32.99 B	1.387
Massese	6.23 A	0.004	5.94 A	0.069	2.09 C	0.057	50.90 A	0,47917	43.55 A	1.205
Sarda	6.16 B	0.004	4.21 C	0.069	2.47 B	0.057	35.84 B	0,51389	30.77 B	1.215

A, B, C : P < 0.01.

Table 3. Least square means of pH and coagulation properties by laboratory.

	pH		CT (min)		K_{20} (min)		a_{30} (mm)		a_{45} (mm)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Lab 1	6.14 Da	0.006	5.06 CB	0.098	2.92 B	0.093	31.06 D	0.974	24.65 Cc	1.966
Lab 2	6.12 Db	0.006	4.45 D	0.098	1.65 Cbc	0.081	57.96 A	0.974	55.25 A	1.949
Lab 3	6.22 B	0.006	4.83 C	0.098	1.84 Cb	0.081	54.27 B	0.974	49.88 AB	1.949
Lab 4	6.28 A	0.006	5.21 AB	0.098	1.58 Cc	0.082	40.83 C	0.974	43.96 B	2.449
Lab 5	6.18 C	0.006	5.48 A	0.098	5.82 A	0.082	31.27 D	0.983	30.56 Cb	1.966
Lab 6	6.08 E	0.006	4.85 C	0.098	2.12 Ca	0.081	29.44 D	1.107	22.60 C	2.077

a,b,c : P<0.05.

A, B, C,D,E : P < 0.01.

Table 4. Least square means of pH and coagulation properties by milk type.

	pH		CT (min)		K_{20} (min)		a_{30} (mm)		a_{45} (mm)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
Whole milk	6.18 a	0.004	4.17 B	0.055	3.02 A	0.049	34.30 B	0.583	30.18 B	1.023
Skimmed milk	6.16 b	0.004	5.79 A	0.057	2.29 B	0.048	47.31 A	0.574	41.83 A	1.036

a,b : P<0.05.
A, B : P < 0.01.

Table 5. Least square means of pH and coagulation properties by milk reconstituted type.

	pH		CT (min)		K_{20} (min)		a_{30} (mm)		a_{45} (mm)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
12 g	6.20 A	0.004	4.05 B	0.056	3.23 A	0.047	35.08 B	0.594	32.55 B	1.051
20 g	6.14 B	0.004	5.91 A	0.056	2.02 B	0.049	46.53 A	0.565	39.46 A	1.010

A, B : P < 0.01.

the curd strength parameters (a_{30} and a_{45}). Moreover, the low reproducibility of the method is a problem requiring further investigation, aiming to avoid rash conclusions while comparing the lactodynamographic results of different labs. The availability of ovine milk samples, adequately freeze-dried and analysed in more operative conditions, could be a useful tool allowing to compare results from different labs by the use of adjustment coefficients properly implemented.

Conclusions

The results obtained in this comparison study carried out by some national labs, on the lactodynamographic properties of ovine milk, highlight the great variability of the parameters evaluated.

Table 6. Repeatability and reproducibility coefficient for the lactodynamographic parameters.

parameters	r	R
CT (min)	0.96	0.58
K_{20} (min)	0.97	0.18
a_{30} (mm)	0.87	0.33
a_{45} (mm)	0.85	0.25

The bulk milk clotting characteristics of the three more important Italian ovine dairy breeds were interestingly compared. Massese milk, although presenting the highest clotting time, showed the best rennet coagulation aptitude as concerns syneresis time and curd strength. A similar trend was shown for skimmed milk, and for milk reconstituted from 20g of freeze-dried powder, comparable to the milk richer in dry matter, typical of some ovine breeds.

The wide variability observed among the different labs indicates that the lactodynamographic method, being scarcely reproducible, is more suitable to compare milk samples within the same lab than among different labs.

To get a through analysis of the problem, the availability of ovine freeze-dried milk to be adequately freeze-dried and used as a reference sample, could be a valid tool for comparing the lactodynamographic parameters obtained by different labs. Finally, the observed pH variability of the reconstituted milk suggests the opportunity to calculate specific adjustment coefficients of the lactodynamographic parameters for the pH of reference samples.

The Commission's members wish to thank the Colleagues E. Cauvin, P. Micari, G. Piredda, M. Rampilli, S. Rapaccini, M.L. Scatassa, A. Sevi, who participated in the workshops as listeners.

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