

Evaluation of ewe colostrum quality by estimation of enzyme activity levels

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SUMMARY

The objective of this study was to assess the potential use of colostrum enzymes for the determination of colostrum quality in ewe. The enzymes gamma glutamyltransferase (GGT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were measured in the colostrum from 11 ewes, milked within six hours after birth, by a dry chemistry system and spectrophotometrically. The quality of colostrum, given by the content of gamma globulins (IgG), was measured by electrophoresis separation of colostrum proteins. The highest activity was found for GGT, followed by LDH and ALP. A very high correlation ($r = 0.83$, $P < 0.001$) between GGT and IgG concentration was shown, suggesting this enzyme can be a good marker for the evaluation of colostrum quality in ewe.

KEY-WORDS : ewe - colostrum - enzymes - gamma globulin - immunity.

RÉSUMÉ

Évaluation qualitative du colostrum de la brebis, par l'estimation du niveau d'activité des enzymes. Par A. ZARRILLI, E. MICERA, N. LACARPIA, P. LOMBARDI, M.E. PERO, A. PELAGALLI, D. d'ANGELO, M. MATTIA et L. AVALLONE.

L'objectif de cette étude a été d'évaluer la possible utilisation des enzymes pour déterminer la qualité du colostrum chez la brebis. Les enzymes gammaglutamyltransférase (GGT), lactate déshydrogénase (LDH) et les phosphatases alcalines (ALP) ont été mesurées dans le colostrum de 11 brebis traitées dans les six heures suivant la mise-bas ou parturition à travers un système chimique à sec et spectrophotométrie. La qualité du colostrum donnée par les globulines gamma (IgG) a été mesurée par la séparation électrophorétique des protéines du colostrum. La plus grande activité a été trouvée dans les GGT, suivi des LDH et ALP. Une grande corrélation ($r = 0.83$, $P < 0.001$) entre les concentrations GGT et IgG a été trouvée, suggérant que cet enzyme peut être un point de référence pour l'évaluation de la qualité des colostrum chez les brebis.

MOTS-CLÉS : brebis - colostrum - enzymes - gammaglobuline - immunité.

Introduction

Ruminants are born lacking of circulating immunoglobulins so, they must absorb maternal immunoglobulins from colostrum during the neonatal period [8]. The ingestion of colostrum for passive immunity must be fast and complete since the intestinal absorption time of immunoglobulins lasts up to 24 hours [4]. The first day after birth is the most critical period for lambs because either an insufficient colostrum intake or a poor colostrum quality lead to a poor immunity. A low concentration of immunoglobulins was shown to be related to many neonatal diseases and mortality [15, 13]. Low concentration of antibodies in colostrum will result in insufficient gamma globulin in the blood and, consequently, in poor immunity. For these reasons, an early determination of colostrum quality is fundamental for a good neonatal management in order to reduce the risk of neonatal diseases. As the measurements of immunoglobulins in the colostrum

are time consuming, costly and of limited use, alternative methods are needed, which are fast, simple and can be performed on the farm by the farmer or veterinary practitioner. When using common methods for immunoglobulin determination, it is possible that by the time the information is obtained, the ability of the calf to absorb the immunoglobulins into blood has been lost. In some large ruminants, the use of colostrometer is a commonly practised procedure [5] but, requiring a large amount of colostrum (250 ml), it is not recommended for ewe colostrum. Moreover, it suffers from poor accuracy and wide variability [12] and, despite its use in cattle, it was shown to be not suitable for other ruminants such as buffalo [10]. Determination of enzymes was shown to be a reliable test to establish colostrum ingestion in several species including sheep [1, 3, 9, 16]. The indirect evaluation of IgG ingestion is a useful tool for a fast therapeutic approach. Further more, the evaluation of colostrum content of IgG can be much more useful for prevention. The aim of

this study was to assess the possible use of enzymes as markers for ewe colostrum quality. As previously shown in other ruminants, the simplicity and speed of enzyme assay by using dry chemistry techniques can make them good parameters for an on farm laboratory marker for colostrum quality.

Materials and methods

Colostrum was milked within 6 hours after lambing from 11 ewes on commercial farms in Puglia region, Italy. The colostrum was first centrifuged at 4000 x g for 15 minutes to remove fat and sediments and the supernatant was then centrifuged at 20000 x g for 30 minutes. The intermediate layer was withdrawn for analysis. Additionally, blood was taken from the jugular vein of lambs at 24h of age, serum was used for analysis.

ANALYSIS

Total protein was determined using the biuret method [7]. Protein fractions were determined by electrophoretic separation using the Hydragel protein kit from SEBIA (France) and was quantified using a densitometer from CGA (Florence, Italy). The enzymes GGT and ALP were measured at 37°C using the dry chemistry system of Boehringer Mannheim «Reflotron» [2]. The enzyme LDH was measured at 37°C using reagents and method from Sclavo, Italy [6].

STATISTICS

Means, standard deviations, correlation coefficients and significances were determined using the general linear model (GLM) procedure of the SAS program [14].

Results

Means and standard deviations of the activities of the enzymes GGT, ALP and LDH, and the concentrations of total proteins and gamma globulines in colostrum are shown in figure 1. The concentration of GGT was the highest 23207 ± 8119 u/l, followed by LDH and ALP, with values of 832 ± 124 and 1011 ± 153 u/l respectively. The results from the electrophoretic separation patterns (figure 2), reveal that the major proteins fraction in the ewe colostrum is gamma globulin, which made up 52 % of the total proteins.

The highest correlation was obtained between IgG and the enzyme GGT (0.83, $P < 0.001$) (figure 3). Lower and insignificant correlations were seen between IgG and the enzymes ALP (0.44). No correlation was found for LDH.

A high correlation ($r = 0.78$, $P < 0.001$) between GGT and IgG was also shown in lambs serum at 24h of age (data not shown).

Discussion

Our results showed that GGT determination is suitable as a marker for colostrum quality in ewe. Lambs are unable to produce immunoglobulins until two weeks of age, therefore, the ingestion of a proper amount of high quality colostrum is necessary for survival. Moreover, like other ruminants, lambs are born with a highly permeable intestinal epithelium, which both passively and selectively allows for the passage of large molecules. Such process is short-lived revealing a rapid decline after parturition, at 48 hours of age the newborn lamb is unable to absorb maternal antibodies. The determination of colostrum quality was shown to be important in other ruminants in order to reduce incidence of diseases and mortality by optimizing neonatal management. Because of the very short time of both the colostrum content of immunoglobulins

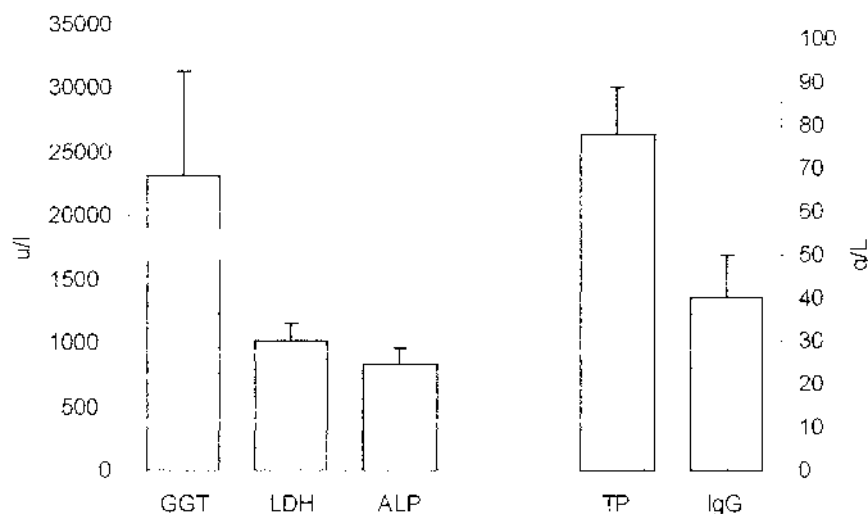


FIGURE 1. — Levels of GGT, LDH, ALP, total protein (TP) and gamma globulin (IgG) in colostrum from healthy ewes within six hours after delivery. The enzyme levels are expressed as U/liter; the protein content (total and IgG) are expressed as g/liter.

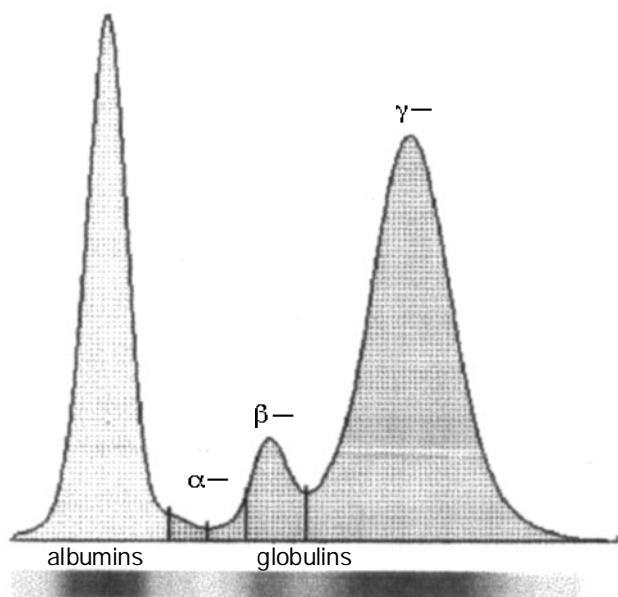


FIGURE 2. — Electrophoretically separated ewe colostrum proteins (representative example).

and the lamb capability to absorb them, fast methods for the determination of IgG content in colostrum and or in the newborn blood were tested to allow the farmer or the veterinary practitioner a fast therapeutic approach. Bovine colostrum quality is estimated by measuring its specific gravity in a Colostrometer. Specific gravity was related to IgG concentration enabling to estimate the quantity on the basis of a statistical correlation. Such method was shown not to be reliable for other ruminants and an enzymatic test based on GGT concentration was shown to be the first choice marker for colostrum quality assay. In ewe colostrum GGT showed a very high concentration and a high correlation with IgG content. A similar result was already described in bovine and buffalo but the levels of GGT were not so high [1, 9]. The reason for the higher concentration of GGT in ewe colostrum is still not clear but, being observed in several species including human being it is guessed that the enzyme is involved in the process of colostrum synthesis. Additionally, as previously shown [3, 16], being correlated with IgG content 24h after birth, GGT confirmed to be a good marker for colostrum ingestion. The availability of dry chemistry tests for GGT assay allows an on farm determination of both colostrum quality and colostrum ingestion, in such a way the farmer can immediately act by feeding the proper amount of high quality colostrum for passive immunity. In conclusion, the high correlation seen between the activity of GGT and the concentration of IgG and the speed and simplicity of its measurement using dry chemistry technology make GGT a good potential test for the evaluation of IgG concentration in ewe colostrum.

References

1. — BOGIN E., YAVIDAR Y., SHENKLER S., ISRAELI B.A., SPIEGEL N. and COHEN R. : A rapid field test for the determination of colostrum ingestion by calves, based on gamma-glutamyltransferase. *Eur. J. Clin. Chem. Clin. Biochem.*, 1993, **31**, 695-699.

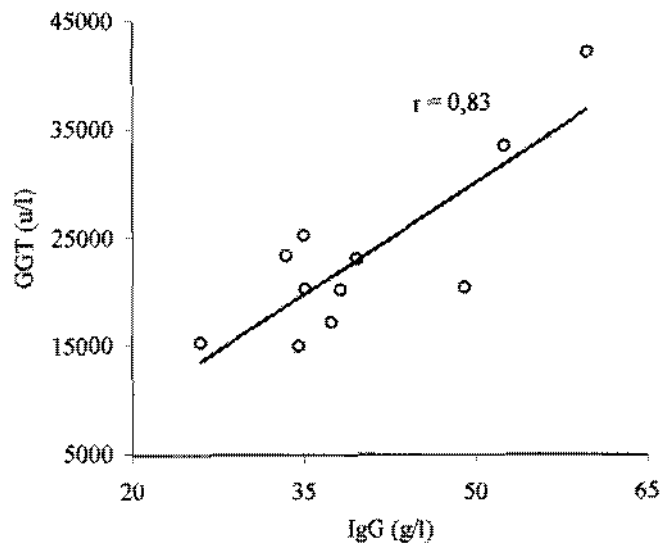


FIGURE 3. — Correlation between GGT and IgG in colostrum from healthy ewes within six hours after delivery.

2. — BOHERINGER-MANNHEIM GmbH Reflotron, Manual, Germany, 1989.
3. — BRAUN J.P., TAINTURIER D., BEZILLE P., RAVIART I. and RICO A.G. : Transfer of gamma-glutamyltransferase from mother colostrum to newborn goat and foal. *Enzyme*, 1984, **31** (4), 193-196.
4. — ESSER D., SCHMIT F.W., VON KORN S. and PETERS K.J. : Immunoglobulins G status of ewes and their lambs. *J. Anim. Breed. Gen.*, 1989, **106**, 120-128.
5. — FLEENOR W.A. and STOTT G.H. : Hydrometer test for estimation of immunoglobulin concentration in bovine colostrums. *J. Dairy Sci.*, 1980, **63**, 973-977.
6. — GERMAN SOCIETY FOR CLINICAL CHEMISTRY : *Ztscher. Klin. Chem. Klin. Biochem.*, 1972, **10**, 281.
7. — GORNALL A.G., BARDAWILL C.J.H. and DAVID D.D. : Determination of serum proteins by means of biuret reaction. *J. Biol. Chem.*, 1949, **177**, 751-766.
8. — HALLIDAY R. : Immunity and health in young lambs. *Vet Rec.*, 1978, **103**, 489-492.
9. — LOMBARDI P., AVALLONE L., d'ANGELO A. and BOGIN E. : γ -Glutamyltransferase and serum proteins in buffalo calves following colostrum ingestion. *Eur. J. Clin. Chem. Clin. Biochem.*, 1996, **34**, 955-968.
10. — LOMBARDI P., AVALLONE L., PAGNINI U., d'ANGELO D. and BOGIN E. : Evaluation of Buffalo Colostrum Quality by Estimation of Enzyme Activity Levels. *J. of Food Prot.*, 2001, **64**, 1265-1267.
11. — MECHOR G.D., GROHN Y.T. and VAN SAUN R.Y. : Effect of temperature on colostrometer readings for estimation of immunoglobulin concentration in bovine colostrums. *J. Dairy Sci.*, 1991, **74**, 3940-3943.
12. — PRITCHETT L.C., GAY C.C., HANCOCK D.D. and BESSER T.E. : Evaluation of the hydrometer for testing immunoglobulin G1 concentrations in Holstein colostrum. *J. Dairy Sci.*, 1994, **77**, 1762-1767.
13. — REHAN A., KHAN A., YAVED M.T. and HUSSAINI I. : The level of immunoglobulins in relation to neonatal lamb mortality in Pak-Karakul sheep. *Veterinarski Archiv.*, 2000, **70** (3), 129-139.
14. — SAS INSTITUTE INC. : SAS user's guide : statistics, SAS Institute Inc., 1985, Cary, NC, NY
15. — SAWYER M. and WILLADSEN C.H. : Passive Transfer and Colostral Immunoglobulins from Ewe to lamb and Its Influence on Neonatal Lamb Mortality. *J. Am. Vet. Med. Association.*, 1977, **171**, 1255-1259.
16. — TESSMAN K.R., TYLER W.J., PARISH M. S., JOHNSON L. D., GANT G. R. and GRASSECCHI. A.H. : Use of age and serum gamma-glutamyltransferase activity to assess passive transfer status lambs. *J. Am. Vet. Med. Association.*, 1997, **211**, 1163-64.