

Assessment of the passive transference of immunity in calves using metabolic indicators

Utilización de indicadores metabólicos en la valoración de la transferencia de inmunidad pasiva en neonatos bovinos

Angie Fairut Carrillo¹, Valentina Loaiza¹, Rómulo Campos G.²

¹Independent professionals. ²Departamento de Ciencia Animal, Universidad Nacional de Colombia, sede Palmira, Author for correspondence: rcamposg@palmira.unal.edu.co

Abstract

Fifteen cows and their calves (from the races Gyr, Brahaman and the cross Gyr x Holstein) were chosen randomly in order to determine the usefulness of the serum activity of Alkaline phosphatase (ALP) and the total serum proteins (TSP) as indicators of transference of immunity in calves through colostrum consumption in their first days of life. 300 ml of colostrum were collected from the cows on the day after delivery to determine total solids, total protein and ash content. Blood samples without anticoagulant were taken from the calves by jugular venipuncture on the 0, 4, 12, and 30th day post partum. The concentration of the TSP was determined using refractometry, while the ALP was determined through the enzymatic colorimetric assay. A high and statistically significant relationship was found between the percentages of crude protein of the colostrum and the total solids, thus, it can be concluded that just one of these measurements is required to study both indicators. The effect of time on the concentrations of TSP as well as on the enzymatic activity was evaluated through the use of ANOVA (Analysis of Variance). In the TSP there was a significant difference ($p < 0.05$) between the first (69 g/dl) and the 30th day (58 g/dl), in which the lowest concentration occurred. Average values, higher than 4.5 g/dl of the TSP were found, which may indicate that the calves developed passive immunity through colostrum consumption. The ALP enzymatic activity decreased progressively during the period of study (1326.4 U/L in the 1st day cf. 599 U/L on the 30th day post partum; $p < 0.05$). However, it was not proved that the ALP can be used as a direct indicator of the transference of passive immunity.

Keywords: Colostrum, passive immunity, maternal immunity, alkaline phosphatase

Resumen

Para determinar la utilidad de la actividad sérica de la fosfatasa alcalina (FA) y las proteínas séricas totales (PST) como indicadores de transferencia de inmunidad en terneros por el consumo de calostro en sus primeros días de vida, en un hato del trópico bajo de Colombia se escogieron al azar 15 vacas de las razas Gyr, Brahaman y el cruce Gyr x Holstein y sus respectivos terneros. En las vacas se recolectaron aproximadamente 300 ml de calostro el primer día del parto para determinar sólidos totales, proteína total y cenizas. En los terneros se tomaron muestras de sangre sin anticoagulante mediante venipunción yugular los días 0, 4, 12 y 30 posnacimiento. La concentración de las PST se determinó por refractometría y la FA mediante colorimetría enzimática. Se encontró relación alta y significativa ($P < 0.05$) entre los porcentajes de proteína total y sólidos totales del calostro, por lo que se concluye que una sola de estas mediciones es válida para evaluar ambos indicadores. Utilizando análisis de varianza se evaluó el efecto del tiempo sobre las concentraciones de PST y la actividad enzimática de la FA. En las PST se presentó una diferencia significativa ($P < 0.05$) entre el primer día (69 g/dl) y el día 30 (58 g/dl) cuando ocurrió la concentración más baja. Se observaron valores promedio de PST mayores que 4.5 g/dl, lo cual indica que los terneros

¹ Animal scientists, independent professionals.

² MV, Ph.D. Associate Professor. National University of Colombia, Palmira campus.

adquirieron inmunidad pasiva por el consumo de calostro. La actividad enzimática de la FA disminuyó durante el período de estudio (1326.4 U/L en el primer día y 599 U/L en el día 30, pos-nacimiento) ($P < 0.05$). No fue posible comprobar si la FA puede ser utilizada como indicador directo de falla en la transferencia de inmunidad pasiva.

Palabras clave: calostro, inmunidad pasiva, inmunidad materna, fosfatasa alcalina.

.Introduction

The high rates of mortality and morbidity during the rearing of calves are due to various factors, including the consumption and the time of consumption and supply of colostrum to the newborn in the first hours post partum. An inadequate offer of colostrum is reflected in low weaning weights, reduced weight gain low growth rates and late puberty, all of which signify low productivity and economic losses for producers.

La naturaleza de la placenta en ruminantes no permite que las inmunoglobulinas (Igs) pasen de la materna a la fetal. Así, el calostro es la única fuente de inmunidad pasiva disponible para la supervivencia del recién nacido. El calostro constituye la primera secreción láctea de los mamíferos post partum, y contiene altos niveles de inmunoglobulinas (70% - 80% IgG, 10% - 15% IgM & 10% - 15% IgA), que son absorbidas intactas durante las primeras 24 a 36 horas después del nacimiento del ternero. Después de este tiempo, los cambios en el tracto intestinal del ternero previenen su absorción. La cantidad, composición, y características físico-químicas del calostro pueden variar con factores como la longitud del embarazo, el período seco de la vaca, el intervalo del parto, el número de lactancias, la raza, la calidad de la alimentación en el período preparto, y la edad del animal. Concentraciones más altas de Igs en el calostro se observan después de tres partos en comparación con vacas más jóvenes (Wattiaux, 2000).

Entre las principales causas de falla en la transferencia adecuada de Igs al ternero están: la baja calidad de la secreción de calostro por la madre, la pobre ingestión por el ternero, una falla en la absorción intestinal debido a la pérdida de permeabilidad intestinal después de 24-36 horas de vida del ternero, junto con la reducción en la concentración de Igs en la leche con el avance de la lactancia (Aricada et al., 2004). Cuando uno o más de estas causas ocurren independientemente o juntas, el neonato presenta un complejo inmunodepresivo, comúnmente llamado una falla en la transferencia de inmunidad pasiva (TPI).

La concentración de Igs es un importante indicador de falla en la transferencia de inmunidad pasiva. El principal factor que influye en esto es el inadecuado manejo de la oferta de calostro en las primeras horas post partum del ternero. Hay una serie de pruebas disponibles para determinar la concentración de Igs, incluyendo: la concentración de proteína total del suero (TSP) usando refractometría, ensayos de turbidez de sodio y sulfatos de zinc, y cuantificación de la concentración de IgG en el suero. Todas estas varían en precisión, costo y adaptabilidad a la práctica veterinaria (Weaver et al., 2000; Çamkerten, 1998; Parish et al., 1997; Tessman et al., 1997).

A number of studies (Parish et al., 1997; Maden et al., 2003) have found a positive correlation ($r = 0.462$) between serum IgG and TSP. It also has been demonstrated that the TSP provides indirect information on the calf immunity state. In some cases, due to the high confidence ($P < 0.05$) of the biological tests, a total protein serum concentration of less than 6 g/dl is an indicator of deficiency ($P < 0.05$) in the TPI (Gungor et al., 2004). Lombardi et al. (2001) suggested that the enzymes present in colostrum could be used to evaluate quality. One of these is Alkaline phosphatase (ALP), which is also found in milk and blood serum, is thermostable and is frequently used in the evaluation of this type of immunity.

In studies in lambs prior to suckling, a high level of ALP has been found in serum at day 0, which subsequently progressively decreased with the ingestion of colostrum, suggesting that this enzyme may be affected by other factors (Maden et al., 2004). Depending on the type of tissue: bone, kidney, liver, placenta, tumor tissue, there are different isozymes of ALP, such that the final activity of this enzyme present in serum may be a result of a mix of the different isozymes originating from the different tissues.

Zarrilli et al. (2003) suggested that the enzyme assay is a secure test to determine the ingestion of colostrum in a number of ruminant species. Recent studies (Maden et al., 2003, 2004; Gungor et al., 2004) have shown the utility of serum activity of gamma-glutamyl transferase (GGT) and ALP as indicators of TPI in calves and lambs.

The present study had the aim to determine whether the serum indices of alkaline phosphatase and total proteins reflected the transfer of passive immunity in calves with free access to colostrum during the first days post partum.

Materials and methods

The study was carried out in a commercial cattle farm, located in low tropical conditions in Colombia (450 m.a.s.l., mean temperature 28 °C , annual precipitation of 700 mm with bimodal distribution). 15 cows of first and second partum were selected randomly from the races Gyr, Brahaman, and the cross Gyr x Holstein. The cows were in the final third of gestation. At the moment of birth, 300 ml of colostrum were collected, and a physical and clinical evaluation of the calves was made.

Using vacuum tubes without coagulant (Vacutainer) and using jugular venipunction, blood samples were taken from the calves at the moment of birth (day 0), and at day 4, 12 and 30 post partum. The serum obtained through coagulation at ambient temperature was stored in plastic bags that were frozen at -20 °C until the moment of analysis.

The concentration of TSP was determined using the direct refractometry method, and the serum activity of ALP using enzymatic colorimetry (Analyzer Chemistry RA-50) using the commercial kit Randox®, Crumlin, IR. The percentage of total solids, total protein and ash in the colostrum was also determined using the proximal analysis of Weende.

The results were analyzed using both descriptive statistics and the Pearson's correlation between total solids and total proteins in the colostrum, as well as between ALP and TSP. The effect of time (days post partum) vs. TSP and ALP enzymatic activity was evaluated using the Duncan test ($P < 0.05$). The program SPSS v 13.0 (SPSS Inc, 2004) was used for the statistical analyses.

Results and discussion

The colostrum contained $22.68\% \pm 5.42$ for total solids and $14.85\% \pm 3.49$ for total protein, being similar to values found by Wattiaux (2000) and Quigley (1998), who found values of 23.9% and 14%, respectively. A high, significant correlation was detected between the proportion of total protein and that of total solids in the colostrum ($r = 0.695$, $P < 0.01$), indicating that only one of these measures is necessary to estimate trends. In this case, the test of total solids is recommended as an indirect method to determine the total protein content of the colostrum, due to its lower cost and rapidity.

The quality of the colostrum may be evaluated through the concentration of Igs, which are proteins of high molecular weight. Parish et al. (1997), on validating the concentration of serum

proteins as an indicator of colostrum consumption, found a positive correlation between the concentration of TSP and the gamma-globulins present in calves.

The highest concentration of TSP was seen at the moment of birth, remaining constant until day 12 (Box 1). This was due to the consumption of colostrum by the calves and the high quality of the colostrum, as the values of ALP in Box 2 show. These results confirm the relationship between total protein concentration and the levels of Igs in the colostrum. Palasz (1996) found values of TSP in bovine fetuses of 1.9 g/dl, suggesting these increase in calves immediately after birth.

Box 1. Concentration of Total Serum Proteins (g/dl) in calves at different times after birth

Age (days)	Calves (n)	Mean	±S.D.	S.E.	Minimum values	Maximum values
0	14	6.9 ^{a*}	1.32	0.3544	4.0	8.6
4	12	6.6a	0.82	0.2376	5.1	8.0
12	12	6.8a	1.01	0.2921	5.4	8.6
30	12	5.8b	0.60	0.1749	5.0	7.2

* Values in the same column followed by identical letters are not statistically different ($P < 0.05$), according to the Duncan test.

Box 2. Enzyme activity of ALP (U/L) in calves at different times after birth.

Age (days)	Calves (n)	Mean	±S.D.	S.E.	Minimum values	Maximum values
0	14	1326.43b	659.28	176.202	258	2316
4	12	734.83a	225.17	64.0	441	1135
12	12	708.33a	391.11	112.9	135	1313
30	12	598.90a	217.70	65.66	301	1048

* Values in the same column followed by identical letters are not statistically different ($P < 0.05$), according to the Duncan test.

In this study the lowest values of TSP in calves was seen at 30 days post partum ($P < 0.05$). Cseh et al. (2000) found that the concentration of this type of protein in calves are low at birth, and increase rapidly after colostrum consumption, reaching the highest levels at 24 hours post partum.

Mean TSP concentrations greater than 4.5 g/l were observed over the durations considered in this study, suggesting that the calves acquired passive immunity through colostrum consumption. In calves, the transfer of this type of immunity is considered to occur when levels of serum proteins greater than 4.5 g/dl are presented in the first day post partum (Perinno et al., 1995).

Sáez et al. (2000) found that the use of refractometry to determine total protein concentrations is a valid method, and simpler and more economic than determining gamma-glutamyl transferase (GGT) to measure the transfer of passive immunity. Cseh et al. (2000) found a high positive correlation between GGT and TSP ($r = 0.83$, $P < 0.0001$). However, Gungor et al. (2004) consider that the use of refractometry to determine TSP could be misleading, as other substances in the plasma such as glucose, urea and creatine may alter the refractive indices. The former seems most logical, as these substances do not have the high specific molecular weights capable of distorting the specific density. Dehydrated calves present high concentrations of TSP, reflecting the water volume in the blood (Kaneko, 1997), a valid observation that is related to clinical boxes and should not be considered a generality of physiological states.

The greatest ALP enzymatic activity occurred during the first day of life of the calves ($P < 0.05$) (Box 2). From this point, the activity diminished until reaching a value of 598.9 g/dl on the 30th day. Maden et al. (2004) found that the ALP activity in lambs decreased on day 3 and 7, from a maximum value seen on the 1st day. Sáez et al. (2000) also found serum activity of ALP in calves to increase on the 1st day, after the first consumption of colostrum, and thus the presence of ALP suggests the consumption of this food. Healy et al. (1975) considered that ALP originated principally from the intestinal tract, although, in contrast Saez et al. (2000) confirmed that high values of ALP on the day of birth come from colostrum, as the group of lambs that did not receive colostrum presented significantly lower levels at 24 hours. Maden et al. (2004) using a multiple lineal model found a negative relationship between IgG concentration and the ALP activity in serum. These authors mention the fact that the results from the ALP activity are not strictly correlated with the IgG concentration in the serum, nor in the colostrum, and so this biochemical marker has some limitations in being considered as an indicator for the state of absorption of IgGs in experimental animals, assuming a low value on being used in the diagnosis and the detection of failures in transfer of passive immunity. Moreover, Zarrilli et al. (2003) found a low correlation between IgG and the enzymatic activity of ALP ($r = 0.44$). Despite the anterior observations, Kaneko (1997) confirms that the ALP presents good potential for use, mainly because its high levels allow observation of changes in activity originating in the intestinal epithelium, particularly in the absorption of nutrients and globulins.

The neonate calf suffers severe physiological changes in the first hours of life, involving different tissues. These changes are probably responsible for the increases in serum levels of ALP, given the presence of the enzyme, however this is yet to be confirmed.

In the preset study no association was found between the serum activity of the ALP and the transfer of passive immunity ($r = 0.09$). This indicates the need to perform measures of both

indicators, as each one, in isolation or together, indirectly supports the determination of the degree of transfer of passive immunity associated with the consumption of colostrum in the first few hours of life of the calf.

Conclusions

From the results of the present study it is possible to conclude:

- The determination of total solids in the colostrum allows the inference of the total protein content, due to the high correlation exists between the two measures ($r = 0.695$, $P < 0.05$).
- The total serum protein content (TSP) presented high values during the experimental period, indicating the colostrum consumed by the calves was of high quality.
- The enzymatic activity of ALP reduced with time, however, in this study it was not possible to confirm that this marker is useful as an indicators to determine failures in the transfer of passive resistance.

References

- Aricada, H. J.; Bedoya R.; García, A. del P.; Heredtía. C.; Maldonado A. M.; Peláez C.; y Ceballos A. 2004. Competencia inmunológica en la primera semana de vida en terneros mantenidos bajo dos sistemas de producción de leche. *Rev. Col. Cienc. Pec.* 17 (2):167-174.
- Çamkerten, I. 1998. The importance of serum immunoglobulin concentrations in healthy and diarrheic calves. Konya, Turkiye. Selcuk University. The Inst. Med. Sci.
- Cseh, S.; Soler, J.; Lloveras, M.; y Peralta, M. 2000. Empleo de la gama-Glutamil-Transferasa para determinar el grado de ingestión de calostro en terneros. (Disponible en: <http://www.exopol.com/general/circulares/159.html>) 07-15-07.
- Gungor, O.; Bastan A.; y Erbil, M. K. 2004. The usefulness of the γ Gamma-glutamyltransferase activity and total proteinemia in serum for detection of the failure of immune passive transfer in neonatal calves. *Revue Méd. Vét.* 155 (1):27-30.
- Healy, P. J. 1975. Isoenzymes of alkaline phosphatase in serum of newly born lambs. *Res. Vet. Sci.* 19:127-130.
- Kaneko, J. J. 1997. *Clinical biochemistry of domestic animals*. 5ed. San Diego, CA. Academic Press.
- Lombardi, P.; Avallone, L.; Pagnini, U.; D'angelo, D.; y Bogin, E. 2001. Evaluation of buffalo colostrum quality by estimation of enzyme activity levels. *J. Food Prod.* 64, (8):1265-1267.
- Maden, M.; Birdane, F. M.; Altunok V.; y Dere, S. 2004. Serum and colostrum/milk alkaline phosphatase activities in the determination of passive transfer status in healthy lambs. *Rev. Méd. Vét.* 155(11):565-569.
- Maden M.; Altunok V.; Birdane F. M.; Aslan; V.; y Nizamlioglu M. 2003. Blood and colostrum/milk serum γ -Glutamyltransferase activity as a predictor of passive transfer status in lambs. *J.*

- Vet. Med. B. 50:128-131.
- Palasz A. T. 1996. Cultivo de embriones bovinos: recientes avances en el desarrollo de sistemas de cultivos definidos. En: II° Simposio Internacional de Reproducción Animal. Instituto de Reproducción Animal, Córdoba. Argentina. p. 185-194.
- Parish, S. M.; Tyler J. W.; Besser T. E.; Gay C. C.; y Krtytenberg D. 1997. Prediction of serum IgG1 concentration in Holstein calves using serum gamma glutamyltransferase activity. J Vet. Int. Med. 11:344-347.
- Perinno L. J; Wittum T.E.; y Ross G. S. 1995. Effects of various risk factors on plasma protein and serum immunoglobulin concentrations of calves at postpartum hours 10 and 24. Am. J. Vet. Res. 56 (9): 1144-1148.
- Quigley, J. 1998. Colostrum Feeding. A primer on colostrum immunoglobulins. (Disponible en: [http:// www.americanprotein.com/calf /calfnotes/APCCNO3.htm](http://www.americanprotein.com/calf/calfnotes/APCCNO3.htm))_09-01-01).
- Sáez, G. T. 2002. Patología y manejo del cordero recién nacido. En: Congreso de la Sociedad Española de Medicina Interna Veterinaria. ISBN 84-7719-810-1. Universidad de León. 2002. p. 63-65.
- Sáez, T.; Ramos J.J. y Fernández, A. 2000. La actividad enzimática en suero de corderos durante sus primeros días de vida y su relación con la toma de calostro. Méd. Vét. 17(2):32-37.
- SPSS, Inc. 2004. SPSS 16.0 para Windows. Chigago, Illinois.
- Tessman, R. K.; Tyler, J. W.; Parish, S. M.; Johnson, D. L.; y Gant, G. R. 1997. Use of age and serum g-Glutamyltransferase activity to assess passive transfer status in lambs. Am. J. Vet. Med. Ass. 211:1163-1164.
- Wattiaux, A. M. 2000. Importancia de alimentar con calostro. p. 109-112. (Disponible en: [http/ www.produccion-animal.com.ar/ informacion_tecnica/cria_amamantamiento/03- importancia_alimentar_con_calostro.html](http://www.produccion-animal.com.ar/informacion_tecnica/cria_amamantamiento/03-importancia_alimentar_con_calostro.html) 07-18-07).
- Weaver, D. M.; Tyler, J. W.; Marion, R. S.; Wallace, L. M.; Nagy, J. K.; y Holle, J. M. 2000. Evaluation of assays for determination of passive transfer status in neonatal llamas and alpacas. J. Am. Vet. Med. Ass. 216:559-563.
- Zarrilli, A.; Micera E.; Lacarpia N.; Lombardi P.; Pero M. E.; Pelagalli A.; D'angelo D.; Mattia M.; y Avallone, L. 2003. Evaluation of ewe colostrum quality by estimation of enzyme activity levels. Rev. Méd. Vét. 154(8-9):521-523.