Transfer of passive immunity and serum proteinogram in the first six months of life of Criollo Lageano and Black and White Holstein calves

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ABSTRACT.- The objective of this study was to evaluate and compare the transfer of passive immunity and the proteinogram in Criollo Lageano (CL) and Black and White Holstein (BWH) calves. Two groups were utilized with 13 Criollo Lageano and 10 BWH calves. Blood samples were collected for the measurement of total serum protein, electrophoresis of serum proteins, activity of the gamma glutamyl transferase, and concentration of IgG by the method of the zinc sulfate turbidity in periods between 24 and 36 hours of life, 15, 30, 60, 90, 120, 150 and 180 days. Statistical analysis was performed by ANOVA and Tukey test at 5% significance level, and correlations between variables were calculated. Variations of serum proteins followed a pattern of physiological behavior over the first six months of life and production of immunoglobulins was active earlier in BWH calves and slower in the Criollo Lageano, without causing any impact on their health. Gamma globulin in the first days of life (24-36h) was correlated with IgG (r=0.87 for CL and r=0.89 for BWH), PTS (r=0.91 for CL and r=0.92 for BWH), Glob (r=0.99 for CL and r=0.98 for BWH) and GGT (r=0.14 for CL and r=0.83 for BWH). It was concluded that there was no failure in the transfer of passive immunity in Criollo Lageano calves but this failure occurred in the BWH calves. IgG values estimated by the zinc sulfate turbidity and serum proteins were considered good indicators of the transfer of passive immunity in calves between 24 and 36 hours of life.

INDEX TERMS: Immunoglobulins, bovine, serum proteins, native breeds.

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Passive immunity and serum proteinogram in the first six months of life of Criollo Lageano and Black and White Holstein calves

INTRODUCTION

Brazil has several cattle breeds that have developed from breeds brought by the Portuguese settlers after the discovery. Unlike other countries in the Americas Brazil received Spanish cattle breeds, the first ones to step into the New World, as well as breeds of Portuguese origin, whose introduction was first made 34 years after the discovery of Brazil (Mazza et al. 1994, Primo 2000). These breeds have been submitted to natural selection in different environments, and developed specific adaptive traits (Egito et al. 2002, Cardellino 2005). The Criollo Lageano breed originated from cattle introduced to Rio Grande do Sul by the Jesuits (Araújo 1990). It is probable that many animals have been lost along the way, and looking for shelter in the woods of the Plateau of Santa Catarina, began to form herds in open fields of Lages. With the colonization of the Santa Catarina Plateau, the settlers brought cattle, which probably crossed with cattle already existing in the region, leading to the formation of the Criollo Lageano breed. By the beginning of last century, The Criollo Lageano was the predominant breed in the fields of Lages, and of the Plateau of Santa Catarina (Mariane & Cavalcante 2000, Camargo & Martins 2005, Martins et al. 2009), acquiring unique adaptive traits to the ecological niches where they have developed (Primo 1993, Mariane et al. 1990). In Brazil, few studies have been conducted using naturalized cattle breeds (Egito et al. 2007, Spritze et al. 2003, Serrano et al. 2004, Bianchini et al. 2006). The study of these breeds can assist in developing future programs of animal breeding and of animal conservation (Egito et al. 2002, Serrano et al. 2004). Some breeds may contain alleles that confer resistance to diseases or adaptation to less favorable environmental conditions, which may be extremely important for genetic improvement (Woolliams et al. 1986). With the development of the cattle industry in Brazil, neonatal mortality has a great importance in dairy and beef herds and may affect between 6 and 85 of the newborns (Rogers et al. 1985, Petterson et al. 1987), but this percentage may reach 25 or even 50 % of all newborns (Radostits et al. 1994). In dairy herds, approximately 75% of deaths of animals under twelve months of age occur in the first months of life (Radostits et al. 1994). Feitosa (1998) followed 40 Holstein calves from birth to one year of age and observed a mortality rate of 22.5%. According to the observations of Goncalves et al. (1993) and Benesi (1996), mortality of calves in Brazil is mainly caused by diarrhea and pneumonia, and the mortality rate may reach 25%. According to Norheim & Simensen (1985), the rate of calves presenting hypogammaglobulinaemia is high, showing a high rate of failure in the transfer of passive immunity. In some herds, these values reach 40% of the born animals (McGuire & Adams 1982, Barragry 1997). McGuire et al. (1976) and Wittum & Perino (1995) reported that the failure of passive transfer of immunity (FPTI) is the leading cause of death in neonates. According to Feitosa et al. (2010) the failure of passive transfer of immunity varies from 10 to 20% in Holstein and Nellore breeds, depending on the blood component and the standard values chosen for their estimation and interpretation. The physiology of the bovine placenta differs from other species, because there is no transfer of immunoglobulins through the placenta. This fraction of the serum protein is responsible for the humoral defense of the neonate against infectious agents, and should be absorbed soon after birth by the ingestion of colostrum. The colostrum is rich in immunoglobulins, which suffer greater absorption up to the first 12 hours of the calf’s life (Zanetti et al. 1982). The absorbed colostral immunoglobulins have a short half-life (20 days for IgG) in the circulatory system of the calf (Besser & Gay, 1994). Generally, after the peak of serum concentration at 24 hours of life, there is an increasing reduction up to 30 or 45 days of age, when a new upward trend is established, which is due to the active synthesis of antibodies by the immune system (Ribeiro et al. 1983, Moraes et al. 1997, Fagliari et al. 1998, Feitosa 1998, Costa 2000, Paulletti et al. 2003). The objective of this work was to study the physiological aspects related to the transfer of passive immunity and serum proteinogram in Criollo Lageano calves, and compare the results with the ones found for Black and White Holstein calves.

MATERIALS AND METHODS

The project was developed by a multidisciplinary team that involved different institutions: the University of the State of Santa Catarina (UDESC), the Agricultural Research and Rural Extension Corporation of Santa Catarina (EPAGRI), the Brazilian Agricultural Research Corporation (Embrapa), the State University of Londrina (UEL) and the Brazilian Criollo Lageano Breeders Association (ABCL). The protocol was approved by the Ethics Committee on Animal Experiments of UDESC (CETEA) under the registration number 1.19.08. During this experiment 13 Criollo Lageano and 10 Black and White Holstein calves were used. The animals were randomly selected, and were healthy at birth and at the first days of life. The Criollo Lageano calves were obtained from three different farms: Bom Jesus do Herval and Canoas, both located in Ponte Alta - SC, and Grande, located in Paimel/SC. The management practiced in these three farms was extensive, under conventional conditions with individual control of the animals and good nutritional and health standards. The Black and White Holstein calves were obtained from the Agricultural Research
and Rural Extension Corporation of Santa Catarina (EPAGRI), located in Lages/SC. The management at EPAGRI was semi-intensive, with good individual control and adequate nutritional and health management. During the experimental period there were two daily observations to identify the occurrence of births. The calves remained in constant contact with their mothers and did not receive any aid for ingestion of colostrum. In their first day of life, the calves received an ear tag, and had their navel healed with an iodine-based product (2% tincture of iodine). During the six months of the experiment, the calves did not receive any vaccine nor had any change in management. Blood samples were collected by jugular vein puncture into vacuum tubes without anticoagulant (Vacuum II®, Labnew, Sao Paulo, Brazil) in the peri-colonial period. Blood samples were drawn with known concentrations of bovine IgG (Single Radial Immunodiffusion Kit; VRMD, USA), as follows: zero (fetal bovine serum), 400, 800, 1600 and 3200mg/dL. Samples that exceeded the estimated value of 3200mg/dL were processed again after the dilution of the serum in saline solution at a 1:1 ratio. The statistical analysis was performed by means of the analysis of variance for repeated measures (ANOVA) and Tukey test for comparison between means, assuming an error probability of 5%. Correlation coefficients were calculated among variables (Curi 1998).

**RESULTS AND DISCUSSION**

The results of total serum protein concentration, electrophoretic profile of serum proteins in agarose gel, relation albumin: globulin, IgG concentration by the method of the zinc sulfate turbidity, and activity of gamma glutamyl transferase (GGT) are shown in Table 1. During the conduction of this work two calves have been culled, one of the Criollo Lageano breed, due to transfer of his mother to another property, for management reasons, and the second one was a Black and White Holstein calf that came to death due to the failure on the passive immunity transfer (FPIT). The value of total protein of the calf was 4.4g/dL from 24 to 36 hours, being considered a failure on the passive immunity transfer (FPIT) according to Donovan et al. (1998), that consider that a minimum value of 5.0g/dL. During the experiments the failure on the passive immunity transfer (FPIT) was considered, the calves were not given any aid to transfer colostrum, they remained in constant contact with their mothers and did not receive any vaccine.

<table>
<thead>
<tr>
<th>Time</th>
<th>Pts (g/dL)</th>
<th>Alb (g/dL)</th>
<th>Alfa1 (g/dL)</th>
<th>Alfa2 (g/dL)</th>
<th>Beta (g/dL)</th>
<th>Gama (g/dL)</th>
<th>Glob (g/dL)</th>
<th>A:G</th>
<th>IgG (g/dL)</th>
<th>GGT (UI/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-36 hours</td>
<td>9.87±1.20 Ab</td>
<td>6.45±1.31 B</td>
<td>0.48±0.10 Bbc</td>
<td>0.52±0.09 Abc</td>
<td>0.99±0.19 Bbc</td>
<td>4.12±1.57 Bbc</td>
<td>6.40±1.51 Bbc</td>
<td>0.44±0.22 Bbc</td>
<td>3.12±1.20 Ba</td>
<td>1060±559.66 Abc</td>
</tr>
<tr>
<td>15 days</td>
<td>7.44±1.09 Ab</td>
<td>5.89±0.58 Ab</td>
<td>0.59±0.10 Bc</td>
<td>0.50±0.09 Abc</td>
<td>1.03±0.20 B</td>
<td>1.80±0.55 Bbc</td>
<td>3.99±0.77 Bbc</td>
<td>1.03±0.20 B</td>
<td>2.28±0.53 Bbc</td>
<td>87.3±61.66 Abc</td>
</tr>
<tr>
<td>30 days</td>
<td>6.09±0.58 Abc</td>
<td>6.07±0.52 Abc</td>
<td>0.62±0.10 Abc</td>
<td>0.59±0.10 Abc</td>
<td>0.80±0.17 Bbc</td>
<td>1.02±0.36 Bbc</td>
<td>2.98±0.60 Abc</td>
<td>1.30±0.17 Abc</td>
<td>1.68±0.36 Abc</td>
<td>24.0±12.9 Abc</td>
</tr>
<tr>
<td>60 days</td>
<td>5.98±0.47 Abc</td>
<td>5.17±0.73 Abc</td>
<td>0.62±0.10 Abc</td>
<td>0.62±0.09 Abc</td>
<td>0.60±0.07 Abc</td>
<td>0.65±0.08 Abc</td>
<td>2.23±0.29 Abc</td>
<td>2.13±0.34 Abc</td>
<td>1.41±0.22 Abc</td>
<td>24.0±12.9 Abc</td>
</tr>
<tr>
<td>90 days</td>
<td>6.47±0.72 Abc</td>
<td>5.81±0.70 Abc</td>
<td>0.52±0.07 Abc</td>
<td>0.62±0.09 Abc</td>
<td>0.58±0.06 Abc</td>
<td>0.58±0.06 Abc</td>
<td>2.50±0.46 Abc</td>
<td>2.13±0.34 Abc</td>
<td>0.79±0.22 Abc</td>
<td>14.9±11.8 Abc</td>
</tr>
<tr>
<td>120 days</td>
<td>6.74±0.56 Abc</td>
<td>6.14±0.09 Abc</td>
<td>0.72±0.17 Abc</td>
<td>0.62±0.09 Abc</td>
<td>0.51±0.06 Abc</td>
<td>0.54±0.06 Abc</td>
<td>2.80±0.70 Abc</td>
<td>2.13±0.34 Abc</td>
<td>1.14±0.05 Abc</td>
<td>10.9±1.9 Abc</td>
</tr>
<tr>
<td>150 days</td>
<td>6.95±1.97 Abc</td>
<td>6.22±0.62 Abc</td>
<td>0.72±0.18 Abc</td>
<td>0.62±0.09 Abc</td>
<td>0.50±0.08 Abc</td>
<td>0.56±0.07 Abc</td>
<td>2.89±0.94 Abc</td>
<td>2.13±0.34 Abc</td>
<td>1.07±0.09 Abc</td>
<td>10.9±1.9 Abc</td>
</tr>
<tr>
<td>180 days</td>
<td>6.63±0.56 Abc</td>
<td>5.99±0.74 Abc</td>
<td>0.64±0.29 Abc</td>
<td>0.57±0.06 Abc</td>
<td>0.50±0.08 Abc</td>
<td>0.54±0.08 Abc</td>
<td>2.60±0.39 Abc</td>
<td>2.13±0.34 Abc</td>
<td>1.07±0.09 Abc</td>
<td>10.9±1.9 Abc</td>
</tr>
</tbody>
</table>

a Equal capital letters represent no significant difference (P>0.05) between groups.

b Equal lowercase letters represent no significant difference (P<0.05) between times.

**Table 1.** Mean values and standard deviations (x ± s) of the concentration of total serum protein (TSP), albumin, alpha 1, alpha 2, beta and gamma globulin, relation albumin: globulin (A/G), immunoglobulin G (IgG), and activity of gamma-glutamyl transferase (GGT) of Criollo Lageano and Black and White Holstein calves at 24-36h (M1), 15 (M2), 30 (M3), 60 (M4), 90 (M5), 120 (M6), 150 (M7) and 180 days (M8) of life.
IgG is considered critical in calves, and is identified as an indicator of success in the transfer of immunity, ranging from 1000 mg/dL (Radostits et al. 2002) to 1600 mg/dL (McGuire & Adams 1982). Values between 500 and 1500 mg/dL (Selim et al. 1995) and from 600 to 1600 mg/dL (McGuire & Adams 1982) may be indicative of a partial FPIT. Considering the individual results only two Black and White Holstein calves presented a partial failure in the transfer of passive immunity (806 mg/dL and 831 mg/dL). The mean values of IgG in both Criollo Lageano and Black and White Holstein calves were much larger than those identified as critical, and the Criollo Lageano calves showed higher values when compared to Black and White Holstein ones. According to Guk-Hyun et al. (2003), when comparing calves of beef and dairy breeds, there is a high failure rate in the transfer of passive immunity due to delays in feed intake and low ingestion in dairy breeds. There is a difference between feeding methods of beef and dairy cattle, influencing serum concentration of immunoglobulins, with the beef calves presenting higher concentrations than the dairy ones. The highest concentration of immunoglobulins was observed at 24-36h of age, indicating the maximum absorption of colostral immunoglobulins, which is in agreement with Costa et al. (2008). The sampling occurred only after the first 24 hours of life, thus avoiding the upward curve that precedes the peak serum concentration (Fagliari et al. 1996, Borges 1997, Feitosa et al. 2001b, Leal et al. 2003, Machado Neto et al. 2004). No samples were collected before ingestion of colostrum to prevent rejection of newborn calves by their mothers and by the difficult management of Criollo Lageano calves, raised extensively. According to Feitosa (1999), dairy cattle have a higher rejection rate than beef cattle, but during this experiment no rejection of calves was observed by Holstein or Criollo Lageano cows. The concentration of immunoglobulins after a peak at 24-36h of age is followed by a continuous decline due to degradation and its combination with antigen, and presented minimum values at 60 days of age in both studied breeds. This behavior is described as a classic curve of this physiological process observed in other cattle breeds (Feitosa et al. 2001b, Amorim 2002, Leal et al. 2003, Machado Neto et al. 2004). IgG concentration in the Black and White Holstein calves reached the lowest value at 30 days of age, differing from Criollo Lageano that presented the lowest IgG at 60 days of age. These data agree with reports by Ribeiro et al. (1983), Borges (1997), Moraes et al. (1997), Costa (2000), Feitosa et al. (2001b) and Pauletti et al. (2002), working with dairy cattle. This difference may be due to breed factors and to the different farming systems. The intensive management of the Holstein calves can lead to an early contact and a greater exposure to infectious agents, leading to a rapid consumption of antibodies. Extensively managed beef calves are, in turn, subjected to natural challenges of lesser magnitude (Costa et al. 2008). The elevation of gamma globulin after its decrease at 60 days of age began gradually at 90 and at 120 days, reaching its maximum concentration at 150 days, decreasing afterwards, at 180 days of age. This behavior was similar for the calves of the two breeds and is similar to the results reported by Borges (1997), Costa (2000), Feitosa et al. (2001b) and
Costa et al. (2008). However, when analyzing the curve of IgG concentration it was noted that in the Holstein calves the lowest values were observed at 30 days of age, and that the increase occurred earlier than in the Criollo Lageano calves. This result resembles the one found by Machado Neto et al. (2004) when studying Canchim and Nellore calves. These findings show that the active synthesis of IgG in Criollo Lageano calves may occur later. However, this does not necessarily imply that these animals are more subjected to infection during this period. However, it is assumed that the calf is more susceptible to infections at this critical age, that is marked by the time interval in which the serum concentrations of immunoglobulins are at their lowest, and is the period between the end of the degradation process of passively transferred immunoglobulins and their active production by competent immune system (Radostits et al. 2002). The change in the ratio albumin:globulin (A:G) is usually the first sign of change in the normal protein profile (Costa et al. 2008). According to Leal et al. (2003) the largest variations occur from birth to the first month of life and are a result of alterations on the two types of protein. In the Black and White Holstein calves it was observed that the highest values of the albumin:globulin ratio were identified at 30 days of age, while in the Criollo Lageano this peak occurred at 60 days of age. Thereafter the values decreased but remained higher for the Criollo Lageano. The albumin concentration was lower at birth and rose until the 60th day of life in Holstein and until the 90th day in the Criollo Lageano calves (Table 1). The reversal in the concentrations of albumin and globulin led to an increase in the ratio A:G. This relationship tends to decrease after the onset of the endogenous production of immunoglobulins. The alpha1 and alpha2 fractions, as well as beta globulins showed little variation during the experimental period and were similar in both breeds. The gamma glutamyl transferase (GGT) is a liver enzyme present in high concentration in the bovine colostrum and is absorbed by intestinal cells of newborn calves (Thompson & Pauli 1981, Braun et al. 1982, Fagliari et al. 1996, Zanker et al. 2001). One can consider that the activity of the serum GGT serves as an indirect indicator of the absorption capacity of immunoglobulins of the neonate (Feitosa et al. 2001a). In the studied breeds, GGT activity showed maximum serum activity from 24 to 36 hours of life, decreasing rapidly to reach physiological values at 60 days of age (Table 1). The highest values of total protein and serum GGT activity were observed between 24h and 36h of life and declined significantly at day 15. These values are related to the activity of intestinal absorption of the colostrum within the first 24 hours of life. Within the first hours of life occurs the largest absorption of immunoglobulins, which present a good correlation with the activity of GGT (Costa et al. 2008). Considering the values obtained in the first days of life (24-36h) and assuming the values of gamma globulin as a standard for comparison, there were high positive correlations between immunoglobulins and IgG (r=0.87 for CL and r=0.89 for BWH), PTS (r=0.91 for CL and r=0.92 for BWH) and Glob (r=0.99 for CL and r=0.98 for BWH), reinforcing earlier assertions that these variables are indicators of the transfer of passive immunity in calves (Naylor & Kronfeld 1977, Pfeiffer et al. 1977, Moraes et al. 1997, Feitosa et al. 2001a, Machado Neto et al. 2004, Costa et al. 2008). Although significant, the correlation between serum GGT activity and gamma globulin was weakly positive in the Criollo Lageano calves (r=0.14), but high in the Holstein calves (r=0.83). The correlation found in the Criollo Lageano contrasts with the observations of Fagliari et al. (1996) and Feitosa et al. (2001a). According to Borges (1997) and Costa et al. (2008) this may be due to the large variability observed in the values of enzyme activity and the fact that, starting at 24 hours of life, the decline of GGT activity anticipates the decline of concentration of gamma globulins. The closer to the 24 hours of life of the calves samples are collected, the stronger the correlation will be. In this study, similarly to what was reported by Costa et al. (2008) due to operational difficulties and because many births occurred at night, the collection of a large number of blood samples took place after the calves had completed 30 hours of age. For the Holstein calves due to the proximity of the pens to the property, sampling occurred closer to the 24 hours of age of calves, increasing the correlation. Feitosa et al. (2010) point out that using a specific value for any blood component, in order to establish whether a newborn calf is immunodeficient or not, one must be cautious. Moreover, it is important to note that the serum concentrations necessary to confer protection to newborn calves are influenced by various environmental factors, by the pathogen load to which the animals are exposed and by the concentration of specific immunoglobulins (Garry et al. 1993, Guy et al. 1994, Soares Filho et al. 2001, Feitosa et al. 2010).

**CONCLUSIONS**

It can be concluded that unlike the Black and White Holstein, there was no failure in the transfer of passive immunity in Criollo Lageano.

Variations of serum proteins followed a pattern of physiological behavior over the first six months of life and the active production of immunoglobulins appeared earlier in Holstein calves and later in the Criollo Lageano ones, without causing any health impact on the animals.

IgG values estimated by the zinc sulfate turbidity and by the total serum protein levels were good indicators of the transfer of passive immunity in calves between 24 and 36 hours of life.

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