QUALITY OF COLOSTRAL PASSIVE IMMUNITY AND PATTERN OF SERUM PROTEIN FLUCTUATION IN NEWBORN CALVES

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ABSTRACT: Immunity acquired by newborn animals is known as passive immunity, and for ruminants, antibody acquisition depends on the ingestion and absorption of adequate amounts of immunoglobulins from colostrum. This study relates different initial levels of acquired passive protection and serum total protein (TP) and immunoglobulin G (IgG). Serum immunoglobulin concentration and total protein were evaluated for female Holstein calves in the first sixty days of life. Animals were separated into three groups according to their initial level of passive immunity: group 1- animals with a low level of passive immunity (below 20 mg mL^{-1}); group 2- animals with a medium level (between 20 and 30 mg mL^{-1}), and group 3- animals with a high level (above 30 mg mL^{-1}). Serum total protein was determined through the biuret method and IgG was determined by radial immunodiffusion. Data were analyzed as a completely randomized, split-plot statistical design. Fluctuation of the variables along the experimental period was determined through non-linear regression by the DUD method (PROC NLIN – Non Linear SAS). Animals with low antibody acquisition started to produce antibodies earlier, reflecting a compensatory synthesis. On the other hand, animals having adequate levels exhibited an extended period of immunoglobulin catabolism and the beginning of the endogenous phase was delayed. Regardless initial levels, the fluctuations in IgG contents occurred around adequate physiological concentrations, ranging from 20 to 25 mg mL^{-1}.
Key words: colostrum, dairy calves, antibodies

QUALIDADE DA IMUNIDADE PASSIVA COLOSTRAL E PERFIL DE VARIAÇÃO DE PROTEÍNAS SÉRICAS EM BEZERROS NEONATOS

RESUMO: A imunidade adquirida pelos recém-nascidos é denominada passiva e no caso dos ruminantes a aquisição de anticorpos depende da ingestão e absorção de quantidades adequadas de imunoglobulinas do colostro. Este trabalho relaciona diferentes teores iniciais de proteção passiva adquirida com o comportamento das variáveis proteína total (PT) e imunoglobulina G séricas (IgG). Os teores séricos de IgG e PT foram avaliados em bezerras da raça Holandesa do nascimento até 60 dias de idade. Os animais foram agrupados de acordo com a concentração inicial de imunoglobulinas séricas adquiridas: grupo 1- animais com baixo nível de imunidade passiva (até 20 mg mL^{-1}); grupo 2- animais com nível médio de imunidade passiva (de 20 a 30 mg mL^{-1}) e grupo 3- animais com alto nível de imunidade passiva (acima de 30 mg mL^{-1}). As amostras séricas foram analisadas quanto a proteína total pelo método de biureto e para quantificação de IgG foi utilizado o método de imunodifusão radial. Os teores séricos de IgG e PT foram avaliados em um delineamento experimental inteiramente casualizado em parcelas subdivididas no tempo. Para determinar o comportamento dessas variáveis ao longo do período experimental, foram traçadas regressões não-lineares pelo método de DUD, procedimento PROC NLIN do SAS. Nos animais com baixa aquisição inicial de anticorpos verificou-se uma produção de anticorpos mais precoce. Já para os animais com níveis adequados de anticorpos verificou-se uma fase de catabolismo prolongada das imunoglobulinas e um início mais tardio da síntese endógena de anticorpos. Independente dos níveis iniciais, as flutuações dos teores de IgG ocorreram em intervalo de concentrações fisiologicamente adequadas, ou seja de 20 a 25 mg mL^{-1}.
Palavras-chave: colostro, bezerros leiteiros, anticorpos

INTRODUCTION

Survival and healthiness of newborn animals directly depend upon the acquisition of maternal antibodies. In the case of ruminants, the newborn relies on the ingestion of colostrum to obtain immunoglobulins (Brambell, 1958; Jeffcott, 1972).

The serum immunoglobulin content found in calves after receiving colostrum is quite variable. The absorption of antibodies by newborn calves can be affected by many factors, particularly by the time elapsed from birth to the first ingestion of colostrum and by their concentration in immunoglobulins (Edwards et al., 1982; Besser et al., 1991; Quigley et al., 1995; Ramin et al., 1996; Machado Neto et al., 1997; Morin et al., 1997).

After the absorptive period, the animal undergoes a transitional stage during which stops benefiting from mother-acquired antibodies and starts to respond actively to environment challenges. However, animals presenting different initial levels of antibodies acquired from colostrum respond differently to the endogenous production (Husband & Lascelles, 1975; Machado Neto & Packer, 1986; Baracat et al., 1997; Aldridge et al., 1998).

The objective of this work was to establish a relation between different initial levels of acquired passive protection and variables serum total protein (TP) and immunoglobulin G (IgG), and to define behavior patterns for their curves, during the first 60 days of life, aiming to facilitate the development of newborn calf management alternatives.

MATERIAL AND METHODS

Fifty nine female Holstein calves were utilized in this study, distributed in three groups according to their initial concentration of acquired serum immunoglobulins at 24 hours after birth: group 1- animals with low level of passive immunity (up to 20 mg mL^{-1} IgG); group 2- medium level (between 20 and 30 mg mL^{-1} IgG); and group 3- high level (above 30 mg mL^{-1} IgG).

Blood samples were collected from calves external jugular vein, at the following ages: 1; 5; 10; 15; 17; 20; 25; 30; 35; 40; 45; 50 and 60 days after birth. The serum, separated after centrifugation, was maintained at -20°C until analysis. Serum samples were analyzed for serum total protein (TP) by the biuret method (Reinhold, 1953); serum immunoglobulin G (IgG) was quantified through the radial immunodiffusion method described by Mancini et al. (1965).

A completely randomized experimental design with split-plots in time was adopted, and the effect of the initial immunoglobulin levels was attributed to plots, with the measurements repeated in time considered as subplots. Correlation, Pearson and Sperman analyses were carried out to verify associations of interest between the TP and IgG variables. Non-linear regressions were plotted for the groups to determine the behavior of variables TP and IgG during the experimental period; these regressions proved to be more suitable for this type of variable; one curve was obtained for each group. The chosen model was: **Y=L+U*(R-ID)+V*(ID-R)**, where **Y**= variable's concentration, **ID**= animal age (days), **L**= point of minimum concentration, **U**= slope during the period that preceded the point of minimum concentration, **V**= slope during the period after the point of minimum concentration, **R**= age at the point of minimum concentration.

The DUD method, an iterative process to reach the convergence criterion, was then applied by means of the PROC NLIN (NonLinear) SAS (1999) procedure. The general curve model selected corresponded to that which best fitted the serum variable data. Since it is a non-linear model, it was necessary to utilize an iterative process to solve the equations. It requires the selection of initial values as starting point to iterate according to some pre-established criterion (Diaz, 1992). However, no algorithm exists to determine adequate initial values, and the body of knowledge suggested by previous research had to be resorted to. There were no values available in the literature for the proposed model, so the initial values for the parameters were selected based on the mean values obtained for each group.

RESULTS AND DISCUSSION

Animals presenting adequate concentrations of passive immunoglobulins modulate the immune response by delaying the production of endogenous immunoglobulin, while those having low contents will speed up their active production as a response to environmental challenges (Husband & Lascelles, 1975; Logan et al., 1974; Ribeiro et al., 1983; Machado Neto & Packer, 1986). The decrease in IgG concentration during the first month after birth for groups 2 and 3 (Table 1; Figure 1), resulted from the catabolism of proteins passively acquired from colostrum. The age at the point of minimum concentration (R) was 22.290 and 26.019 day for groups 2 and 3, respectively; the confidence interval analysis indicated that the groups did not differ in relation to age at the point of minimum concentration for the variable IgG. Many studies report that the endogenous production of antibodies does not contribute towards increasing the amounts of serum antibodies until two weeks after birth, since this fraction is lower than the rate of catabolism of maternal antibodies (Husband et al., 1972; Daniele et al., 1994a; Rajala & Castren, 1995; Baracat et al., 1997).

For group 3, parameter V was non significant by the confidence interval analysis. This result indicates the occurrence of a plateau between the date of minimum concentration R=26.019 and the end of the experimental period. This fact is a consequence of the high initial concentration of acquired immunoglobulin, thus delaying the endogenous production of antibodies. For group 1, however, there was no initial peak of IgG followed by a reduction, and this can be justified by the initial condition, which determined a reduced catabolic activity and an early endogenous production of antibodies. Animals of high serum IgG contents, correlated with high concentrations of total serum protein, would delay the endogenous synthesis of immunoglobulins, while animals of low initial values would accelerate their production (Logan et al., 1974; Husband & Lascelles, 1975; Machado Neto & Packer, 1986).

Table 1 - Mean predicted values of immunoglobulin concentrations for the experimental groups.

Age	Group 1	Age	Group 2	Age	Group 3
day	mg mL^{-1}	day	mg mL^{-1}	day	mg mL^{-1}
1	10.321	1	26.403	1	37.819
5	10.321	5	24.707	5	35.041
10	10.321	10	22.587	10	31.567
15	10.321	15	20.467	15	28.093
17	10.321	17	19.619	17	26.704
19.592*	10.321	20	18.347	20	24.620
20	10.441	22.290*	17.376	25	21.146
25	11.914	25	17.934	26.019*	20.438
30	13.387	30	18.963	30	20.438
35	14.860	35	19.992	35	20.438
40	16.333	40	21.021	40	20.438
45	17.807	45	22.049	45	20.438
50	19.280	50	23.078	50	20.438
60	22.226	60	25.136	60	20.438

Group 1: low level = up to 20 mg mL^{-1} IgG
Group 2: medium level = between 20 and 30 mg mL^{-1} IgG
Group 3: high level = above 30 mg mL^{-1} IgG.
*Age at the point of minimum concentration (R)

Table 2 - Mean predicted values of serum total protein concentration in the experimental groups.

Age	Group 1	Age	Group 2	Age	Group 3
day	g 100 mL^{-1}	day	mg mL^{-1}	day	mg mL^{-1}
1	6.259	1	7.617	1	8.998
5	6.259	5	7.485	5	8.613
10	6.259	10	7.320	10	8.132
15	6.259	15	7.155	15	7.651
17	6.259	17	7.089	17	7.458
20	6.259	20	6.990	20	7.169
25	6.259	22.845*	6.896	20.358*	7.135
29.999*	6.259	25	6.956	25	7.211
30	6.259	30	7.095	30	7.292
35	6.398	35	7.233	35	7.373
40	6.538	40	7.372	40	7.455
45	6.678	45	7.511	45	7.536
50	6.818	50	7.649	50	7.617
60	7.098	60	7.927	60	7.780

Group 1: low level = up to 20 mg mL^{-1} IgG
Group 2: medium level = between 20 and 30 mg mL^{-1} IgG
Group 3: high level = above 30 mg mL^{-1} IgG

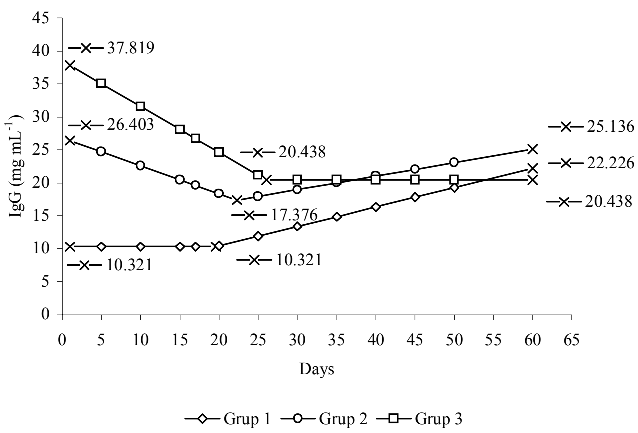

Figure 1 - Average fluctuation of seric IgG concentration, with adjusted values.

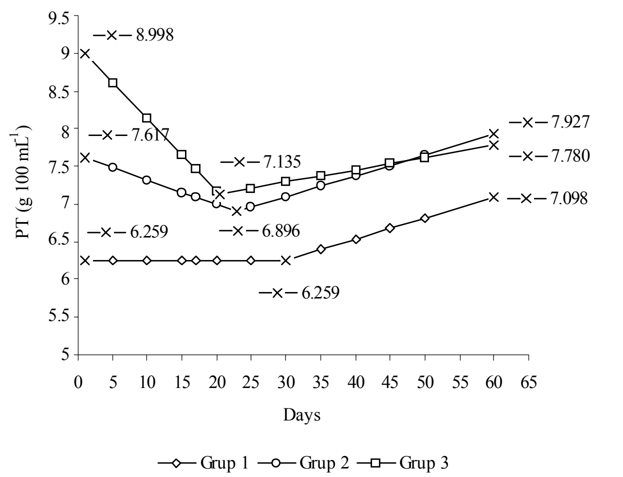

Figure 2 - Average fluctuation of total protein concentration, with adjusted values.

The serum TP and IgG variables presented similar behavior ($P < 0.0001$), considering the entire experimental period, with r=0.788; this was also observed by other authors (Daniele et al., 1994a; Baracat et al., 1995). For groups 2 and 3 there was a decrease in serum TP concentration in the 1st month after birth (Table 2, Figure 2), and this was a consequence of the catabolism of antibodies passively acquired from colostrum over the group of serum proteins, indicating a protein catabolic process, a fact already observed by several authors (Machado Neto & Packer, 1986; Daniele et al., 1994b; Baracat et al., 1995; Bessi, 1996).

The point of minimum concentration of TP (L) for group 2 was 6.896 g 100 mL^{-1} while for group 3 L was 7.135 g 100 mL^{-1}. The age at the point of minimum concentration (R) for group 2 was 22.845 day whereas for group 3 R was 20.358 day. The range of variation of L and R values, expressed by the confidence interval, demonstrated that the groups did not differ among themselves. In group 1, however, no point of minimum concentration for TP was detected. This variable increased through the experimental period as a consequence of the compensatory synthesis of IgG for animals with low initial concentration of serum antibodies. The greater the initial value of serum IgG, the longer the period of proteic catabolism tends to last. Consequently, the inflexion point follows the same trend, since colostrum-acquired immunoglobulins are the most important fraction that determines fluctuation in the serum TP variable, at the beginning of animal's life.

Regardless the initial levels, fluctuations in IgG contents occurred toward physiologically adequate concentrations, i.e., between 20 and 25 mg mL^{-1}. In addition, the initial levels of IgG are directly related to the beginning of the endogenous production of antibodies and influence the behavior of serum IgG and TP of animals in their first months of life.

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