INDICATORS OF PASIVE IMMUNITY AND HEALTH STATUS OF CALVES

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The aim of this study was the determination of immunoglobulin (IgG, IgM, IgA), total serum protein content and activity of enzyme GGT in sera of 71 dairy calves up to the age of four weeks. We investigated to which extent the sera profiles correlated with total immunoglobulin level in the administered colostrum. The results were related to the health status of the calves. One of the goals of the study was also to asses the applicability of these indicators of colostrum supplied in Slovenian herds. In the serum samples the concentration of IgG, IgA, IgM, TSP and activity of GGT were measured. In colostrum samples the total Ig level was estimated. The correlations between the colostral Ig content and the concentration of IgG, TSP and activity of GGT were significant up to the 4th week of age. Only on the base of the values of the investigated parameters no direct conclusion about the foreseen health status of calves is possible. The investigated indicators are suitable to assess colostrum supply in calves at least up to the 3rd week of age. Indirect indicators such as TSP and GGT are more reliable to ascertain if the colostrum was absorbed as for estimation of immunoglobulin levels in the calf's serum.

Key words: calves, gamma glutamyltransferase, immunoglobulin, serum protein

INTRODUCTION

The newborn calves receive immunoglobulin (Ig) with passive transfer from the colostrum. Sufficient colostral immunity is important for the calves' health because colostral Ig provide systemic as local protection in the intestine. The immunoglobulin transport through the intestinal mucosa follows the mechanisms of receptor transmitted transocytosis. According to recent research, variations in the allel for a particular protein sequence of bovine neonatal IgG-Fc receptor are suspected to have also an impact on the immunoglobulin level of newborn calves (Laegreid et al., 2002). The intestinal epithelium loses its capacity to take up colostral antibodies on average at the 24th hour of life (Bush and Staley, 1980). The ability to absorb IgM is lost prior to the ability to absorb IgA and IgG (Stott, 1979; Kim and Schmidt, 1983).

514   **Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.**
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

Passive immunity is influenced not only by the quality of the colostrum, but also with the way in which colostrum is supplied. The calves which received colostrum of good quality, had higher serum IgG concentration in comparison with calves which got low quality colostrum or were suckling their dams (Nocek *et al*., 1984; Franklin *et al*., 2003; Jaster, 2005). Immunoglobulin concentration in colostrum, is of same importance for sufficient passive immunity as early administration of colostrum. The serum Ig concentration is more influenced by colostrum quality than by quantity (Schmidt, 1985). Some studies established a positive influence of substances added to the colostrum like mineral adsorber zeolite, on absorption of IgG in calves (Fratrić *et al*., 2007; Gvozdić *et al*., 2008).

Efficiency of immunoglobulin transfer can be assessed with different methods. The passive immunity can be assessed directly by measuring Ig concentration in calves' serum. There is a significant correlation between Ig concentration in colostrum and in calves' serum (Burton *et al*., 1989). The indirect methods for estimation of passive immunity are measuring of total serum protein (TSP) and of activity of enzyme gamma glutamyltransferase (GGT). Immediately after birth calves have very low TSP concentration which in general increases significantly after colostrum consumption (Stefen *et al*., 1997; Schäfer *et al*., 1998; Kirovski *et al*., 2002). The concentration of TSP is a good indicator of colostrum intake and of risk for disease (Tyler *et al.*, 1998; Tyler *et al*., 1996; Selim *et al*., 1995; Rea *et al*., 1996). The enzyme GGT is accumulated in increased amounts in the colostrum (Zanker *et al*., 2001) and is absorbed through the intestinal wall after colostrum intake. So the activity of GGT in the serum of newborn calves is increased in this period and can be used for indirect estimation of colostrum intake (Bostedt, 1983; Schlerka and Bucher, 2003). In different studies the association between IgG concentration in calves' serum in the first days of life and activity of enzyme GGT was established (Parish *et al*., 1997; Wilson *et al*., 1999; Tyler *et al*., 1999; Perino *et al*., 1993).

Calves which did not receive enough colostrum or received colostrum with low concentration of Ig grow more slowly and are more prone to disease or to death compared with the calves which received colostrum of good quality (Nocek *et al*., 1984; Quigley *et al*., 1995). In calves with low serum Ig concentration, the incidence of respiratory disease was twice as high, as in calves with higher concentration of Ig (Virtala *et al*., 1999). There are differences in average serum Ig concentration between herds, as well as in the part of hipogamaglobulinemic calves (Ig<5 mg/mL) (Hancock, 1985).

The objective of this study was to measure the concentrations of immunoglobulins (IgG, IgM, IgA), TSP and activity of GGT in calf sera up to the age of four weeks. We investigated to which extent the sera profiles correlated with total immunoglobulin level in the administered colostrum. The results were related to the health status of the calves. Furthermore the relations between different indicators of passive immunity were examined and their usefulness in the evaluation of passive immunity in newborn calves in Slovenian herd management conditions.

Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

515

MATERIAL AND METHODS

Seventy one dairy calves and sixty eight cows were included in the research. The calves and cows were Holstein Friesian breed. After calving, the calves received 1 to 1.5 litre of colostrum from their mother via teat bottle. The first four days of life the calves were administered mothers' colostrum and milk three times a day. Blood samples from the calves were taken from the jugular vein in evacuated tubes. The first sample was taken at the age of 2 to 3 days, later on, the samples were taken once a week up to the age of 4 weeks. Pending analysis the serum samples were stored at -22°C. In blood serum samples the concentrations of IgG, IgA and IgM with quantitative ELISA (Bovine IgG (IgA, IgM) ELISA Quantitation Kit, Bethyl laboratories, UK) were measured. The concentration of TSP and activity of GGT were measured with the biochemical analyzer Cobas Mira (Hoffmann-La Roche). In the first milking colostrum samples the concentration of total Ig was measured with colostrometer (Bergophor, Germany). The measurements were performed at room temperature (22°C). Health status of calves was monitored throughout the research period. Diarrhoea was defined as feces with a consistency that was looser than normally observed in calves continuing for at least one day. Respiratory disease was defined as increased respiratory sounds at lung auscultation with systemic signs of disease which was treated. The calves were divided in two groups with regard to health status. First group consisted of calves which were healthy and in the second group were calves which diseased at least once in the investigated period. The data were processed with statistical program SPSS (Ver 15). Descriptive statistics for investigated parameters with regard to health status was calculated. Because the IgM, IgA and GGT data were not normally distributed the median value was calculated instead of mean. Before the Pearson's correlation coefficients were calculated the logarithmic transformation was done. The total Ig content in colostrum samples between groups was compared with Students t-test.

RESULTS

The average concentration of Ig in colostrum samples was 88.11 ± 31.47 g/L, it ranged between 20–155 g/L. Colostrum of bad quality (concentration of Ig below 50 g/L) was established in 7 (10.3 %) cows. The mean concentration of Ig in colostrum samples administered to the calves which remained healthy was 88.5 ± 32.7 g/L and in samples administered to the diseased calves was 86.5 ± 30.2 g/L, the difference was not significant (P = 0.800). In most cases the calves received colostrum soon after birth, in average 1.7 hours after delivery.

The values of indicators of passive immunity revealed that majority of calves were well supplied with colostrum. The time of the first colostrum intake was not significantly associated with the concentrations of Ig in calves' serum.

The disease was observed in 26 (36.6 %) calves in the investigated period. Predominant disease was diarrhoea. The disease was observed in 8 calves in the 1st week, in 7 calves in the 2nd, in 11 calves in the 3rd and in 6 calves in the 4th week of age. In 5 calves the disease was observed two times.

516   **Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.**
                                              Ježek J *et al*.: Indicators of pasive immunity
                                                          and health status of calves

In figures (1 – 5) the mean values of IgG and TSP and median values of IgA, IgM and GGT with regard to the health status of calves are presented. The greatest differences between groups were observed in the 1st week of age except for the concentration of IgM. It is interesting that concentration of IgM in healthy calves was higher in the 2nd than in the 1st week of age. The concentrations of IgG and TSP differed more evidently between groups. No statistically significant correlations were obtained between health status and investigated indicators of passive immunity.
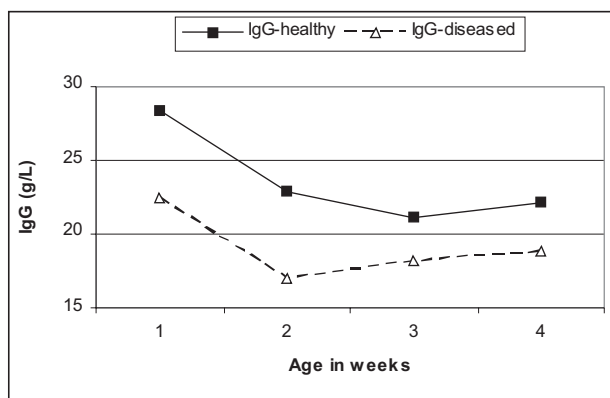

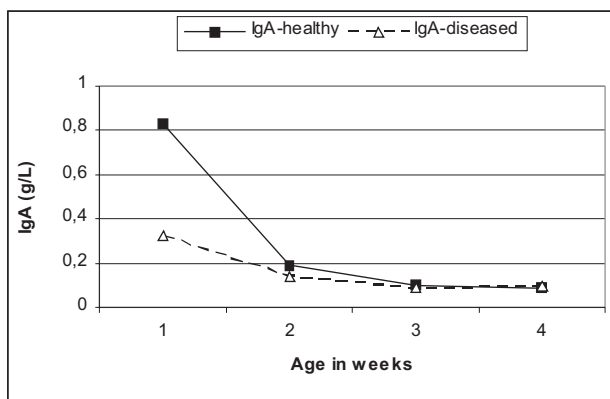Figure 1. IgG concentrations in healthy and diseased calves

Figure 2. IgA concentrations in healthy and diseased calves

Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.
Ježek J *et al*.: Indicators of pasive immunity
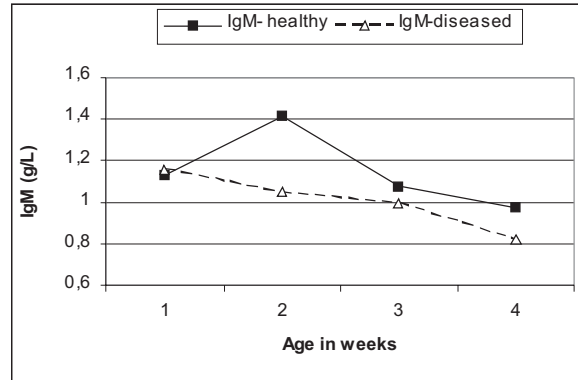and health status of calves
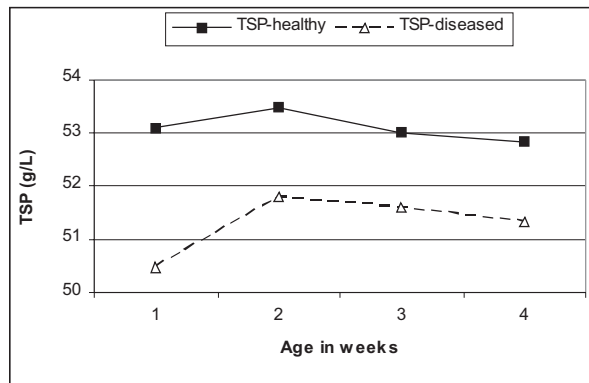
517

Figure 3. IgM concentrations in healthy and diseased calves

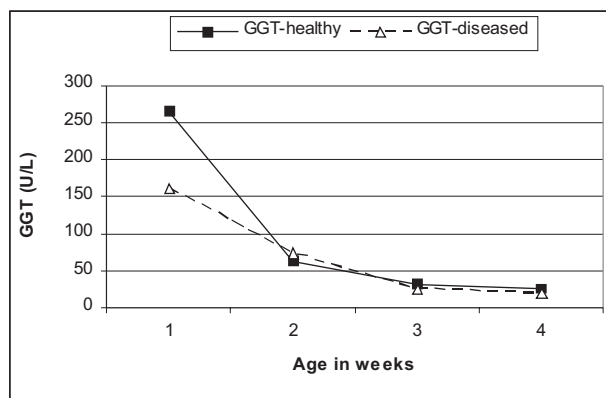Figure 4. TSP concentrations in healthy and diseased calves

Figure 5. The activity of GGT in healthy and diseased calves

518   Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

The statistically significant positive correlations between concentration of Ig in the colostrum and indicators of passive immunity in calves sera were established (Table 1).

Table 1. Correlation coefficients between concentration of Ig in colostrum and indicators of passive immunity in calves' sera in the first week of life

	logIgM	logIgA	Colostrum	logGGT	TSP
IgG	0.385**	0.569***	0.335**	0.398**	0.447***
logIgM	1	0.495***	0.284*	0.237*	0.431***
logIgA		1	0.376**	0.528***	0.478***
Colostrum			1	0.436***	0.596***

*p<0.05; **p< 0.01; ***p<0.001

The correlations between concentration of Ig in colostrum and IgG (r=0.352; p=0.005), TSP (r=0.467; p<0.001) and activity of GGT (r=0.420; p=0.001) in calves sera remained statistically significant up to the 4th week of age. The correlation between colostrum quality and concentration of IgA was statistically significant to the 3rd week of age (r=0.259; p=0.037). The correlation between colostrum quality, and IgM was statistically significant only in the 1st week.

DISCUSSION

The concentration of Ig in colostrum was in most cows considered to be adequate (above 50 g/L). The results agree with findings of Rajala and Castren (1995) who measured an average concentration of 76.2 g/L in colostrums of Finish Ayrshire cows. Lambrecht *et al*. (1982) established an average concentration of IgG 53.63 g/L, IgA 9.46 g/L and IgM 4.78 g/L in colostrums of German Holstein Friesian cows. In Swedish dairy cows the average concentration of Ig, measured with a colostrometer was 61.9±31.2 g/L (Svensson and Liberg, 2006). In the research of Franklin *et al*. (1998) the average concentration of Ig measured with a colostrometer was 77.6 g/L, 83.7 g/L and 89.2 g/L for Holstein cows in the 1st, 2nd and 3-6th lactation.

The correlation between time of first colostrum intake and concentration of Ig (IgG, IgA, IgM) in calves serum in our research was not statistically significant at first sampling (Table 1). The calves were supplied with colostrum at average 1.7 hours after birth. These findings are consistent with results of Burton *et al*. (1989) who found no correlation when calves that received colostrum within 6 hours after birth.

It is interesting that IgM level in healthy calves was higher in the 2nd than in the 1st week. Bender and Bostedt (2008) studied the dynamics of IgG and IgM in calves in the first 10 days of life and in some calves established the maximal IgG or IgM concentration was a few days after the last colostrum feeding. To the authors'

Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.   519
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

opinion it is possible that after resorption, IgG and IgM are stored in the vacuoles in the enterocytes and they pass into the blood circulation with delay. We think that to be the possible explanation for higher IgM level in the 2nd week is also that it is the primary immune response on new antigens which were introduced to the calf.

With regard to the health status, in the calves which remained healthy slightly higher values of investigated indicators of passive immunity were observed as in diseased ones, especially in the 1st week, with exception of IgM. No significant correlations were ascertained between health status and values of investigated parameters. The majority of diseased calves had lower values of indicators of passive immunity. Diarrhea in the 1st week was more frequently observed in calves with very low IgA concentration in comparison to those with high IgA. The finding is in accordance with the role of IgA to protect mucosal sites. There were also some exceptions; some calves with very low IgG concentrations did not diseased and some calves with very high concentration (also the calf with the highest IgG concentration) diseased in investigated period. Bender and Bostedt (2008) also found no significant association between disease incidence and concentrations of IgG and IgM in calves. The literature data about association between health status and immune status of calves rather differ. Caldow *et al*. (1988) found no statistically significant relationship between plasma IgG1 and disease incidence. Rea *et al*. (1996) found that calves with lower passive transfer values had increased risk of death. Selim *et al*. (1995) established higher morbidity rate in calves up to the age of 60 days with low concentration of TSP measured at the age of 3 days. Tyler *et al*. (1998) and Tyler *et al*. (1999) observed increased mortality up to the age of 10 weeks in calves with low TSP concentration. Stefen *et al*. (1997) found no association between TSP and GGT after colostrum intake and later incidence of disease. The variation between studies is not surprising because the disease incidence is influenced also by other factors like management, environment, etc.

The quality of colostrum was positively correlated with concentrations of IgG, IgA and IgM in the calves serum in the 1st week of life (Table 1). A similar correlation between concentration of IgG in the colostrum and in the serum was found also by Erhard *et al*. (1999). Dolenc (1998) ascertained a good association between IgG1, IgG2 and IgM in the colostrum and in the blood of calves (r=0.91-0.97), and a lower correlation for IgA. Burton *et al*. (1989) found a significant correlation between concentrations of Ig in colostrum and in calves serum at the age of 24 and 36 hours for all classes of Ig. The correlation was significant up to the 5th week for IgG, to the 3rd week for IgA and only 36 hours for IgM. Besser *et al*. (1985) established an association between the concentration of IgM respectively IgG in colostrum and serum of calves after colostrum intake (r=0.595, r=0.751 respectively), but they found better absorption efficiency in calves which got colostrum with lower concentration of IgG or IgM. Significant correlation between IgG and IgM in colostrum and in calves' serum at 24 hours found also Bender and Bostedt (2009). However, by checking the individual data they observed that administration of colostrum with maximum or minimum immunoglobulin concentration does not necessarily result in the respective sera immunoglobulin

520   **Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.**
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

concentration. Similarly, was found also in our study. This finding can be attributed to interindividual differences between calves in their ability for resorption of immunoglobulins and other molecules after birth, and possibly partly to the minor differences in the amount of colostrum ingested. The time of first colostrum intake was very short in most cases and could not have much influence on efficiency of resorption. A statistically significant correlation was established between colostrum quality and activity of GGT, as between GGT and other indicators of passive immunity (Table 1). The enzyme GGT is absorbed through the intestinal wall after colostrum intake; therefore serum activity of GGT in calves is increased in this period, and can be used for indirect estimation of colostrum supply (Bostedt, 1983). In our study the correlation coefficient between activity of GGT and concentration of IgG in calves serum in the 1st week of life was 0.398 (p=0.001). Similar correlation coefficient calculated Wilson *et al*. (1999) in calves at the age of 8 days (r=0.438). A higher correlation was established by Parish *et al*. (1997) (r=0.63) between activity of GGT and concentration of IgG1 in serum of calves at the age of 3 to 10 days. In reviewing our data of individual calves we observed that the activity of GGT not always agreed completely with concentration of IgG in calves. There were some calves with over average IgG concentration which had an activity of GGT under average and *vice versa*. The results of different studies indicated that calves are sufficiently supplied with colostrum, if their serum activity of GGT is above 50 U/L (Tyler *et al*., 1999; Parish *et al*., 1997). For Perino *et al*. (1993) the boundary value is 200 U/L. With regard to the results of our study is considered that the activity above 50 U/L in the 1st week of age is more appropriate.

The correlation coefficient between concentration of TSP and IgG in serum of investigated calves in the 1st week of age was 0.447 (p<0.001) (Table 1). Brand *et al*. (1996) established a good correlation (r=0.87) between concentrations of TSP and serum gamma globulins in calves. The authors considered the method to be appropriate for assessment of passive immunity in calves up to the age of 6 days. Nocek *et al*. (1984) established good correlation between concentration of TSP and IgG in calves at the age from 1 to 4 days (r=0.84), the correlation decreased with the age. At the age of 11 days the correlation coefficient was 0.69. Bender and Bostedt (2009) obtained a significant correlation (r=0.642) between the sum of IgG and IgM concentration and total plasma protein concentration. The findings of some studies indicate that TSP concentration in calves with sufficient passive immunity is above 55 g/L, the result below 49 g/L TSP indicates failure of passive transfer (Tyler *et al*., 1998; Tyler *et al*., 1996; Selim *et al*., 1995). For other authors the boundary concentration of TSP is 45 g/L and 42 g/L, respectively (Rea *et al*., 1996; Perino *et al*., 1993). This is considered more appropriate regarding our results.

The calves which diseased had lower values of indicators of passive immunity especially in the 1st week of life, but no significant correlation could be determined. So only on the base of values of the investigated parameters no direct conclusion about later health status of calves is possible. The results indicate association between colostrum quality and indicators of passive immunity IgG, GGT and TSP up to the 4th week of age. Therefore, we are of the

Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.   521
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

opinion that the mentioned indicators are suitable to assess colostrum supply in calves at least up to the 3rd week of age. The indirect indicators such as TSP and GGT are more reliable to ascertain if the colostrum was absorbed as for prediction of immunoglobulin levels in the calf's serum.

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522

Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

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Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 513-523, 2010.
Ježek J *et al*.: Indicators of pasive immunity
and health status of calves

523

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POKAZATELJI PASIVNOG IMUNITETA I ZDRAVSTVENO STANJE TELADI

JEŽEK JOŽICA, NEMEC MARIA, MALOVRH T i KLINKON MARTINA

SADRŽAJ

Cilj ovog ispitivanja je bio da se odredi koncentracija imunoglobulina (IgG, IgM, IgA) i ukupnih serumskih proteina, kao i aktivnost enzima GGT u serumu 71 teleta do uzrasta od 4 nedelje. Ispitivan je stepen korelacije parametara serumskog profila i ukupne koncentracije imunoglobulina u kolostrumu kojim su telad napajana. Rezultati su povezani sa zdravstvenim stanjem teladi. Jedan od ciljeva rada je bio i da se utvrdi mogućnost primene ovih pokazatelja za utvrđivanje snabdevenosti teladi slovenačkih rasa kolostrumom. U uzorcima seruma je određivana koncentracija IgG, IgA, IgM, ukupnih serumskih proteina i aktivnost GGT. U uzorcima kolostruma njihovih majki određivana je koncentracija ukupnih imunoglobulina. Korelacija između sadržaja imunoglobulina u kolostrumu i koncentracije IgG, ukupnih serumskih proteina i aktivnosti GGT je bila značajna do uzrasta od četiri nedelje. Samo na osnovu vrednosti određivanih parametara ne može se doneti zaključak o kasnijem zdravstvenom stanju teladi. Ispitivani pokazatelji su pogodni za ocenu stepena snabdevenosti kolostrumom teladi do uzrasta od najmanje 3 nedelje. Indirektni pokazatelji kao što su koncentracija ukupnih serumskih proteina i aktivnost GGT su pouzdaniji u proceni da li je kolostrum resorbovan i za utvrđivanje nivoa imunoglobulina u serumu teladi.