C-Reactive Protein and Antibacterial Activity in Blood Plasma of Colostrum-Fed Calves and the Effect of Lactulose

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ABSTRACT

Several milk proteins are very important for immunological defense and can be absorbed in the intestine of calves in the first hours after birth. The influence of colostrum intake and the effect of additional lactulose application on the concentration of C-reactive protein (CRP) in blood were investigated. The CRP is known as a mediator of innate immunity. Results were compared to the bovine acute phase protein haptoglobin, and to lactalbumin, lactoferrin, and immunoglobulins in plasma from calves. After colostrum intake, the concentration of most proteins were strongly increased. The data show, for the first time, a significant increase of CRP in the blood of calves 1 d after colostrum intake (nonlactulose group, $n = 10$), and an even more significant increase in CRP concentration (1 d postpartum) was measured in the group of animals with additional application of lactulose (lactulose group, $n = 10$) when compared to the nonlactulose group. In an in vitro assay with the plasma of these animals, an increased bactericidal activity was detected against *Morganella morganii* (1 d postpartum) in both groups, but again a higher activity occurred in the lactulose group. The results of these investigations emphasize the importance of colostrum intake during the first hours after birth for the defense potential of newborn calves. In addition, lactulose may have a positive effect in the period of passive transfer of colostrum proteins and in the immune defense.

(**Key words:** colostrum, lactulose, C-reactive protein, bactericidal activity)

Abbreviation key: BCPBA = blood cell-plasma-bactericidal activity, CRP = C-reactive protein, HBSS = Hanks-balanced salt solution, FCS = fetal calf serum,

GGF = gamma-globulin fraction, REU = relative ELISA units.

INTRODUCTION

The passive transfer of colostrum proteins in calves such as immunoglobulins is very important for the health of the newborn animal (Holloway et al., 2002). In 1994, C-reactive protein (CRP) was detected in bovine milk. It was found in higher concentrations in animals suffering from mastitis (Schroedl et al., 1995). C-Reactive protein is a major acute-phase protein in human and some other species and is involved in various immunological reactions (Steel and Whitehead, 1994). It is able to bind to some microbial compounds, such as phosphorylcholine and several sugar-determinants and can stimulate the elimination of the pathogens by opsonizing and activating the complement system (Volanakis, 2001; Du Clos and Mold, 2001). Many investigations to its function have been carried out with human CRP or with CRP in laboratory animals. Latest investigations show a protective effect of human CRP in transgenic mice against infection with *Salmonella enterica* serovar *typhimurium* (Szalai et al., 2000). Xia and Samols (1997) determined a protective effect of rabbit CRP in transgenic mice to endotoxemia. Various studies with other bacteria strains, especially *S. pneumoniae*, proved the antibacterial and protective effects of CRP (Mold et al., 2002).

Only a few published results with regard to bovine CRP show its isolation and concentration values in blood (Maudsly et al., 1987; Sarikaputi et al., 1991). Morimatsu et al. (1991) found a positive relationship between the CRP concentration in blood serum of cows and the daily milk yield. The special functions of CRP in cattle are almost unknown (Cheryk et al., 1996); however, its importance as a major component of the bovine innate immune system is suspected.

The application of prebiotics in veterinary medicine, and its versatile use play a more and more important role (Vanbelle et al., 1990). The semisynthetic disaccharide lactulose is a compound that is chemically well

Received January 21, 2003.

Accepted May 6, 2003.

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characterized and does not occur naturally. Lactulose cannot be hydrolyzed by endogenous enzymes of enterocytes because of its specific structure (4-O- β -D-galactopyranosyl-D-fructose). It is poorly absorbed from the small intestine and is a suitable substrate for some bacteria in the gut, especially in the colon (Clausen and Mortensen, 1997; Schumann, 2002). The reduction of the ammonia concentration in human blood and the production of short-chain fatty acids, especially of acetate, have been regarded as important effects after the application of lactulose (Wrong and Vince, 1987; Mortensen et al., 1990; Mortensen and Clausen, 1996). The lactulose can have laxative effects in humans, depending on the individual (Clausen et al., 1998). But also some immunomodulatory, antiendotoxic, antibacterial, or receptormodulatory effects of lactulose have been published (Vendemiale et al., 1992; Gardiner et al., 1995; Bovee-Oudenhoven et al., 1997). Lactulose is applied in the treatment of constipation and hepatic encephalopathy in human medicine (Clausen and Mortensen, 1997). The lactulose could also be of importance for farm animals, like sows and piglets (Krueger et al., 2002).

The objective of these investigations was to determine the influence and effects of a daily lactulose application on the CRP level in blood of colostrum-fed newborn calves and other selected colostrum and blood proteins. With regard to a possible function of the bovine CRP as an opsonine, the bactericidal activity against *Morganella morganii* was investigated with an in vitro assay by using the same plasma samples.

MATERIALS AND METHODS

Experimental Design

Twenty newborn calves (German black and white breed $\times$ Holstein-Friesian breed, sbHF) were used for the investigations. Ten newborn calves were orally given 15 ml of lactulose syrup (approx. 10 g of lactulose, Solvay Pharmaceuticals GmbH, Hannover, Germany) before the first application of colostrum and then 15 ml/d (lactulose group). The remaining 10 calves represented the control group (nonlactulose group), chosen on a random selection basis. In the first 5 d, the calves were bottlefed with 1 to 2 L of colostrum (or milk) from their dam twice daily. Thereafter, each calf received mixed milk three times daily.

Blood Collection

The first blood samples were collected before the first application of colostrum and lactulose. The other blood samples were taken 1 and 10 d postpartum. Blood samples were obtained from the jugular vein into heparin-

ized tubes. Tubes were then centrifuged at $3000 \times g$ at room temperature for 15 min to separate the plasma and were stored at -25°C before completing all analyses.

Analysis

The parameters lactalbumin, lactoferrin, haptoglobin, CRP, IgG, IgM, and IgA, and the IgG- and IgA-anti-lipopolysaccharides (LPS) from *Escherichia coli* J5 (IgG- and IgA- anti-LPS, J5) were determined in plasma with ELISA.

Common ELISA-Steps

All ELISA were done with ELISA-plates (microtiter, 96-well, high binding, Greiner, Frickenhausen, Germany). All plates were incubated at room temperature for 1 h on a microtiter plate shaker (400 rpm) with a volume of 100 μl in the well. The coating buffer was 0.1 M NaHCO_3 and the wash buffer was Dulbecco-PBS without Ca and Mg (pH 7.35) and with 0.1% (vol/vol) Tween 20 (Sigma-Aldrich, Taufkirchen, Germany). The plates were then washed three times with the wash buffer (PBS with Tween 20) by using the Nunc-Immuno-Washer 12 (NUNC, Wiesbaden, Germany). The horseradish peroxidase was determined on the solid phase with the substrate 3,3',5,5'-tetramethylbenzidine as described by Gallati and Pracht (1985). The substrate reaction was stopped with 1 M H_2SO_4 (50 μl /well). The optical density was measured with microplate-ELISA-reader at 450 nm. For all ELISA, the intraassay variation was less than 10%, and the interassay variation was less than 15%.

Antiserum

The gamma-globulin fraction (GGF) was prepared from antiserum by precipitating it twice with 40% (vol/vol) ammonium sulfate. The precipitate was dissolved with PBS. After the dialysis against PBS (3×500 ml), the protein concentration was measured with the spectrophotometer MBA 2000 and the integrated software (Perkin-Elmer, Norwalk, CT).

ELISA for Alpha-Lactalbumin

The coating antibody was GGF from rabbit-antiserum anti-bovine alpha-lactalbumin (Riedel de Haen, Seelze, Germany) with 6 $\mu\text{g}/\text{ml}$ (protein concentration). The standard was bovine alpha-lactalbumin from bovine milk (Fluka, Buchs, Switzerland), ranging from 0.04 to 10 ng/ml. The samples were diluted 1:100 and 1:1000. The assay buffer for dilution of the standard

and plasma samples was PBS (Dulbecco), 0.1% Tween 20 (vol/vol), 10 mM EDTA, and 0.5% BSA. The detection antibody was GGF from rabbit antiserum anti-bovine alpha-lactalbumin (Riedel de Haen) conjugated with horseradish peroxidase (1044 units/mg, Fluka) according to Wilson and Nakane (1978). The detection antibody was diluted 1:10,000 in assay buffer with 0.1% rabbit normal serum.

ELISA for Lactoferrin

The coating antibody was affinity purified IgG (goat) anti-bovine lactoferrin (Bethyl Laboratory, Inc., Montgomery, TX) with 1 μ g/ml. The standard was lactoferrin from bovine colostrum (Sigma-Aldrich), ranging from 0.6 to 40 ng/ml. The samples were diluted 1:200. The assay buffer for dilution of the standard and plasma samples was 50 mM Tris/HCl (pH 8.0), 0.15 M NaCl, 10 mM EDTA, 0.1% Tween 20 (vol/vol). The detection antibody was affinity purified IgG (goat) anti-bovine lactoferrin conjugated with horseradish peroxidase (Bethyl Laboratory, Inc.) in a dilution of 1:40,000 in assay buffer with 1% goat normal serum.

ELISA for Haptoglobin

The coating antibody was the Ig-fraction from rabbit-antiserum anti-human haptoglobin (DAKO, Hamburg, Germany) with cross-reactive antibodies anti-bovine haptoglobin, which was diluted 1:3000. The standard was a bovine plasma, in which the haptoglobin concentration was determined with a standardized colorimetric assay for bovine haptoglobin (Tridelta Development Ltd., Greystones, Co., Wicklow, Ireland). The standard concentration ranged from 3 to 200 ng/ml. The samples were diluted 1:2000 and 1:20,000. The assay buffer for dilution of the standard and plasma samples was 50 mM Tris/HCl (pH 8.0), 0.15 M NaCl, 10 mM EDTA, 0.1% Tween 20 (vol/vol) and 0.2% bovine casein (Sigma-Aldrich). The detection antibody was the same polyclonal Ig-fraction for the coating, but conjugated with horseradish peroxidase (1044 units/mg, Fluka) as described by Wilson and Nakane (1978). The detection antibody was diluted 1:10,000 in assay buffer.

ELISA for C-Reactive Protein

The coating antibody was the Ig-fraction (from rabbit-antiserum) anti-human CRP (DAKO) with cross-reactive antibodies anti-bovine CRP, which was diluted 1:2000. The standard was bovine pooled serum in which the CRP-concentration was measured with affinity purified bovine CRP as standard. C-Reactive protein was purified according to the method described by Sarika-

puti et al. (1991). The standard concentration ranged from 1 to 100 ng/ml. The samples were diluted 1:1000. The assay buffer for dilution of the standard and plasma samples was PBS, 5 mM Tris/HCl (pH 8.0), 0.1% Tween 20 (vol/vol), and 10 mM EDTA. The detection antibody was Ig-fraction (rabbit) anti-human CRP conjugated with horseradish peroxidase (DAKO), which was diluted 1:2000.

ELISA for IgG

The coating antibody was affinity purified IgG (goat) anti-bovine IgG-Fc (Dianova, Hamburg, Germany; producer: Jackson Immuno Research, West Grove, PA) with 2.4 μ g/ml. The standard IgG-range in the ELISA was 1 to 100 ng/ml. Bovine reference serum (Bethyl Laboratory, Inc.) was used as standard. The samples were diluted 1:10⁴, 1:10⁵, and 1:10⁶. The assay buffer for dilution of the standard and plasma samples was PBS, 0.1% Tween 20 (vol/vol), 10 mM EDTA. The detection antibody was affinity purified IgG (goat) anti-bovine IgG conjugated with horseradish peroxidase (Dianova), diluted 1:40,000 in assay buffer.

ELISA for IgM

The coating antibody was affinity purified IgG (sheep) anti-bovine IgM (Bethyl Laboratory, Inc.) with 1.0 μ g/ml. The standard IgM-range was 1 to 100 ng/ml. Bovine reference serum (Bethyl Laboratory, Inc.) was used as standard. The samples were diluted in the range between 1:10³ and 1:10⁵. The assay buffer for dilution of the standard and plasma samples was PBS, 0.1% Tween 20 (vol/vol), 10 mM EDTA, 0.2% bovine casein (wt/vol). The detection antibody was affinity purified IgG (sheep) anti-bovine IgM conjugated with horseradish peroxidase (Bethyl Laboratory, Inc.), diluted 1:20,000 in assay buffer.

ELISA for IgA

The coating antibody was affinity purified IgG (sheep) anti-bovine IgA (Bethyl Laboratory, Inc.) with 1.0 μ g/ml. The standard IgA-range in the ELISA was 1 to 100 ng/ml. Bovine reference serum (Bethyl Laboratory, Inc.) was used as standard. The samples were diluted in the range between 1:10³ and 1:10⁵. The assay buffer for dilution of the standard and plasma samples was PBS, 0.1% Tween 20 (vol/vol), 10 mM EDTA. The detection antibody was affinity purified IgG (sheep) anti-bovine IgA conjugated with horseradish peroxidase (Bethyl Laboratory Inc.), diluted 1:10,000 in assay buffer.

ELISA for IgG- and IgA-anti-LPS (*E. coli* J5)

The ELISA-plate was coated with 100 μ l/well of 5 μ g/ml LPS, *E. coli* J5 in 0.15 M NaCl and 0.2% (wt/vol) trichloroacetic acid (Hardy et al., 1994) and was incubated overnight. The plates were washed twice with PBS with Tween 20 and then 150 μ l/well of 0.15 M NaCl-solution with 2% bovine casein (wt/vol) was added. After 1 h of incubation, 50 μ l/ml of the 1:100 (IgG-anti-LPS, J5) or 1:10 (IgA-anti-LPS, J5) diluted samples and a diluted pooled bovine plasma taken arbitrarily from more than 200 cows of the same herd as a reference value (internal standard) was added to the 150 μ l/well, without washing. The internal standard was defined to 100 relative ELISA units (REU)/ml. The REU/ml were calculated for each plasma sample in relation to the internal standard taking also the dilution factors into account. The samples and the standard-pool plasma were diluted with PBS (Dulbecco; four times concentrated), and with 0.4% Tween 20 (vol/vol). After 1 h of incubation (see common ELISA-steps) and several washing steps, 100 μ l/well of affinity purified IgG (goat) anti-bovine IgG conjugated with horseradish peroxidase (Dianova) were diluted 1:10,000 and added to the plates. The IgA was detected with affinity purified IgG (sheep) anti-bovine IgA conjugated with horseradish peroxidase (Bethyl Laboratory, Inc.), diluted 1:2000. PBS (Dulbecco), 0.1% Tween 20 and 0.1% bovine casein were used as the dilution buffer.

Blood Cell-Plasma-Bactericidal Activity (BCPBA) Assay Bacteria Suspension

A field strain of *M. morganii* and *E. coli* (Institute of Bacteriology and Mycology, Leipzig, Germany) were cultivated (at 37°C, overnight) on nutrient agar I (SIFIN, Berlin, Germany). The colonies were harvested and washed in sterile Hanks-buffered saline solution (HBSS) without phenol red (Biochrom, Berlin, Germany) three times by centrifuging $3000 \times g$ for 30 min. The bacteria cells were suspended in HBSS. The cell numbers of 4×10^6 bacteria/ml were calculated by a spectrophotometer (as described above) at 620 nm (number of bacteria $\times 10^7$ /ml = $E_{620\text{nm}}^{1\text{cm}} \times 24.47$).

Bovine Blood Cells

Heparinized (10 units/ml) blood (*V. jugularis*) from a healthy cow was washed three times with HBSS without Ca, Mg, and phenol red (Biochrom) and with heparin (10 units/ml) by $1250 \times g$ for 30 min at 4°C. The washed blood cell pellet was resuspended in HBSS with 10 units of heparin/ml for a 50% suspension (vol/vol).

Assay Procedure

The 50% suspension of blood cells (50 μ l/well), or for the control 50 μ l/well HBSS with heparin, and 25 μ l/well plasma samples, or for the control 25 μ l/well HBSS with heparin, and 25 μ l/well bacteria suspension were added into sterilized 96-well cell culture plates (Nunc) and carefully mixed. After 2 h of incubation at 37°C the assay samples were diluted 1:10 in sterile distilled water and incubated for 5 min. All assay samples were then diluted in sterile PBS (Dulbecco) in log₁₀-steps. Then, 10 μ l from each dilution was plated on nutrient agar I (SIFIN). After overnight incubation at 37°C, the cfu/ml was determined. The percentage of bactericidal activity was calculated in relation to the cfu/ml of the control (without cells and without plasma).

Statistical Analysis

The statistical analysis was conducted with the software SigmaStat (SPSS Science Software GmbH, Erkrath, Germany). The figures were made with the software SigmaPlot (SPSS Science Software GmbH) and show box plots with the median (solid line within the box), the 25th and 75th percentiles (top and bottom solid lines of the box) and the 10th and 90th percentiles (small lines outside the top and bottom of the box). The level of significance was calculated with the nonparametric Mann-Whitney rank sum test, because in most statistical analysis a failed normality in the Kolmogorov-Smirnow-test ($P < 0.01$) was shown (automatic check before running a test with the SigmaStat software), and high value ranges were available. The correlation coefficient was determined with the Spearman rank order correlation.

RESULTS

Determinations in Plasma Before Colostrum Intake

Before the initial intake of colostrum IgG values ranged from 0.030 to 0.867 mg/ml, IgM values ranged from 0.013 to 0.170 mg/ml, and lactoferrin values ranged from 0.248 to 4.365 μ g/ml in all blood samples of calves. In 70% of all the blood samples, lactalbumin was detected, and values ranged from 0 to 147 ng/ml. In 35% of all blood samples haptoglobin was detected, and values ranged from 0 to 23 μ g/ml. In 85% of all blood samples, IgA was detected, but values were lower than 1 μ g/ml in 15% of samples. The IgG-anti-LPS, *E. coli* J5 was detected in 50% of blood samples, and IgA-anti-LPS, *E. coli* J5 was measured in 85% of blood samples. The CRP was quantified in all blood samples with values ranging from 1.8 to 7.7 μ g/ml (mean = 4.473 μ g/

Table 1. Median (10th and 90th percentiles) of the blood plasma investigations before the first application of colostrum, 1 d postpartum, and 10 d postpartum and the influence of daily oral lactulose application.

Parameter	Before colostrum and lactulose ¹		1 d Postpartum		10 d Postpartum	
	Group 1 (NLG ²)	Group 2 (LG ³)	Group 1 (NLG ²)	Group 2 (LG ³)	Group 1 (NLG ²)	Group 2 (LG ³)
LA ⁴ (ng/ml)	0.21 (0.00–47.31)	3.59 (0.53–31.16)	798.40 (25.30–2307.18)	276.68 (22.65–1398.34)	7.28 (3.37–44.88)	10.79 (6.21–30.03)
LF ⁵ (μg/ml)	0.63 (0.37–3.40)	0.61 (0.27–1.51)	1.12 (0.43–2.96)	0.71 (0.49–2.41)	0.39 (0.23–2.14)	0.33 (0.26–0.54)
Hpt ⁶ (μg/ml)	0.00 (0.00–1.35)	0.00 (0.00–11.63)	0.05 (0.00–1.21)	1.40 (0.10–6.80)	0.28 (0.00–124.49)	1.87 (0.43–100.81)
IgG (mg/ml)	0.12 (0.06–0.64)	0.15 (0.05–0.71)	9.24 (2.42–17.42)	12.48 (3.89–37.52)	9.29 (5.07–24.58)	8.48 (2.63–28.18)
IgM (mg/ml)	0.03 (0.02–0.05)	0.03 (0.01–0.11)	0.88 (0.12–1.77)	0.75 (0.23–2.21)	0.92 (0.52–1.33)	0.57 (0.38–1.00)
IgA (mg/ml)	0.00 (0.00–0.07)	0.01 (0.00–0.05)	0.44 (0.11–1.71)	0.81 (0.32–2.30)	0.13 (0.07–0.39)	0.08 (0.03–0.37)
IgG-anti-LPS ⁷ (REU ⁸ /ml)	0.00 (0.00–7.57)	2.36 (0.00–5.50)	45.96 (8.66–98.34)	52.19 (7.31–157.60)	54.44 (12.36–116.91)	19.12 (4.68–92.70)
IgA-anti-LPS ⁷ (REU ⁸ /ml)	3.02 (0.05–52.19)	9.24 (0.00–58.31)	151.84 (24.65–228.84)	89.57 (29.77–858.96)	27.10 (10.12–46.16)	51.16 (1.48–253.49)

¹After birth and before the first application of lactulose and colostrum.²NLG = nonlactulose group.³LG = lactulose group.⁴LA = lactalbumin.⁵LF = lactoferrin.⁶Hpt = haptoglobin.⁷LPS (*E. coli*, J5).⁸REU = relative ELISA units.

ml; median = 4.259 μg/ml; 10th percentiles = 2.082 μg/ml; and 90th percentiles = 6.609 μg/ml).

Determinations in Plasma after Colostrum Intake

The values measured are summarized in Table 1. One day after birth and intake of colostrum, the concentration of most measured proteins increased ($P < 0.01$), but values of lactoferrin and haptoglobin were unchanged. Decreases occurred in concentrations of IgA ($P < 0.05$), lactalbumin ($P < 0.01$), and IgA-anti-LPS (*E. coli* J5; $P < 0.01$) in the blood of the calves of the nonlactulose group from 1 to 10 d postpartum.

Application of Lactulose

In relation to the nonlactulose group, a significantly higher concentration of the CRP was present in the plasma of calves with additional oral application of lactulose at 1 and 10 d (Figure 1). The lactulose group did not have a significant decrease of IgA anti-LPS (*E. coli* J5) from 1 to 10 d postpartum.

Bactericidal Activity

The results of the investigations with the in vitro bactericidal assay using *M. morganii* as target bacteria

are summarized and shown in Figure 2. A significantly higher bactericidal activity against *M. morganii* was detectable at 1 d postpartum in the lactulose group. In both groups, a significant increase of the *M. morganii*-bactericidal activity was seen compared with the time before colostrum intake (lactulose group: $P < 0.005$,

Figure 1. C-Reactive protein (CRP) concentration in plasma of calves before and after (days postpartum) application of colostrum (white boxes) and of lactulose and colostrum (grey boxes).

Figure 2. Bactericidal activity against *Morganella morganii*, tested with washed blood cells and the plasma samples from calves before and after (days postpartum) application of colostrum (white boxes) and of lactulose and colostrum (grey boxes).

nonlactulose group: $P < 0.05$). No significant differences were seen for the bactericidal activity against *E. coli* (1 d postpartum).

Relationship Between CRP and in Vitro Bactericidal Activity

The Spearman rank order correlation was conducted with all measured values from the blood samples taken before the application of colostrum and lactulose ($n = 20$). The values of the in vitro bactericidal activity against *M. morganii* were positively correlated (coefficient of 0.447; $P < 0.05$) with the CRP plasma concentrations, but the correlation tendency to each of the other plasma proteins was not significant ($P < 0.05$).

A possible relationship between CRP and the in vitro bactericidal activity against *M. morganii* was tested in an additional in vitro investigation. Nonheat inactivated fetal calf serum (FCS) with a low CRP concentration of 1 $\mu\text{g/ml}$ was investigated before and after the addition of 10 μg of purified bovine CRP/ml in the BCPBA assay in relation to a heat-inactivated (at 56°C for 1 h) FCS as the reference value (= 0% bactericidal activity). In the FCS, with the added CRP concentration a fivefold increase in bactericidal activity against *M. morganii* could be seen in vitro. Furthermore, a plasma sample from a dairy cow with a high CRP concentration (230 $\mu\text{g/ml}$) showed the highest bactericidal activity. The same undiluted plasma sample (1 ml) was incubated with immobilized phosphorylcholine agarose (0.5 ml of gel washed three times with 10 ml of HBSS, Pierce, Rockford, IL) for 1 h at room temperature. After centrifugation and sterile filtration (0.2 μm), the CRP concentration was 179 $\mu\text{g/ml}$, and the bactericidal activ-

Figure 3. Bactericidal activity against *Morganella morganii* using washed blood cells (see Materials and Methods) and the following samples: fetal calf serum (FCS) with 1 $\mu\text{g/ml}$ C-reactive protein (CRP) (FCS⁽¹⁾) and with 10 $\mu\text{g/ml}$ added purified bovine CRP (FCS⁽²⁾), cow plasma (CowP) with a high CRP-concentration of 230 $\mu\text{g/ml}$ (CowP⁽¹⁾) and the same cow plasma after 1 h of incubation with immobilized phosphorylcholine agarose (Pierce, Rockford, IL) with 179 $\mu\text{g/ml}$ CRP (CowP⁽²⁾). The means and standard error of the means ($n = 4$) are shown. The bactericidal activity in % relates to the control with heat-inactivated FCS (at 56°C for 1 h).

ity in the BCPBA assay was reduced from 70 to 38.6% (Figure 3).

DISCUSSION

The passive transfer of proteins from colostrum to the blood in calves is well established (Talukder et al., 2002). The maternal nonspecific and specific factors of the immune system are important for the health of the calf after birth and the defense of pathogens and several environmental factors. The objective of our study was to investigate especially the CRP level in blood plasma of calves before and after colostrum intake, as well as the role of an additional application of lactulose in comparison to the kinetics of known colostrum proteins. Some years ago (Schroedl et al., 1995) we detected CRP in colostrum and milk from cows, but no investigations have been conducted regarding a possible passive transfer from colostrum into the calf blood. In the current study, CRP could be measured in all blood plasma samples of the calves before the first colostrum intake, and a significantly higher concentration in the blood was measured 1 d after colostrum application. It is assumed that the increase of the CRP concentration (1 d postpartum) is affected by the passive transfer of CRP from colostrum to blood because the measured concentrations of the immunoglobulins and the lactalbumin were characteristically and significantly increased. C-Reactive protein is a component of the innate host resistance.

It disposes of opsonic properties and can bind to Fc-receptors, but it also activates the classical complement cascade (Volanakis, 2001). It was our objective to investigate the plasma-mediated bactericidal activity against *M. morganii* in vitro and to put it into relation to the measured CRP concentrations.

Phosphocholine (phosphorylcholine) is a typical ligand of CRP and part of the cell membrane in some bacteria species like *S. pneumoniae*, *P. aeruginosa*, *Haemophilus influenzae*, and also in *M. morganii* (Volanakis, 2001). The *M. morganii* strain used in these investigations was successfully agglutinated with a monoclonal antibody anti-phosphorylcholine (TEPC-15, Sigma-Aldrich). With the plasma samples taken after colostrum intake (1 d postpartum), a significant increase of the in vitro bactericidal activity against *M. morganii* was determined, but not in case of *E. coli*.

The result of a possible positive correlation between CRP and the bactericidal activity against *M. morganii* was confirmed in an additional in vitro experiment with FCS supplemented with purified bovine CRP. A fivefold increase of the in vitro bactericidal activity against *M. morganii* was determined after having added 10 µg of purified bovine CRP/ml to the FCS. The highest bactericidal activity against *M. morganii* could be seen with plasma from a cow that contained a high concentration of CRP (230 µg/ml). The decrease of the plasma-mediated bactericidal activity against *M. morganii* from 70 to 38.6% after incubation of the cow plasma with phosphocholine immobilized agarose speak for a possible phosphocholine-specific effect.

Summarizing all results, it is suggested that bovine CRP can contribute to the elimination of some bacteria species and could be another important factor for the immune defense in cattle and especially in newborn calves. Further investigations are necessary to characterize the special functions of the bovine CRP.

Based on earlier observations of individual animals, the possible influence of the oral and daily application of lactulose on the passive protein transfer was investigated in colostrum-fed, newborn calves during the first 10 d after birth. After the application of lactulose and colostrums, a greater increase of the CRP plasma concentration and also a significantly higher bactericidal activity against *M. morganii* was determined in comparison to the nonlactulose group.

No other investigated plasma protein was significantly affected by the additional lactulose application on 1 d postpartum. The median of IgG and IgA concentration tended to be higher in the lactulose group 1 d postpartum, but was not significant statistically. To measure specific antibodies against a typical compound of gram-negative bacteria, especially of *E. coli*, the LPS (*E. coli* J5 strain) was selected as solid-phase antigen.

The levels of antibodies against LPS (*E. coli* J5) were also increased after colostrum intake. It is an indication for the antibodies still being active after the passive transfer. There was no significant difference between the lactulose and nonlactulose group. The results indicate a possibly selective or stronger influence of lactulose on the passive transfer of the CRP from colostrum into the calf blood. It is possible that lactulose can stabilize the complex protein structure (pentamer) of CRP. It might also have receptor-modulating effects or stimulate the solubilization of colostrum CRP and can positively promote the transfer of the functional active protein. This could also be a reason for the differences measured in some of the other plasma proteins from 1 to 10 d postpartum. The daily oral application of one dose of 15 ml of lactulose syrup (equivalent to approx. 10 g of pure lactulose) per calf did not show any clinical signs for enteral disorders or other diseases. Also, in both groups (with and without lactulose) no significant increase of the haptoglobin plasma concentration was measured from 1 to 10 d postpartum. Haptoglobin is a major acute-phase protein in cattle, since its concentration can increase dramatically (10- to 1000-fold) after infection or complications with induced inflammatory reactions (Horadagoda et al., 1999; Schroedl et al., 2001). The low concentrations of haptoglobin in the blood of the calves after birth could therefore be a good basis for evaluating possible complications such as intrauterine infections.

The lactulose with a prebiotic potential could contribute to animal health, particularly to the development of the endogenous microbial flora and the immune defense. In our investigations, the analyses were concentrated on the aspect of a possible influence of lactulose on selected plasma protein levels in newborn calves after birth. Many investigations with lactulose have been conducted in human medicine or with laboratory animals, but thus far no investigations have been made to the effects of lactulose in cattle. Further research is necessary to find out immunomodulatory, and other, effects of lactulose that could be important for animal health.

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