# The influence of passive colostrum transfer on humoral immunity to selected antigens of *Mannheimia haemolytica* in calves

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**Abstract**

The aim of the study was to evaluate the effectiveness of colostral immunity against aetiological agents of bovine respiratory disease complex (BRDC), to assess the suppressive effect of colostral antibodies against *Mannheimia haemolytica* on immunity, and to analyse acute phase proteins in calves. Holstein-Friesian and Simmental cows and calves were immunized with *M. haemolytica* leukotoxin (Lkt) and outer membrane proteins (OMPs) at 6 and 4 weeks before parturition. Sera and colostrum were collected from the cows directly after calving. Sera from calves were obtained before colostrum intake and 48 h after birth. Calves from vaccinated and unvaccinated cows were placed in the feedlot and immunized with Lkt or OMP on days 10 and 24. Calves were tested for serum antibodies against respiratory viruses. Trachea and lung samples were collected for bacteriological examination from all calves that had died with BRDC. The results indicated high colostrum values and IgG transfer in calves at 48 h (> 12.5 g/l) and a high concentration of anti-BVD antibodies in calves at 48 h (> 33–45 mg/ml). Similar values were observed for bovine herpes virus BHV-1 and bovine respiratory sycytil virus BRSV. Immunoglobulin concentrations were highest for antibodies specific to parainfluenza PI-3 and adenoviruses. The lowest antibody levels were detected against *M. haemolytica* antigens in all experimental groups of calves (< 50 mg/ml in calves of cows vaccinated with *M. haemolytica* antigens and < 25 mg/ml in calves of unvaccinated cows). The findings indicate the need for early immunization of calves, which is often precluded by stress associated with transport and adaptation to the new conditions in the feedlot.

*BRDC, cattle, immunoglobulins, immune response*

Respiratory disease in calves is a major health problem in beef and dairy cattle. The condition was previously referred to as haemorrhagic septicaemia (pulmonary form), transit or shipping fever, pulmonary pasteurellosis, or fibrinous pneumonia. These terms have currently been replaced with 'bovine respiratory disease complex' (BRDC) (Autio et al. 2007). Economic losses, which exceed the combined expenditures associated with all other diseases in calves, result from increased morbidity and delayed growth of animals, ranging from 16% to 30%. Morbidity varies between 65% and 80%, and mortality in unvaccinated calves reaches 30–70% (Barrett 2000). The total costs in the USA reach 3 billion dollars a year, in the EU they are estimated at 576 million euro (Schroedl et al. 2003; Snowder et al. 2007). In the complex aetiology of BRDC, an important role is played by viruses – parainfluenza virus type 3 (PI-3), bovine respiratory syncytial virus (BRSV), bovine viral diarrhoea and mucosal disease virus (BVD/MD known as BVD-bovine viral diarrhoea virus), and herpesvirus (BHV-1 known as IBR); and bacteria (*Mannheimia haemolytica*, *Pasteurella* sp., and *Histophilus somni)* and mycoplasmas (Autio et al. 2007; Srikumaran et al. 2008). A key role is attributed to a complex of predisposing factors, such as concentrations of large numbers of animals, transport stress, an unsuitable microclimate and poor nutrition. The immunosuppressive effect resulting from viral interference in macrophages and lymphocytes has a fundamental role and prepares the way

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for the development of pathological changes, most often caused by bacteria (Hodgson et al. 2005). Despite incomplete knowledge of the mechanisms of general and local immunity against infection, many authors present the view that immunostimulation of calves is the most effective way to protect them against infection (Woolums 2014). Most commercial inactivated *M. haemolytica* vaccines show negligible efficacy against infections. One reason for the low effectiveness of this group of vaccines is the lack or inadequate concentration of the antigenic components responsible for inducing the immune response (Windeyer et al. 2012). A much better effect is obtained using live modified *M. haemolytica* vaccines. In field conditions, the use of a live vaccine is not recommended when antibiotic therapy is used and is also limited in animals exposed to stress factors (Rice et al. 2008).

The virulence factors of *M. haemolytica*, including leukotoxin (Lkt) and outer membrane proteins (OMPs), are the most important vaccine components used in immunoprophylaxis of BRDC. Many studies have shown a close relationship between high titres of serum antibodies against Lkt and OMPs and resistance to *M. haemolytica* infection (Confer et al. 1997; Ayalew et al. 2010). A key role in the immune response to *M. haemolytica* antigens is ascribed to IgM, IgG and IgA synthesized locally in the respiratory system. Antibodies against *M. haemolytica* have been found in the colostrum of cows and in the sera of newborn calves 48 h after birth. However, immunization of calves with antigens of these bacteria is believed to provide much more effective protection than colostral antibodies. For BRDC prophylaxis in calves < 2 months of age, it is possible to exploit passive immunity obtained through the colostrum, in which antibodies are induced by administering the vaccine to cows twice, six and three weeks before parturition. The antibodies received by the calves in the first hours after birth provide effective protection against infection for up to 6 weeks. High levels of colostral antibodies may provide passive immunity in calves at the age of 3–4 months (Chamorro et al. 2014). A disadvantage of this is that the high titres of agglutinating antibodies obtained via colostrum may interfere with active immunization with *M. haemolytica* antigens (Woolums 2014). Immunization of cows with a commercial *M. haemolytica* vaccine has been found to significantly increase serum antibody titres in calves from 2 to 7 days of age (Hodgins and Shewen 2000). Additional vaccination of cows against Infectious Bovine Rhinotracheitis (IBR), BVD, BRSV and PI-3 between month 2 and 1 before calving increases antibody production to titres ensuring immunity in the first few weeks of life (Woolums 2014). The solutions proposed for immunoprophylaxis of BRDC in calves in terms of vaccine construction and methods and dates of vaccination, are not satisfactory for veterinarians or cattle breeders. This is due to factors associated with the organization of cattle production, complex aetiology, and inadequate understanding of immune mechanisms against infection and disease (Taylor et al. 2010).

Given the broad scope of the health problem of BRDC, the aim of the study was to assess the efficacy of colostral transfer of resistance to BRDC in calves and the suppressive effect of passive antibodies against *M. haemolytica* on the effectiveness of immunity, as well as to carry out a comparative analysis of selected acute phase proteins to assess the health status of calves.

## Materials and Methods

The research was carried out on a randomly selected group of 30 Holstein-Friesian (HF) and 30 Simmental dairy cows at the age of 3–4 years and their calves. In total, 60 cows were used in the experiment. Thirty cows were immunized with experimental vaccines with Lkt or OMPs, with a commercial adjuvant (Montanide, IMS, Germany), at 6 and 4 weeks before parturition (Mikucki et al. 2006).

Calves from cows vaccinated and unvaccinated with *M. haemolytica* antigens were immunized with identical formulations containing Lkt or OMPs on days 10 and 24 in the feedlot, by subcutaneous injections in the neck fold. The control consisted of unimmunized calves from vaccinated and unvaccinated cows (n = 10 each). The immunization scheme was carried out according to Wernicki et al. (2002). A 2-ml dose of vaccine contained 1 ml of OMPs (2 mg/ml) or 1 ml of Lkt at 10 μg/ml in 1 ml of adjuvant. The experimental design is presented in Table 1.

Table 1. Experimental design.

| Group of calves (n = 10) | Vaccinated cows (n = 30) | | Unvaccinated cows (n = 30) | |
|---|---|---|---|---|
| | HF | S | HF | S |
| Control 1 | 5 | 5 | - | - |
| Control 2 | - | - | 5 | 5 |
| Group 1 | 5 | 5 | - | - |
| Group 2 | - | - | 5 | 5 |
| Group 3 | 5 | 5 | - | - |
| Group 4 | - | - | 5 | 5 |

HF - Holstein-Friesian; S - Simmental
Control 1 (5 Holstein-Friesian and 5 Simmental) - unvaccinated calves born of unvaccinated cows; Control 2 (5 Holstein-Friesian and 5 Simmental) - unvaccinated calves born of vaccinated cows; Group 1 - calves born of vaccinated cows, immunized with vaccine containing Lkt; Group 2 - calves born of unvaccinated cows, immunized with vaccine containing Lkt; Group 3 - calves born of unvaccinated cows, immunized with vaccine containing OMPs; Group 4 - calves born of vaccinated cows, immunized with vaccine containing OMPs.

Blood for sera was collected from all cows before calving, and colostrum was collected immediately after calving. Blood serum was collected from all calves before colostrum intake, at 48 h of life, and then at 3, 7, 14, 21, 35 and 42 days of feedlot. New-born calves received 2 l of colostrum in the first 2 h after birth, and the total amount of colostrum in the first 12 h of life was 10% of the calf's weight. In the following days, the calves received a milk replacer *ad libitum* and were fed solid feed from 85 kg body weight. Calves weighing about 200 kg were placed in the feedlot in conditions complying with EU Council Directive 2008/119/EC (2008). The research was approved by the 2nd Local Ethics Committee in Lublin (approval no. 57/2011).

Calf sera obtained at 48 h from birth were tested for the presence of serum antibodies against BRSV, BVD, PI-3 and BHV-1 using a commercial ELISA kit (Cypress Diagnostics, Belgium) according to the manufacturer's instructions. Similar tests were carried out in sera obtained from cows. The total Ig level in the colostrum was determined with a colostrometer (ProAnimali, Wrocław, PL), and the concentration of IgG and IgM was determined using a commercial ELISA kit (Cusabio, China) according to the manufacturer's instructions. The protein content in the calf sera was determined by the Bradford method (Sigma, Germany). Haptoglobin (Hp) and serum amyloid A (SAA) concentrations in the sera were measured using EIA assays (Tridelta, Ireland) according to the manufacturer's instructions. The results are presented in g/l for Hp and mg/l for SAA.

From calves that died (n = 2) in the first weeks of feedlot with respiratory symptoms, trachea and lung samples were collected for bacteriological analysis. Material from the remaining calves (n = 48) was collected in the form of swabs from the nasal cavity. Cultures were run on a standard agar medium with a 5% addition of sheep blood and on selective differential media (Sigma, PL) MacConkey agar (Sigma, PL); m-FC Agar for *Klebsiella* and Columbia blood agar containing 5% bovine blood plus antibiotics (CBAA) for *Pasteurellaceae* (Jaworski et al. 1993). The samples were also tested for the presence of *Mycoplasma* according to Byrne et al. (2001).

ELISA test for detection of antibodies specific for the *M. haemolytica* antigens was performed according to Bowersock et al. (1994). Flat-bottom plates (Nunc-Immuno MaxiSorp plates) coated with Lkt at 50 µg/ml or OMPs at 1 mg/ml were used as a solid phase. In the first antibody 100 µl each of 1:100 dilutions of the sera in phosphate-buffered saline buffer was placed in the wells. After overnight incubation at 4 °C the plates were centrifuged at 200 × *g* for 10 min at 4 °C and washed 3 ×, after which anti-bovine IgG rabbit horseradish peroxidase-conjugated antibodies (Bio-Com, PL) were added to each microplate well. After 3 h of incubation, the plates were washed and each well was filled with 100 µl of chromogen (ABTS) (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid diammonium salt, BioRad, PL). Then the plates were incubated for 20 min at room temperature. Absorbance was read using a 650 nm filter.

### Statistical analysis

The results were analysed statistically using Statistica 10.0 software. One-way analysis of variance (ANOVA) to compare differences between the breeds of calves and groups was performed using Kruskal-Wallis test.The *post hoc* effect between the groups was also determined using Dunnett test. Correlation analysis was performed using the Pearson correlation coefficient. Significance of differences was defined at $P \leq 0.05$.

## Results

Bacteria of the species *M. haemolytica*, *Pasteurella multocida*, *Trueperella pyogenes* and *H. somni* were isolated from calves with clinical signs of respiratory disease (a total

of 13 calves – eight from the control groups and five vaccinated calves from unvaccinated cows) and from calves that died with symptoms of BRDC (two calves from the control groups). We did not obtain any *Mycoplasma* isolates.

The mean density of colostrum ranged from 1.06 to 1.07 which indicates an Ig level from 80 to 118 g/l and thus high-quality colostrum. No significant differences were found in the density of colostrum obtained from HF and Simmental cows (Table 2).

Table 2. Average colostrum quality in cows used in the experiment.

| Group of cows | Colostrum density | | |
|---|---|---|---|
| | Holstein-Friesian | Simmental | *P* |
| Vaccinated | 1.07 ± 0.01 | 1.07 ± 0.01 | 0.4 |
| Unvaccinated | 1.06 ± 0.02 | 1.07 ± 0.01 | |

Analysis of the total protein concentration in the calf sera showed significant differences ($P \leq 0.05$) after birth and at 48 h in HF and Simmental calves. There were also significant differences between the sera of HF and Simmental calves at 48 h (Plate I, Fig. 1). A significant correlation was found between serum protein levels before colostrum intake and at 48 h (r = 0.52; Plate I, Fig. 2). The serum IgG concentration in calves showed a significant ($P \leq 0.05$) increase at 48 h of age, up to an average of 12.4 g/l, relative to values obtained before colostrum intake. It is worth noting the significant differences ($P \leq 0.05$) in the IgG concentration in individual calves, which ranged from 7.5 g/l to 22.7 g/l (Fig. 3). The IgM values were 0.89 g/l before colostrum intake and 2.2 g/l at 48 h; these differences were significant ($P \leq 0.05$) in the case of calves obtained from vaccinated cows. In the case of IgG, significant differences ($P \leq 0.05$) were also noted between the groups of calves from vaccinated vs unvaccinated cows (Fig. 3). The level of Hp and SAA in the calf sera obtained before and after colostrum intake showed no significant differences ($P \leq 0.05$) between any experimental groups of calves or in comparison to the control group. The values for these indices in the sera of calves of different breeds of cattle were not significant either ($P = 0.3$ for SAA and $P = 0.46$ for Hp) (Table 3).

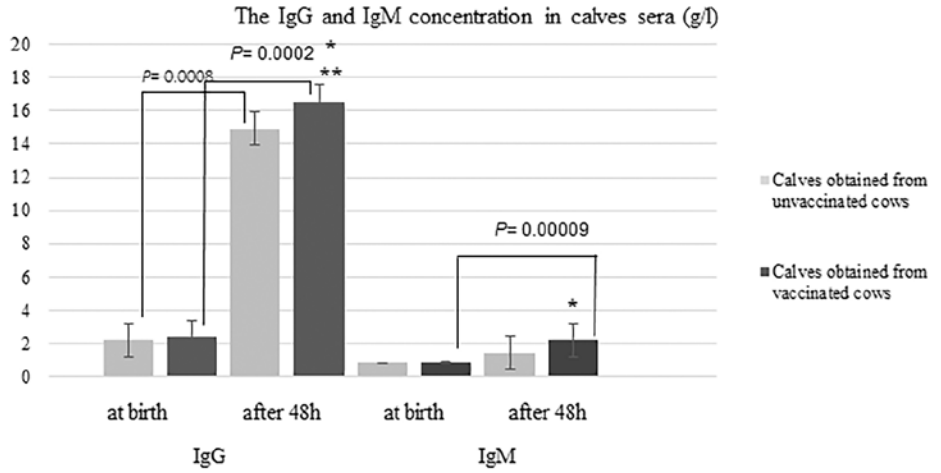


Fig. 3. Average concentration of IgG and IgM in calf sera immediately after birth and at 48 h of age

*Significant differences ($P \leq 0.05$) in comparison to calves at birth; **Significant differences ($P \leq 0.05$) in comparison to calves from unvaccinated cows

Table 3. Mean concentration of haptoglobin and serum amyloid A in calf sera.

| Group of calves | Haptoglobin mg/l | | | | | SAA mg/l | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Calf sera before colostrum intake | | Calf sera 48 h after colostrum intake | | | Calf sera before colostrum intake | | Calf sera 48 h after colostrum intake | | |
| | HF | S | HF | S | *P* value | HF | S | HF | S | *P* value |
| Control 1 | 0.56 ± 0.32 | 0.52 ± 0.40 | 0.94 ± 0.50 | 0.87 ± 0.50 | 0.20 | 89.03 ± 40.90 | 95.30 ± 33.40 | 90.20 ± 33.20 | 101.15 ± 29.30 | 0.32 |
| Control 2 | 0.56 ± 0.19 | 0.60 ± 0.20 | 0.90 ± 0.40 | 0.80 ± 0.30 | 0.19 | 76.90 ± 33.30 | 95.30 ± 33.40 | 90.20 ± 33.20 | 101.15 ± 29.30 | 0.26 |
| Group 1 | 0.59 ± 0.19 | 0.56 ± 0.53 | 0.90 ± 0.50 | 0.99 ± 0.49 | 0.27 | 74.60 ± 39.00 | 100.40 ± 41.20 | 84.50 ± 35.10 | 86.90 ± 31.40 | 0.46 |
| Group 2 | 0.76 ± 0.20 | 0.50 ± 0.53 | 0.99 ± 0.49 | 1.06 ± 0.46 | 0.40 | 77.30 ± 44.00 | 102.60 ± 36.40 | 82.40 ± 33.20 | 89.60 ± 29.40 | 0.32 |
| Group 3 | 0.62 ± 0.20 | 0.50 ± 0.35 | 0.89 ± 0.49 | 0.90 ± 0.40 | 0.10 | 76.10 ± 36.50 | 99.40 ± 39.00 | 85.50 ± 34.00 | 89.20 ± 35.00 | 0.29 |
| Group 4 | 0.59 ± 0.30 | 0.50 ± 0.35 | 0.80 ± 0.30 | 0.89 ± 0.35 | 0.29 | 75.00 ± 35.00 | 100.40 ± 30.00 | 86.00 ± 35.00 | 90.20 ± 33.00 | 0.30 |

HF - Holstein Friesian, S - Simmental
Control 1 (n = 10; five Holstein Friesian and five Simmental) – unvaccinated calves; Control 2 (n = 10) – unvaccinated calves born of vaccinated cows,; Group 1 (n = 10) - calves born of unvaccinated cows, immunized with vaccine containing Lkt; Group 2 (n = 10) - calves born of vaccinated cows, immunized with vaccine containing Lkt; Group 3 (n = 10) - calves born of unvaccinated cows, immunized with vaccine containing OMPs; Group 4 (n = 10) - calves born of vaccinated cows, immunized with vaccine containing OMPs.

Determination of the concentrations of antibodies against IBR-BHV1, BVDV, PI-3, adenovirus 3 and *M. haemolytica* confirmed the presence of various levels of antibodies against respiratory aetiological factors in colostrum and sera obtained from cows and calves at 48 h. The highest concentrations were observed for antibodies against adenovirus 3 (ADENO 3) and PI-3 (>150 mg/ml). The lowest antibody levels were observed for IBR-BHV1, BVD and *M. haemolytica* (< 50 mg/ml) (Plate II, Fig. 4).

Analysis of the concentration of antibodies against the tested antigens in the sera of calves from vaccinated and unvaccinated cows at 48 h showed significant differences ($P \leq 0.05$) only for *M. haemolytica* antibodies. Significantly lower antibody values were observed in the group of calves from unvaccinated cows. In other cases, despite slight differences in absolute values, the level of antibodies was not significant (Fig. 4). In the case of vaccinated and unvaccinated cows, the differences in the concentration of antibodies against the aetiological factors were also non-significant.

Analysis of the concentration of anti-Lkt and anti-OMP antibodies in immunized calves showed a significant ($P \leq 0.05$) increase in the group of calves from unvaccinated cows at 21 and 35 d in the feedlot. In the case of calves from vaccinated cows, a significant level of humoral immunity for Lkt was obtained at 14 d in the feedlot, and it remained at a significant level up to 35 d. In calves immunized with vaccines containing OMPs (Group 3), a significant ($P \leq 0.05$) increase in Ig concentration was obtained as early as the third day in the feedlot and persisted until day 35 (Plate II, Fig. 5). An increase in antibody concentration was obtained in the control group as well, which was due to resistance induced in the calves by the presence of *M. haemolytica* in their environment.

## Discussion

The present study confirmed the presence of the bacteria *Pasteurellaceae* in all experimental groups of calves, but not that of *Mycoplasma* spp. The most commonly isolated bacteria were *M. haemolytica*, which confirms the common occurrence in the upper respiratory tract in cattle.

Colostral antibodies obtained from cows ensure adequate passive immunity in calves in their first weeks of life, resulting in very low morbidity in the initial rearing period. In the present study, the level of colostral IgG antibodies observed in calves at 48 h of age confirms the high value of the colostrum and adequate transfer of passive immunity. The high correlation ($r = 0.5$) between the total protein content in the sera of calves before colostrum intake and after 48 h confirms proper absorption of Ig in the colostrum. A total Ig concentration below 10 g/l in calf sera at 48 h of life indicates low transfer of immunity, which is reflected in their health and productivity (Poulsen et al. 2010). To ensure effective passive immunity that reduces BRDC incidence, the antibody level in calf sera on the second day of life should be 10 to 15 g/l. The high average IgG value in calf sera obtained in our study at 48 h of life is the result of appropriate application of a high quality colostrum, in which the average IgG level was > 80 g/l. In a study by Pritchett et al. (1991), the average IgG1 concentration in the colostrum of H-F cows was 48.2 g/l and ranged from 20 to over 100 g/l in individual cows. Proper transfer of immunoglobulins can significantly improve the immune function, as well as daily weight gains (Soberon and Van Amburgh 2013). In the case of dairy cattle, vaccination with commercial viral preparations has no significant effect on the incidence of BRDC or mortality in calves in the first five weeks of life (Windeyer et al. 2012). The situation is quite different in beef breeds. A high level of colostral antibodies on the day of birth and high sera IgG values persisting even up to four months of age translate into increased immunity to respiratory disease (Prado et al. 2006). Owing to high Ig levels in calves, guaranteeing protection against disease for up to three months and the ability to produce antibodies between 60 and 90 days of life following contact with a pathogen, active immunization can begin at four months, i.e. about 3–4 weeks before weaning (Otomaru et al. 2013). Fulton et al. (2004) showed that colostral antibodies in calves begin to disappear as early as the first week of life, but the age at which they become undetectable depends on individual and environmental factors, including the quantity of maternal antibodies absorbed by the new-born. In research by Munoz-Zanzi et al. (2002), the period during which antibodies specific for BVDV 1 persisted in calves ranged from 139 to 143 days of age, while antibodies for serotype 2 were undetectable on days 110 and 118. In the study of Chamorro et al. (2014) the BVDV-1 and BVDV-1 virus-antibody levels was observed in calves aged 5 months of age.

In the present study, the average concentration of anti-BVDV antibodies in calves coming from vaccinated cows at 48 h of life was comparable in comparison to calves born of unvaccinated cows. Similar results were observed for anti BHV-1 (IBR) antibodies, whose mean concentration ranged from > 35 to 40 mg/ml in the group of calves from vaccinated and unvaccinated cows, respectively. In both cases, the level of antibodies for tested antigens in the groups of calves from vaccinated and unvaccinated cows was lowest for antibodies specific for viral antigens.

The duration of passively acquired immunity in calves that receive maternal colostrum at birth is highly variable. A high level of maternal antibodies against respiratory viruses including BVDV, BRSV and BHV-1 was observed in beef calves aged 6 months (Kirkpatrick et al. 2008). However, in another study (Kirkpatrick et al. 2001) the mean time of antibodies presence in calf sera was about 3 months for BVDV-1 antigens and 2 months for BRSV virus.

In the case of *M. haemolytica*-specific antibodies, significant differences were found in the transfer of colostral immunity in calves from vaccinated and unvaccinated cows. The low level of Ig specific for *M. haemolytica* is a problem and may be a factor increasing morbidity in calves in the first weeks of feedlot. This could also be due to the prevalence of these bacteria in cattle herds as an environmental factor reducing the immune response in calves.

It seems very interesting that the highest average concentrations of Ig in the groups of calves from vaccinated and unvaccinated cows were for antibodies against the PI-3 virus and adenoviruses, which confirms the high level of passive immunity to these viral agents. The results indicate a high immunostimulatory effect of these viruses in cows, guaranteeing a high transfer of colostral immunity in calves.

Prado et al. (2006) have reported that vaccination of cows in the final stage of pregnancy with whole-cell or subunit vaccines with OMPs or Lkt resulted in a significantly shorter period of passive immunity in feedlot calves, from 37 to 116 days. The resistance period was significantly shorter than in the case of immunity in calves acquired as a result of vaccination of cows with viral antigens, which lasted up to 3.5 months. Given the short-term nature of passive immunity to *M. haemolytica*, in designing a prophylactic programme it should be remembered that most antibodies are produced in calves at 60 to 90 days of age (Conlon et al. 1995). The low level and short duration of antibodies against *M. haemolytica* observed in the present study may be an indication for early immunization of calves, although this is not always possible due to stress associated with transport and adaptation. However, given the production technology currently adopted by many farms, involving the purchase of animals from diversified breeding environments, there is a need to introduce appropriate logistic solutions in terms of control of the purchase of feedlot calves. Cattle farmers should make also an effort to obtain animals for breeding mainly from their own stock.

Analysis of the induction of a post-vaccination immune response in calves placed in the feedlot confirmed an increase in the production of antibodies against *M. haemolytica* Lkt and OMPs in cattle. A significant ($P \leq 0.05$) increase in anti-Lkt Ig was observed in calves born of vaccinated cows from 21 d in the feedlot, i.e. 18 days after the first dose of vaccine. High values of anti-Lkt antibodies, persisting until the end of the experiment, were also demonstrated in the group of calves from unvaccinated cows. The absence of significant differences in the concentration of anti-Lkt antibodies between the two groups of calves indicates that colostral immunity has a minor stimulating effect on post-vaccination immunity. The equally high, significant ($P \leq 0.05$) values for anti-OMP antibodies observed in the two groups of calves (3 and 4) indicate that colostrum antibodies did not significantly stimulate anti-OMP immunity. It should be noted that in the control group which comprised non-immunized calves, the presence of antibodies and changes in the immune response were observed as well, which could be due to the induction of immunity by the presence of *M. haemolytica* in the environment. Analysis of the dynamics of the post-vaccination immune response on particular days in the feedlot revealed that a small level of passive immunity in calves at more than two months of age does not significantly affect the induction of a humoral immune response resulting from immunization with *M. haemolytica*. The consequence of this is a significant increase in Ig guaranteeing protection against disease (Otomaru et al. 2013). Moreover, immunization of calves from two months of age with live modified vaccines stimulates the production of antibodies at a level that ensures protection. The effectiveness of passive and post-vaccination resistance may also be influenced by breed determinants (Gaspers 2015), which explains the differences observed in the present study in the time antibodies remained in the sera of the HF and Simmental calves.

In the present study, small and non-significant differences in Hp and SAA levels were observed in calves before colostrum intake and on the second day of life. The values

of these proteins in calves immediately after birth may indicate an internal response to stress factors. In a study by Schroedl et al. (2003), the Hp level on the day of birth was comparable with the values at 10 days of age. Seppa-Lassila et al. (2013) found a significant increase in Hp in foetuses before birth and in new-born calves, as well as a gradual decline during the first three weeks of life to the values found in adult cattle. That study showed that serum Hp levels are nearly undetectable in healthy mature individuals, whereas concentrations up to 200 ng/ml are acceptable for healthy animals. Acute phase proteins have been recognized as indicators of disease and as potential indicators of BRDC in feedlot calves (Orro et al. 2011). The lack of significant differences in our study suggests that detection of Hp and SAA in calves in the first days of life cannot be the basis for assessing the immune status of calves.

To sum up, the level of colostral immunity in calves in the first days of life influences post-vaccination immunity induced later. However, the duration of the passive immunity could be dependent on the type of bacterial and viral antigens used to immunize pregnant cows. The results of our research and cited literature indicate that post-vaccination immunity does not always guarantee protection against disease, especially against bacterial antigens. An important problem is the lowest level antibodies against *M. haemolytica* antigens in calves from vaccinated and unvaccinated cows, as well as IBR and BHV, which may be due to stress environmental factors like adaptation, social stress. A positive effect of the immunization of calves was that the presence of colostrum antibodies was shown to have no significant effect on the post-vaccination immune response in calves.

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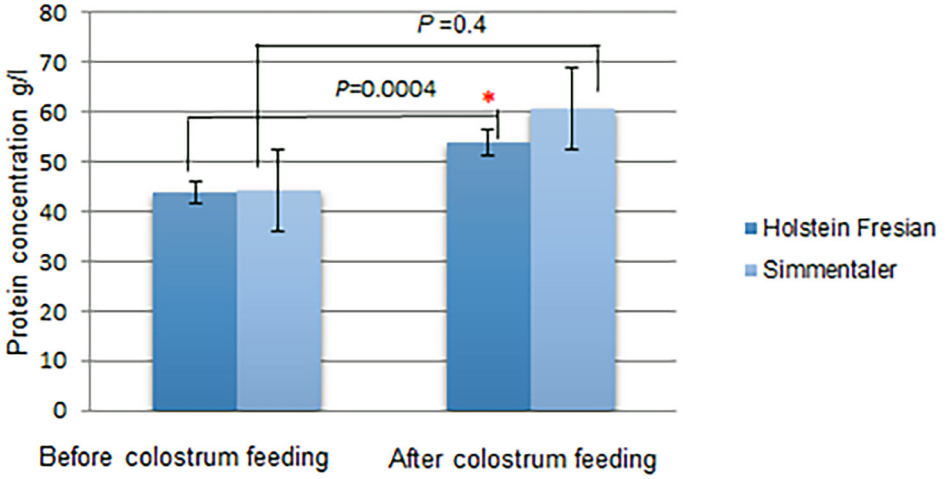


Fig. 1. Total protein concentration in the sera of Holstein Fresian and Simmental calves prior to colostrum intake and at 48 h of age

*Significant differences ($P \leq 0.05$) in comparison to the level observed directly after birth before colostrum feeding

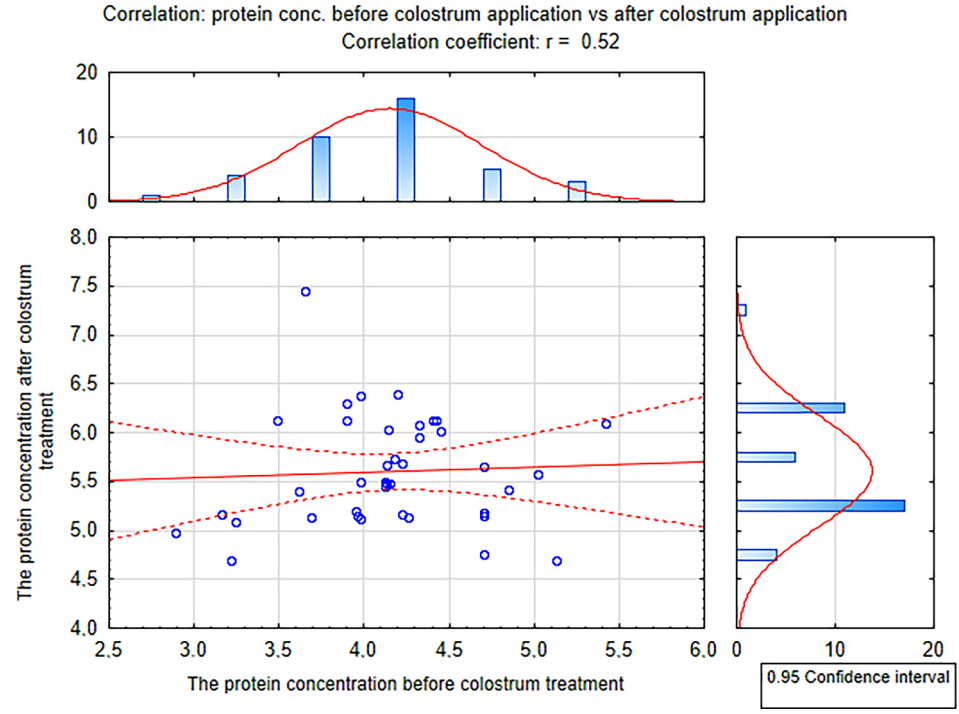


Fig. 2. Correlations between total protein concentration in calf sera before colostrum intake and at 48 h of age

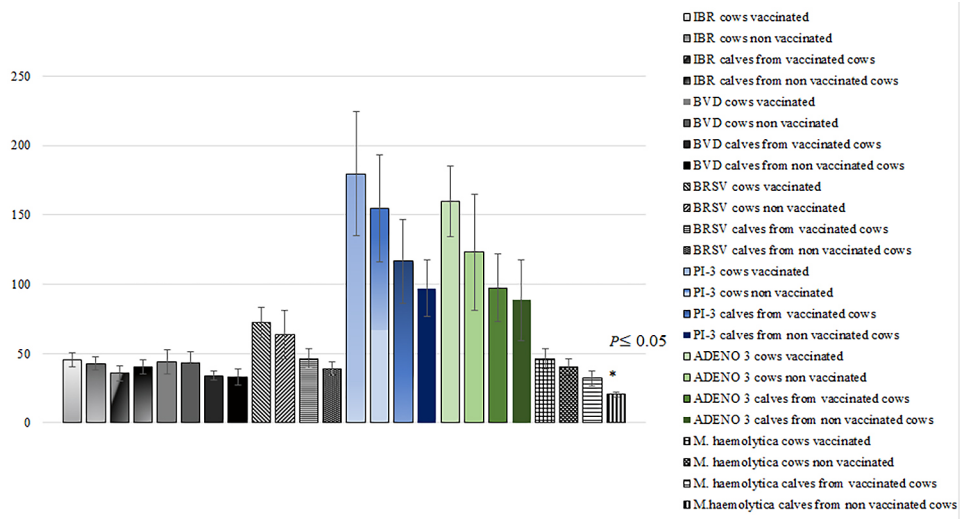


Fig. 4. Concentrations of antibodies against aetiological agents of BRDC in cows and calves 48 h after birth

*Significant differences ($P \leq 0.05$) in comparison to calves coming from unvaccinated cows
ADENO3 - adenovirus 3; IBR- infectious bovine rhinotracheitis virus; PI-3- parainfluenza virus, BVD- bovine viral diarrhoea virus, BRSV- bovine respiratory syncytial virus

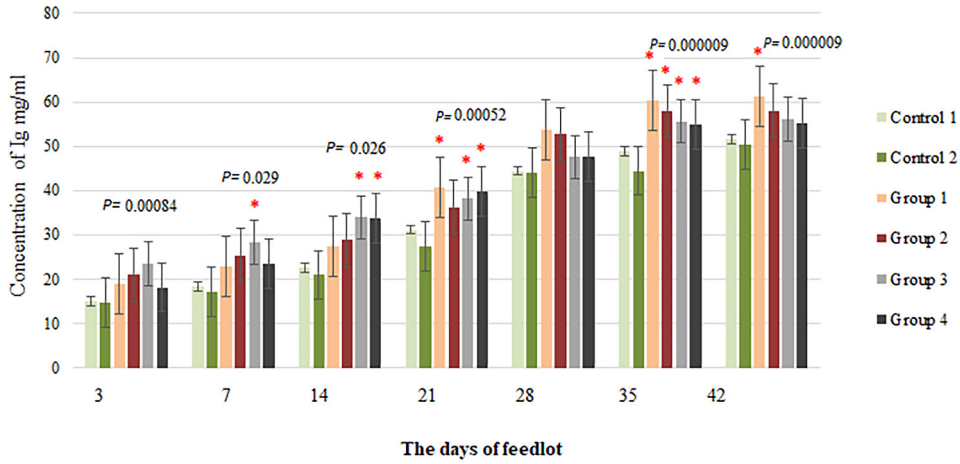


Fig. 5. Concentration of antibodies against *M. haemolytica* leukotoxin (Lkt) and outer membrane proteins (OMP) antigens in calf sera

Control 1 - unvaccinated calves from vaccinated cows; Control 2 - unvaccinated calves from unvaccinated cows; Group 1 - calves born of vaccinated cows, immunized with vaccine containing Lkt; Group 2 - calves born of unvaccinated cows, immunized with vaccine containing Lkt; Group 3 - calves born of unvaccinated cows, immunized with vaccine containing OMPs; Group 4 - calves born of vaccinated cows, immunized with vaccine containing OMPs

*Significant differences ($P \leq 0.05$) relative to the control group