Comparison of immunoglobulin G concentrations in colostrum and newborn calf serum from animals of different breeds, parity and gender

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SUMMARY

Bovine neonatal enteritis is a major cause of losses in cattle production, involving microbial and extra-microbial causative factors. Failure of passive transfer (FPT) plays a critical role in the development of this disease, and colostrum management and quality have a direct impact on FPT. The aim of this study was to investigate the effects of breed, parity and calf gender on colostrum IgG content and newborn calf immunity. Understanding the influence of these factors could be useful in implementing effective strategies to improve calf health. Breed emerged as a major factor affecting colostrum quality and calf immunity. IgG levels were compared in colostrum and newborn calf serum across dairy (Italian Friesian, Reggiana and Bianca Modenese) and cow-calf type (Piemontese and Limousine) breeds. Italian Friesian cattle showed significantly lower IgG levels in both their colostrum and newborn calf serum compared to the other breeds. Parity did not significantly affect overall colostrum quality or FPT prevalence. However, first-calf heifers had a lower prevalence of inadequate colostrum compared to multiparous cows, suggesting their colostrum is suitable for colostrum banks. With regard to gender, the analysis of IgG levels and FPT prevalence in Italian Friesian newborn calves revealed a significant sex disparity. Females exhibited higher IgG levels and lower FPT prevalence compared to males, suggesting potential management practices influencing these outcomes. In conclusion, results revealed significant differences in IgG concentration between colostrum and newborn calf serum samples across breed, parity, and calf gender. Newborn calves are particularly vulnerable and prone to enteritis. Poor colostrum management and breeding practices that prioritize high milk production can lead to lower IgG levels, further compromising calf immunity. While colostrum banks and cow vaccination offer potential solutions, limitations exist. Moreover, effective control requires close cooperation between farmers and veterinarians, which is often lacking.

KEY WORDS

Bovine; colostrum; failure of passive transfer.

INTRODUCTION

Colostrum, the first secretion from the mammary gland after birth, is an important source of immunity and nutrition for the newborn. The neonatal period in cattle - from birth to 28 days of age - is particularly important from a health point of view, as approximately 75% of calf mortality occurs during this period. In particular, the first week of life is considered the most critical period, with more than 50% of losses due to enteric disease, mainly caused by viral, bacterial and parasitic pathogens, alone or in association (1). The main factor influencing calf health and future production is ensuring adequate intake of high quality colostrum as soon as possible after birth. Colostrum quality varies between animals depending on breed, parity, season and pre-partum diet (2). Failure of the neonatal calf to absorb sufficient colostral immunoglobulins (IgG) within the first hours of life results in failure of passive transfer (FPT). FPT in calves depends on the quality of the dam's colostrum as well as postpartum management in terms of the timing and method of colostrum feeding to the newborn calf (3). FPT status compromises the systemic immunity of the calf as well as the local immune protection against enteric pathogens, triggering neonatal enteritis. Neonatal enteritis, when severe, has a negative impact on weight gain during the growth period, milk production and is associated with the onset of severe respiratory disease (4). Because of the role of colostrum in the onset of FPT, it's crucial to establish parameters for assessing its quality, particularly with regard to IgG concentration. The radial immunodiffusion assay (RID) is the gold standard for assessing colostrum and serum IgG (5,6). Un-

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fortunately, RID is a laboratory test that takes approximately 18 to 24 hours to determine results. Colostrometers are well suited for on-farm use because they take only a few minutes to use, even though they are temperature sensitive, fragile and have low sensitivity (7). Brix refractometry and sodium sulphite precipitation tests are accepted to determine IgG concentration in colostrum (8) and calf serum (9) samples, respectively. More recently, on-farm immunoassay kits have become available at relatively low cost and have been widely used to assess IgG concentration in colostrum and calf serum samples. The aim of this study was to investigate the effects of breed, parity and calf gender on colostrum IgG content and newborn calf immunity. Understanding the influence of these factors could be useful in implementing effective strategies to improve calf health.

MATERIALS AND METHODS

The study included animals from 28 cattle herds of different breeds located in the Po Valley (Italy). Dairy breeds, suited for Parmigiano Reggiano cheese production, were Italian Friesian (8 herds), Bianca Modenese (5 herds) and Reggiana (6 herds). Breeds belonging to the cow-calf type were Piemontese (5 herds) and Limousine (4 herds). All study herds were officially tuberculosis, brucellosis and enzootic bovine leucosis free. In addition, all calves sampled were negative to the diagnostic tests used to detect bovine viral diarrea virus (BVDV) persistently infected (PI) animals.
The study included the following actions.

Action A (variable: breed)

Comparison of IgG concentration in colostrum and newborn calf serum samples from cattle belonging to the five different breeds. Mean values of reproductive performance, milk production and quality of the herds included in the study were recorded.
A1. Italian Friesian**.** Sixty colostrum (**A1c**) and 45 newborn calf serum (**A1s**) samples were collected from 7 different herds. Calving/cow: 2.9; milk production cow/lactation: 10780 kg; milk quality - fat: 3.5%; protein: 3.35%; K-casein BB: 21.8%.
A2. Bianca Modenese (native breed). Thirty-five colostrum (**A2c**) and 25 newborn calf serum (**A2s**) samples were collected from 4 different herds. Calving/cow: 4.2; milk production/cow/lactation: 5660 kg; milk quality - fat: 3.44%; protein: 3.45; K-casein BB: 43%.
A3. Reggiana (native breed). Fifty colostrum (**A3c**) and 35 newborn calf serum (**A3s**) samples were collected from 6 different herds. Calving/cow: 4.4; milk production/cow/lactation: 6100 kg; milk quality - fat: 3.72%; protein: 3.40%; K-casein BB: 49%.
A4. Piemontese (native breed). Thirty colostrum (**A4c**) and 25 newborn calf serum (**A4s**) samples were collected from 4 different herds. Calving n./cow: 7.8; life span/cow: 11.2 years.
A5. Limousine (foreign breed, reared in Italy). Thirty colostrum (**A5c**) and 25 newborn calf serum (**A5s**) samples were collected from 3 different herds. Calving n./cow: 8.2; life span/cow: 11.8 years.

Action B (variable: parity)

The concentration of IgG in the colostrum of 30 first-calf heifers (**B1c**) was compared with that of 30 multiparous cows (**B2c**). In addition, the concentration of IgG in 30 serum samples of newborn calves from first-calf heifers (**B1s**) was compared to that in 30 serum samples of newborn calves from multiparous cows (**B2s**). All animals belonged to the Italian Friesian breed and came from 7 different herds.

Action C (variable: gender)

The concentration of IgG in 40 serum samples of newborn female calves (**C1s**) was compared to that in 40 serum samples of newborn male calves (**C2s**). All calves belonged to the Italian Friesian breed and came from 5 different herds.

Assessment of IgG concentration in colostrum

For each enrolled cow, colostrum was collected from the four udder quarters 4-6 h after calving. A 10 ml sample was collected from the pooled colostrum of the four quarters and transferred to a sterile plastic vial. Samples were stored frozen at -20 °C. IgG concentration was assessed indirectly using an optical Brix refractometer with automatic temperature compensation (Kern ORA 6HA, Balingen, Germany). Before each analysis the refractometer was cleaned and calibrated with distilled water at room temperature. The threshold for adequate IgG concentration in colostrum was set at 21 °Bx, corresponding approximately to 50 mg/ml IgG (10) (Figure 1).

Assessment of IgG concentration in newborn calf serum

Blood samples were collected from calves 3-10 days after calving using a BD Vacutainer™ serum tube with clot activator. After clotting, each sample was gently centrifuged for five minutes to obtain serum. Solutions of 14, 16 and 18% sodium sulphite in sterile deionised water were prepared. Sodium sulphite solution induces IgG precipitation resulting in optically detectable turbidity of the solution. For each serum sample, 13 x 100 mm borosilicate test tubes containing 1.9 ml of each solution were prepared. Serum (0.1 ml) was

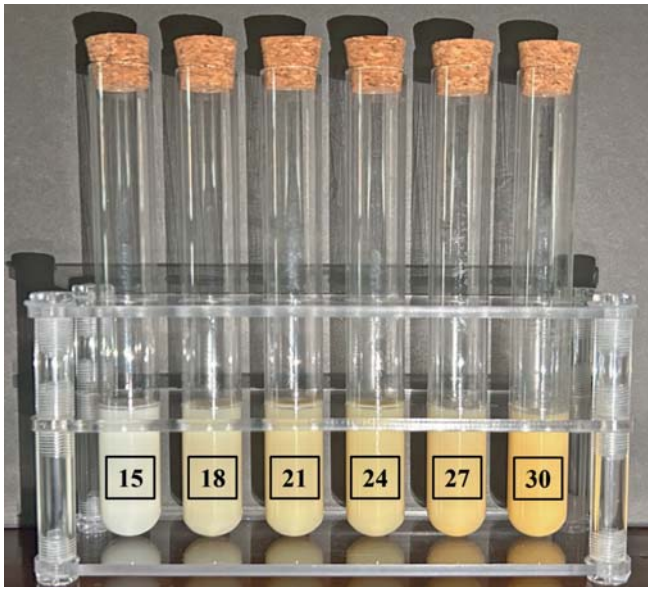

Figure 1 - IgG concentration in colostrum samples expressed in Brix degrees.

Table 1 - Distribution of IgG-deficient colostrum samples and FPT by demographic factors.

Factor	Colostrum samples with inadequate levels of IgG n./tot. (%); 95% CI[1]	Calves with FPT n./tot. (%); 95% CI[1]
Breed		
Italian Friesian	11/50 (22.0); 16.3-27.7	15/45 (33.3); 26.4-40.2
Bianca Modenese	2/35 (5.7); 1.9-9.6	1/25 (4.0); 0.2-7.8
Reggiana	3/50 (6.0); 2.7-9.3	2/35 (5.7); 1.9-9.6
Piemontese	3/30 (10.0); 4.6-15.4	4/25 (16.0); 8.8-23.2
Limousine	3/30 (10.0); 4.6-15.4	3/25 (12.0); 5.6-18.4
Parity		
First-calf	3/30 (10.0); 4.6-15.4	5/30 (16.7); 10.0-23.3
Multiparous	10/30 (33.3); 24.9-41.8	10/30 (33.3); 24.9-41.8
Gender		
Female	-	11/40 (27.5); 20.6-34.4
Male	-	23/40 (57.5); 49.8-65.2

[1] CI = Confidence Interval

added to each of the 3 tubes. The threshold for considering the IgG concentration in newborn calf serum to be adequate is the detection of turbidity in 18% sodium sulphite solution (with no turbidity in 16% and 14% solutions), corresponding approximately to a mean of 1000 mg/dl of IgG (11). Below this threshold the newborn calf was considered to have FPT status (12). The presence of turbidity in 18 and 16% sodium sulphite solutions corresponds approximately to 1400 mg/dl of IgG. Finally, the presence of turbidity in 18, 16 and 14% sodium sulphite solutions corresponds approximately to 1800 mg/dl of IgG (11) (Figure 2).

Statistical analysis

Data on IgG content in colostrum and serum were analyzed by One-Way ANOVA. Homogeneity of variances was assessed by Levene's test and multiple comparisons were performed by Tukey-Kramer test. When homogeneity was not met, Welch and Brown-Forsythe tests were used. The frequencies of colostrum with an inadequate content of IgG and of FPT were analysed by Pearson's chi-squared test. Differences at $p<0.05$ were considered statistically significant. Statistical analysis was performed by SPSS version 29 software (IBM).

RESULTS

Action A

The estimated IgG concentration showed statistically highly significant differences for the breed variable, both with regard to colostrum and calf sera. In particular, Italian Friesian cows (A1c) produced a colostrum with a lower IgG content compared to all the native breeds (A2c, A3c, A4c and A5c), whilst differences among A2c, A3c, A4c and A5c were not statistically significant (Figure 3A). Similarly, Italian Friesian calves (A1s) showed a significantly lower IgG content in blood serum compared to all the native breeds (A2s, A3s, A4s and A5s), while no significant differences were detected among A2s, A3s, A4s and A5s (Figure 3B).

Frequencies of inadequate colostrum and of FPT were com-

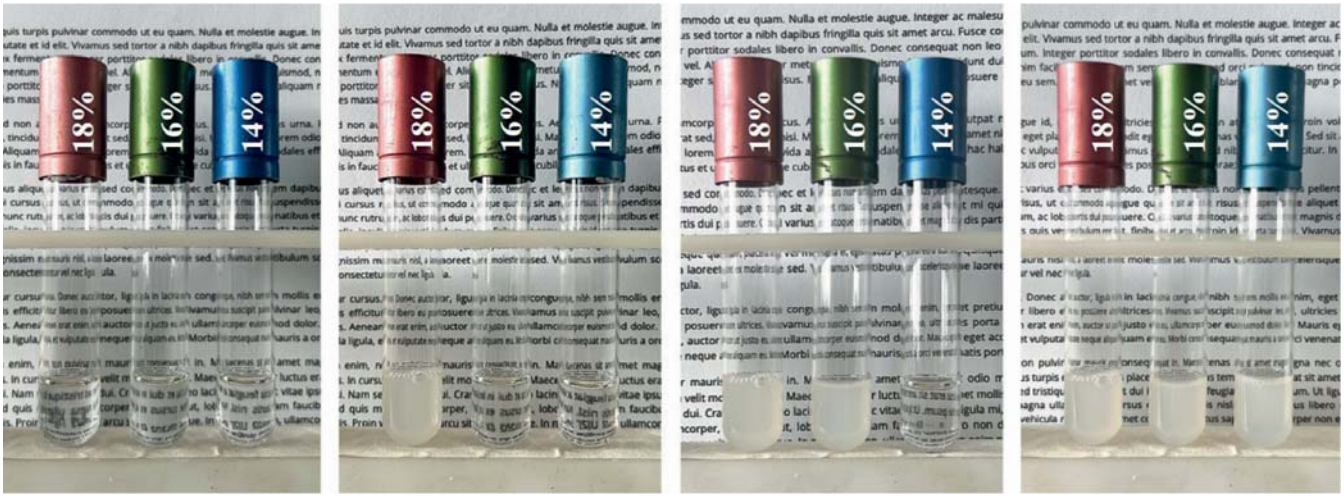

Figure 2 - IgG concentration in newborn calf serum assessed by a precipitation assay using sodium sulphite at different concentrations, with turbidity as the endpoint of measurement.

pared by breed variable (Table 1). Since we found no statistically significant difference between the native breeds (p=0.837 for indadequate colostrum prevalence and p=0.397 for FPT prevalence), the frequency data for the Bianca Modenese, Reggiana, Piemontese and Limousine breeds were merged and compared with those of the Italian Friesian breed. Italian Friesian cows showed highly significant higher values (p=0.005 for indadequate colostrum prevalence and p<0.001 for FPT prevalence) compared to native breeds.

Action B

No statistically significant differences were detected in the mean IgG content in colostrum between first-calf heifers (B1c) and multiparous cows (B2c), see Figure 4A. Likewise, no significant differences in the mean IgG content in newborn calf serum samples were detected for the parity variable (B1s and B2s), see Figure 4B.

Frequencies of inadequate colostrum and of FPT were compared by parity variable (Table 1). Prevalences of inadequate

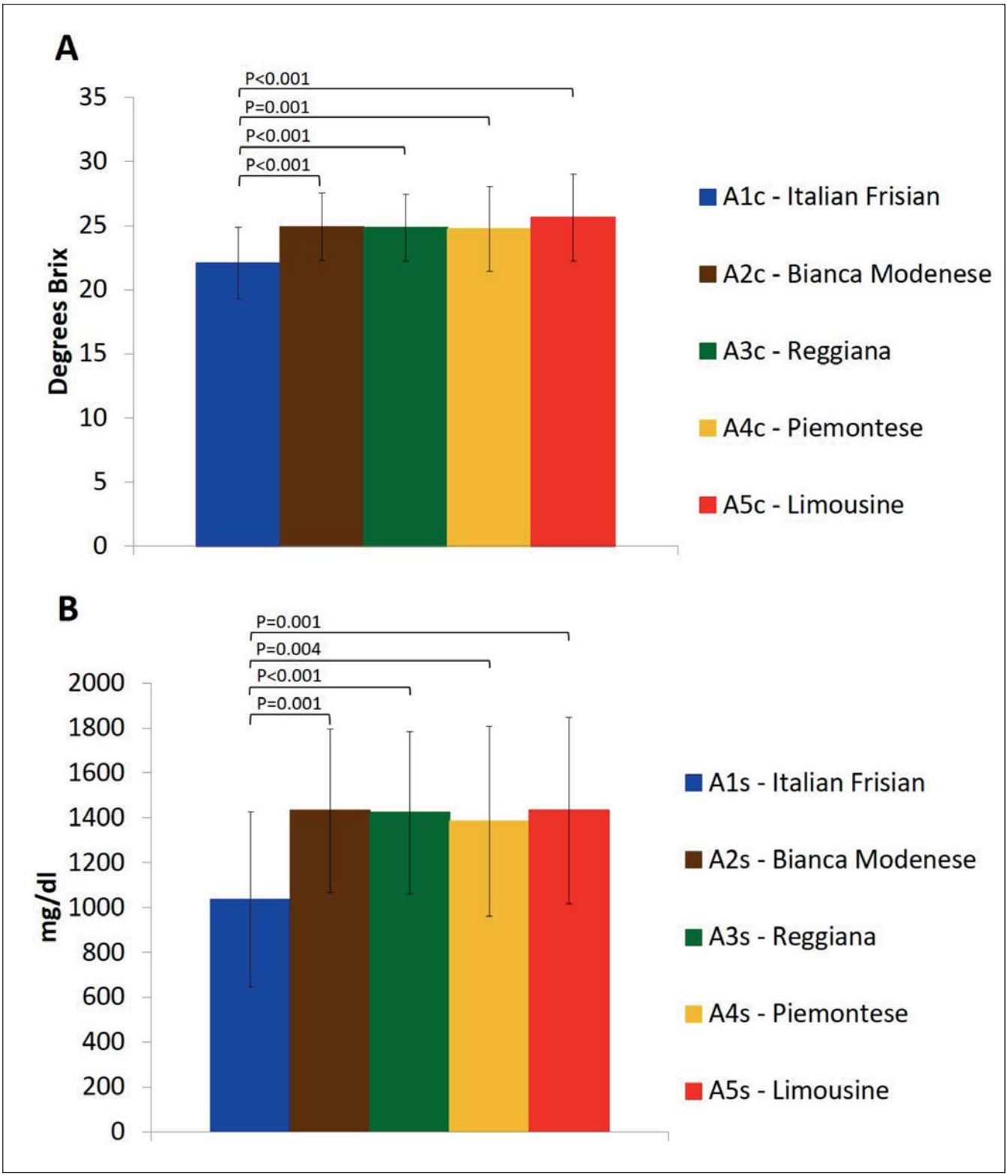

Figure 3 - A) Colostrum quality (Brix) related to breed; B) Serum IgG concentration related to breed. Statistically different means and the corresponding p values are reported above the histogram.

colostrum were significantly lower (p=0.028) for first-calf heifers (B1c) compared to multiparous cows (B2c), but no significant difference (p=0.136) was found regarding FPT prevalences in calves from these different categories (B1s and B2s).

Action C

The mean IgG content in serum samples collected from female calves (C1s) was significantly higher than in male calves (C2s), see Figure 5.

Frequencies of FPT were compared by gender variable (Table 1). The difference in FPT prevalence between female (C1s) and male (C2s) calves was statistically highly significant (p=0.007).

DISCUSSION

The comparative evaluation of the IgG concentration in colostrum and newborn calf serum samples showed a significant difference between the different breeds included in the study. Italian Friesian cows showed a higher amount of colostrum and newborn calf serum samples below the standard value of IgG to be considered adequate. It's worth discussing the comparison of the data concerning the enrolled dairy breeds, namely Italian Friesian versus Reggiana and Bianca Modenese. The results pointed out a significant difference in colostrum quality and FPT prevalence between the Italian Friesian and the other two breeds. The IgG concentration in colostrum and newborn calf serum samples collected from Italian Friesian animals was significantly lower when compared to that recorded in the other two dairy breeds. The reason for this difference could be found in the milk productive characters. In the last twenty years the Italian Friesian breed has been subjected to an effective selective pressure, also thanks to the application of genomics, with the aim of improving milk production, both in terms of quantity and quality. During this period of time, average milk production has increased from 7000 to 9500 kg/cow/lactation (13). Inevitably, today's animal has a faster and higher milk yield than in the past. This has resulted in diluted colostrum with a consistent reduction in IgG concentration per unit volume, but the amount of colostrum fed to the newborn calf is limited, about 4 L in the first 24 hours of life. The result is that around

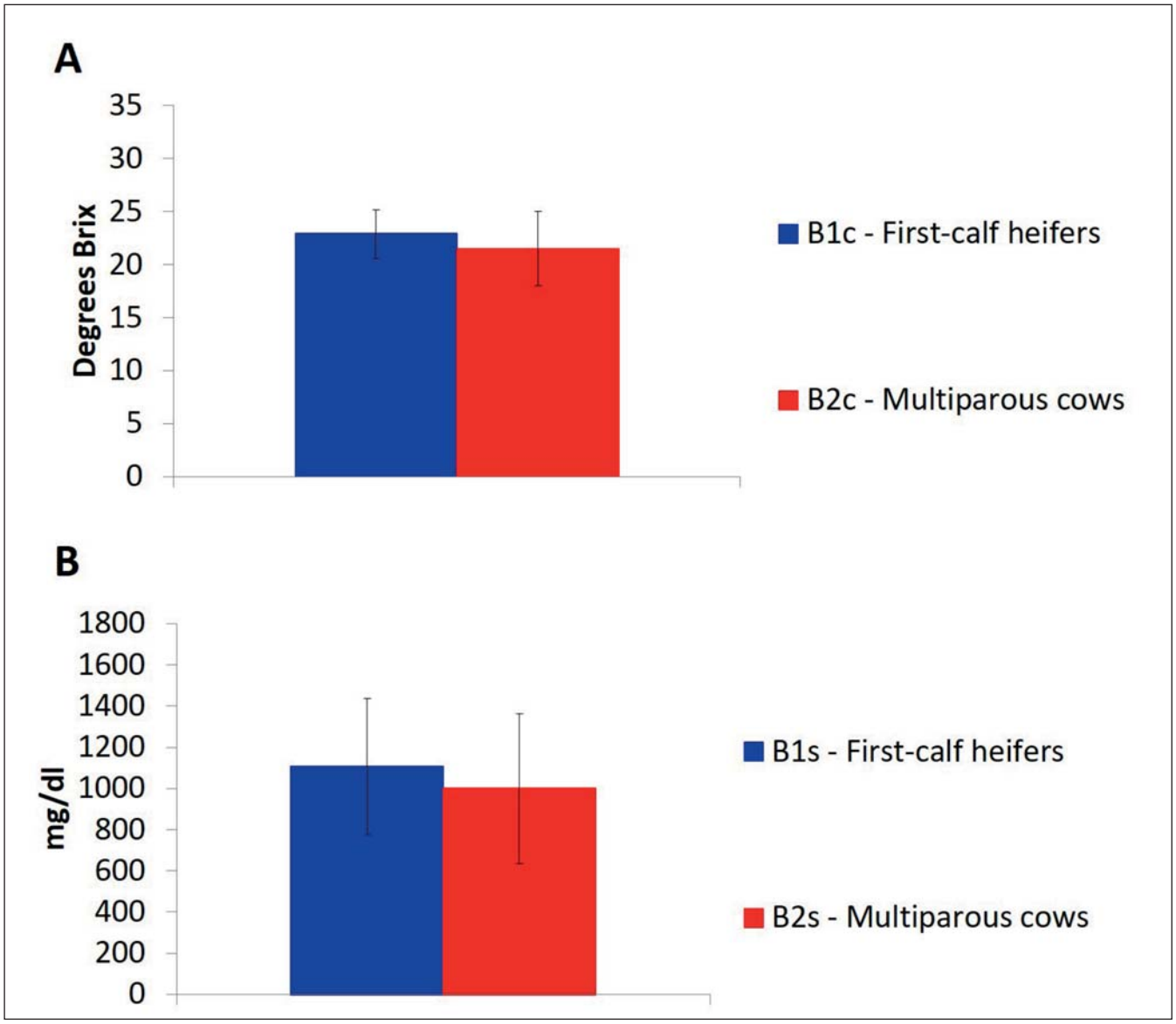

Figure 4 - A) Colostrum quality (Brix) related to parity; B) Serum IgG concentration related to parity. Differences are not statistically significant for both comparisons.

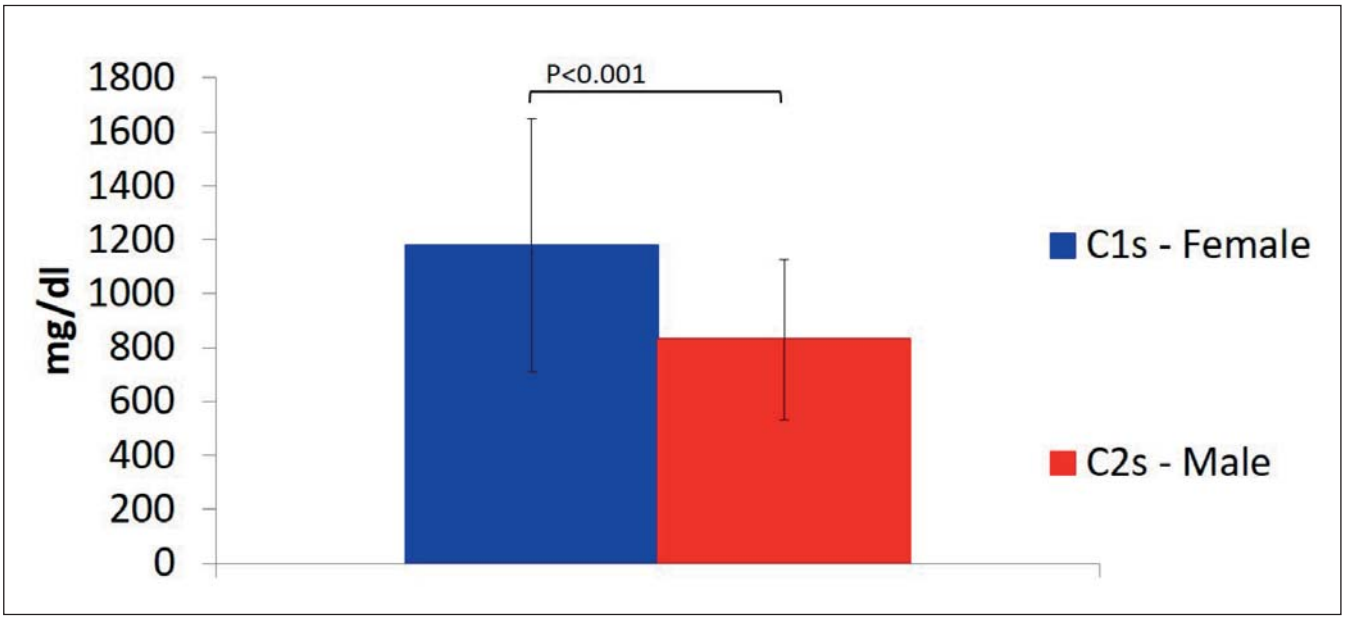

Figure 5 - Serum IgG concentration related to gender. Statistically different means and the corresponding *p* value are reported above the histogram.

30% of newborn calves are affected by FPT, a prevalence that is common in high-production dairy herds. Conversely, the native dairy breeds have maintained a lower milk production over time: an average of 6000 kg/cow/lactation. In this case, the colostrum doesn't suffer from dilution, so that IgG concentration is adequate for the standard parameters. This means less IgG deficit in newborn calf serum and therefore lower FPT prevalence. For the native dairy breeds considered, the lower production of milk compared to Friesian cows is offset by the milk quality in terms of fat and proteins, mainly K-casein BB, which is crucial for the production of Parmigiano Reggiano cheese. In addition, the native dairy breeds have a better longevity, which is a serious and widespread problem today for most of the large Friesian dairy herds, but not only. An added value that improves the longevity and, more generally, the animal welfare of the native dairy breeds under consideration is the size of the herds (30-60 milking cows per herd), which allows for a family management. As they say: «the master's eye fattens the horse», in this case the cow.

In cow-calf breeds, ingestion of colostrum directly from the nipple increases bacterial contamination, particularly coliform bacteria, which reduces the efficiency of IgG intestinal absorption (14). Firstly, physical binding of IgG by microbes within the gastrointestinal lumen blocks its uptake by enterocytes. Secondly, pathogenic bacteria can attach to and damage intestinal cells, reducing their permeability. Thirdly, when these pathogens damage intestinal cells, gut closure is accelerated (14). However, the mean concentration of IgG in colostrum and serum (Figure 3, A and B) and the prevalence of inadequate colostrum and FPT (Table 1) did not differ significantly between native dairy breeds and cow-calf breeds.

High quality maternal colostrum is still the «gold standard» for feeding newborn calves. However, when colostrum supplies are limited and quality is inadequate, colostrum replacers can be a valuable tool to boost calf immunity. These products increase the amount of IgG fed to calves, but cannot replace high-quality colostrum. It is generally accepted that any product capable of increasing serum IgG concentration above 10 mg/ml can be labelled as a colostrum replacer. However, it has been demonstrated that when colostrum replacers are added to low quality colostrum, IgG intestinal absorption is reduced compared to the calf's intake of high quality maternal colostrum (15). Colostrum replacers are usually supplemented with antibodies against *Escherichia coli*, but unfortunately *E. coli* is characterised by a wide antigenic variability. Therefore, these products may not be able to protect calves against the specific bacterial strain found on the farm. In addition, the systematic use of colostrum replacers is expensive and requires a cost-benefit assessment. An effective option is the colostrum bank, which is also included in the guidelines for the control and eradication of infectious diseases, e.g. bovine paratuberculosis.

Regarding the selection of animals for colostrum collection, it's traditionally believed that colostrum from first-calf heifers is inferior in IgG content compared to later lactating cows (5). However, our data did not show a statistically significant difference between first-calf heifers and multiparous cows with regard to the mean IgG concentration in colostrum. Even, the prevalence of samples of colostrum with inadequate IgG content was significantly higher in multiparous cows than in first-calf heifers. This result could be due, for the enrolled animals, to a more uniform IgG content in the colostrum produced by first-calf heifers compared to multiparous cows. Moreover, first-calf heifers have a less rapid and massive milk production compared to multiparous cows and it is biologically plausible that this may, if anything, result in lower colostrum dilution and higher IgG concentration. In conclusion, the observed data suggests that the animals selected for the colostrum bank should include first-calf heifers.

Finally, we found gender inequality in newborn calves. Data from Friesian cattle herds showed a significantly lower mean serum IgG level and a higher prevalence of FPT in male than in female newborn calves. Data showed also a wide variation in the prevalence of FPT in male calves between herds, ranging from 37% to 87%, although these differences were not statistically significant (p=0.319). It has been reported that the sex of the calf may influence blood levels of IgG, which

would be higher in females than in males, but it is unclear whether the sex of the calf may be more related to blood volume than to IgG uptake (16). However, an actual role of sex on IgG absorption has not always been observed (17). The gender difference found could also be due to differences in the way management is carried out. Usually, male dairy calves are collected before weaning, within the first 2 weeks, and housed in white-meat veal farms. There, calves from different herds and with different microbiota are closely housed in groups, regardless of their herd origin. The presence of animals with FPT increases the risk of occurrence of enteric and/or respiratory diseases. The current low price of male newborn calves - around 50 euros per head - partly justifies mismanagement, including incorrect and/or unequal colostrum administration between genders. Certainly, bad practices must be considered incorrect and unacceptable, as they are inconsistent with the European low (18) and recommendations (19) on calf welfare.

CONCLUSIONS

As with any industrial enterprise, the main objective of the bovine dairy industry is economic sustainability. This means producing a sufficient quantity of milk to ensure an acceptable income for the farmer. A dairy herd must be viewed as an integrated production unit. From newborn calves to milking cows, the health of the animals is crucial to achieving satisfactory economic results. The neonatal period of the calf is a period of life that requires special care due to its vulnerability. Enteritis is considered to be the main pathology affecting newborn calves. Microbial and extra-microbial causes are involved in the onset of neonatal enteritis. Among extra-microbial factors, failure of passive treatment (FPT) plays a key role in the induction of neonatal enteritis. To cause FPT, mismanagement of colostrum administration and poor colostrum quality in terms of deficit in IgG concentration are of concern. It has to be considered as a side effect of the genetic selection of dairy cattle aimed at improving milk production. Comment: facing the low milk price payed the farmer, he must produce more milk. To cope with the poor quality of colostrum, bank colostrum is suggested. However, this practice requires a specific herd organisation, which is not easy to set up. Vaccination of cows during the dry period is considered to be an effective tool in the control of microbial agents causing neonatal enteritis. However, there are problems with this practice. In the presence of FPT, even if the cow is well immunised, the newborn calf suffers from a lack of passive immunity. As a result, diarrhoea persists despite correct vaccination of the dam. This can lead to farmer frustration and a loss of confidence in vaccinating cows against enteric pathogens and, more importantly, in vaccination practice in general. Oral vaccination of newborn calves could be an alternative, but even here there are problems. Firstly, there's no vaccine on the domestic market against the microbial agents that cause neonatal enteritis. In addition, the oral vaccine must be administered before colostrum feeding. This requires constant assistance at calving, which is not realistic in the management of a large dairy herd. In conclusion, though dealing with neonatal enteritis is a tough fight, it is a duty to keep fighting. Close cooperation between farmer and veterinarian is not an option, but the right weapon. Unfortunately, it is not the norm today.

Ethical Approval

Ethical review and approval were waived for this study, because the study did not involve a prospective evaluation, did not involve laboratory animals, and only involved routine diagnostic procedures, commonly performed in bovine herds. In particular, the study fell within the cases excluded from the scope of the national law Decreto Legislativo n. 26/14 (art. 2), regarding the execution of Directive 2010/63/EU on the protection of animals used for scientific purposes.

Conflict of Interest Statement

The authors declare no conflict of interest.

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