

Measurement of transfer of colostral passive immunity in dairy calves

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The administration of high quality colostrum reduces preweaning morbidity, mortality and, therefore, economic losses related to replacement animals. It also stimulates and improves calf growth, increasing milk production and longevity of the future dairy cows. The aim of the present study was to evaluate the influence of breed and parity of the dam on colostrum quality, and of breed and gender of the calf, and time from calf birth to the administration of the first colostrum meal on the transfer of passive immunity to the calf by the field test of the Failure of Passive Transfer (FPT) on calf serum. A further objective was to improve the diagnostic accuracy of the field FPT test through a second laboratory phase improving the turbidity evaluation. The amount of IgG fed to calves (IgG concentration multiplied by the volume of colostrum administered) was influenced by dam parity as significant differences ($P < 0.05$) were detected between first- and fourth-parity cows, and between second- and fourth-parity cows. The administration of good quality colostrum (IgG > 50 mg/ml) between 5 and 9 h of life was able to reduce the risk of FPT more effectively than the administration performed within the first 4 h of life. However, further studies on larger sample size is needed to confirm the present findings. The spectrophotometric measurements confirmed the results obtained by the field turbidity test at 14% sodium sulphite dilution. It would be interesting in future to expand the dataset and validate the spectrophotometric method.

Keywords: Failure of Passive Transfer, colostrum, immunoglobulin, breed, gender

1 Introduction

The synepitheliochorial placenta of cows does not allow the transfer of immunoglobulins from the dam to the fetus during gestation, hence calves are agammaglobulinemic at birth (Wooding, 1992, Weaver et al., 2000) and have an immature immune system (Nonnecke et al., 2003). Therefore, a prompt colostrum administration helps to protect calves from diseases during the neonatal period (McGrath et al., 2016). In fact, colostrum contains a wide spectrum of important immunitary and nutritional components, and among them, immunoglobulins of the G isotype (IgG) represent more than 85% of total colostrum immunoglobulins (Ig). Usually, colostrum quality refers to the quantity of IgG present in the first milk (Godden, 2008).

The IgG concentration of colostrum has been reported to differ among parity order in Holstein (Tyler et al., 1996, Moore et al., 2005, Gulliksen et al., 2008), but not in crossbreed cows (Coleman et al., 2015, Hang et al., 2017). Breed may also have an influence on the quality of colostrum. Muller and Ellinger (1981) reported that the highest Ig concentration was detected in Jersey and the lowest in Holstein (IgG) and Guernsey cows (immunoglobulins A and M). Besser and Gay (1994) observed that less than 30% of Holsteins had concentration of colostral IgG greater than 60 mg/ml, whereas 40% of Jersey and 60% of crossbred beef cattle produced colostrum with concentrations of IgG that were greater than this threshold.

The administration of high quality colostrum reduces preweaning morbidity (Donovan et al., 1998), mortality (Robison et al., 1988) and, therefore, economic losses related to veterinary costs and replacement of animals (Dewell et al., 2006). It also stimulates and improves calves growth, allows for

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the passive transfer of the immunity and contributes to increase milk production and longevity of the future dairy cows (Godden, 2008, Atkinson et al., 2017, Turini et al., 2020).

The failure to achieve an adequate level of IgG in calves blood in the immediate neonatal period is described as Failure of Passive Transfer (FPT) and this condition can deeply impair the future career of cattle (Raboisson et al., 2016). In order to establish whether or not FPT has occurred, it is necessary to evaluate the concentration of IgG in the calf serum (Jaster, 2005). In particular, a threshold limit of 10 g/l has been established, referring to a blood sample taken within 48 h from birth. Calves with an antibody level lower than this limit show a mortality approximately double compared to calves with a higher concentration (Quigley et al., 1998). Therefore, testing calves for FPT is an important step in monitoring the successfulness of colostrum management programs (McGuirk, 2005, Godden, 2008).

The method described by Hopkins et al. (1984) represents a field tool to detect the occurrence of FPT. This method is based on protein precipitation by solutions of sodium sulphite at the concentrations of 14%, 16%, and 18%, measured by visual evaluation of the generated turbidity. However, since the solutions could have different degrees of turbidity, this "visual" method has the limitation of expressing the result only within three categories and does not provide an accurate description of the IgG content. Therefore, an improvement of the turbidity reading phase is desirable. On this regard, evidences are provided in the literature concerning the possible use of the spectrophotometer to evaluate the transfer of immunoglobulins, for example in foals (Sedlinska et al., 2005).

The aims of the present study were to: i) evaluate the influence of dam breed and parity on colostrum quality and on the calf FPT; ii) evaluate the influence of calf breed and gender, and of time from calf birth to the administration of the first colostrum meal on the occurrence of FPT adopting the field test; iii) ameliorate the diagnostic accuracy of the field FPT test through a second laboratory phase able to improve turbidity evaluation.

2 Material and methods

2.1 Samples collection

A total of 60 colostrum and 60 serum samples were collected from cows and calves, respectively, in 4 herds (A, B, C, D) located in the province of Parma (Italy): 11 samples of each matrix (colostrum and serum) were collected from herd A, 13 from herd B, 17 from herd C and 19 from herd D. In particular, herds A, B and C were constituted of Holstein cows, whereas herd D had Brown Swiss cows. For each calf, the timing of colostrum administration and the volume administered were recorded. All calves were purebred Holstein or Brown Swiss.

Colostrum. Each colostrum sample was taken at the first milking, performed within 4 h from parturition in non-sterile bottle and stored at -20°C immediately after sampling, until the day of the analysis.

Serum. A blood sample from each calf was collected from the jugular vein using a 10 ml tube (Venosafe Lithium Heparin, Vetefarma Srl, Italy) between 24 and 48 h of life, in order to allow the calf to completely absorb colostrum Ig. Samples were left at room temperature for 12 h and then they were centrifuged at 3,000 rpm for 7 min. Finally, the serums obtained were stored at -20°C until the day of the analysis.

2.2 Quantification of colostrum IgG

The colostrum was thawed in a water bath and then warmed (20°C) as recommended by Quigley (1998). A colostrometer (Colostrometer™, Biogenics, USA) was used to measure the IgG concentration. For this purpose, 250 ml of colostrum were taken from each sample and poured in a graduate cylinder where the colostrometer was placed to float. The colostrometer is characterized by a graduated scale ranging from 0 to 140 mg/ml and divided into red zone (from 0 to 30 mg/ml), yellow zone (from 30 to 50 mg/ml) and green zone (from 50 to 140 mg/ml). The estimation of the concentration of IgG contained in each colostrum was read directly on the graduated scale of the colostrometer.

2.3 Determination of the dry matter of colostrum

Colostrum dry matter content (g/kg) was determined according to the procedure described by Savini (1946) for milk, as a further characteristic for colostrum quality evaluation. For each sample, a muffle porcelain capsule was conditioned at a temperature of 530°C for 6 h. An amount of 10 g of colostrum was weighed in each capsule and added with 10 ml of distilled water and 4 ml of 10% acetic acid. The

capsules were then placed in an oven at the temperature of 70°C overnight (pre-concentration); subsequently the temperature was brought to 100°C for 7 h. The capsules were then cooled in a dryer and re-weighed using an analytical scale; the value obtained was subtracted from the initial weight to obtain the quantity of dry matter.

2.4 Serum analysis

After defrosting in a water bath at 20°C, the IgG were quantified in each serum by the procedure described by Hopkins et al. (1984). This technique is based on protein (and therefore also Ig) precipitation by solutions of sodium sulphite at different concentrations. Briefly, sodium sulphite solutions (14%, 16%, and 18%) were prepared in 3 flasks (0.5 L). For each sample, 0.1 ml of serum and 9 ml of 14% or 16% or 18% sodium sulphite solutions were added in a glass tube. The diluted samples were left at room temperature for 60 min. After incubation, the solutions were examined by illuminating the top and bottom of the tubes placed in front of a dark colored background to reveal the presence of precipitate. For the reading of the results, it was considered that serum samples that formed precipitates with 16% and 18% sodium sulphite solutions contained IgG levels in the range from 5 to 10 mg/ml, whereas serum samples with IgG levels higher than 15 mg/ml formed precipitates with all the 3 concentrations of sodium sulphite.

For the spectrophotometric reading, the instrument was set to a wavelength of 590 nm. Precipitated samples were then homogenised by vortex for about 10 s, and read in the order 14%, 16% and 18% sodium sulphite solutions. Before starting each series, the reference sample, represented by fetal bovine serum at the serial dilution employed in the FPT test was inserted as the zero test.

2.5 Statistical analysis

An ANOVA using PASW statistics (Version 26.0, SPSS Inc., Chicago, IL, USA) was performed to investigate if parity and breed of the dam were significant factors affecting the variation of colostrum IgG concentration, colostrum dry matter, and total amount of IgG fed to the calf, calculated as the product of the IgG concentration of each sample and the volume of colostrum administered. The model included the fixed effects of parity, breed and herd nested within breed, and the Tukey multiple comparison test was used for the *post-hoc* evaluation of the parity effect. The chi-square test was applied to compare the number of calves with FPT among dam parity levels.

One-way ANOVA was performed for the spectrophotometer measurements obtained on the three immunoglobulin levels (< 5 IgG/ml, < 10 IgG/ml, > 15 IgG/ml) determined by visual turbidity test grouped within the three sodium sulphite dilutions (14%, 16%, 18%). The Tukey *post-hoc* multiple comparison test was used to investigate if means differed significantly ($P < 0.05$). Differences of frequencies of FPT for calf breed (Brown Swiss vs. Holstein), gender (male vs. female), and time from calf birth to the administration of the first colostrum meal (1 to 4 h vs. 5 to 9 h) were evaluated through the z-test (<https://www.socscistatistics.com/tests/ztest/>).

Finally, a principal component analysis (PCA) was performed with the same software on the matrix of the spectrophotometric measurements obtained at the three sodium sulphite dilutions (14%, 16%, 18%) for 60 serum samples. The matrix was constructed with the correlation method. Two components were extracted and plotted in a 2D score plot where the samples were marked with labels of different colors based on the positive or negative classification deriving from the visual turbidity test.

3 Results and discussion

Since only good quality colostrum, if properly administered, allows the calves to achieve a sufficient immune coverage of maternal origin, it is of great importance to establish the quality of this secretion before administering it to the newborn. On-farm protocols usually recommend that an appropriate absorption of IgG in calves can be achieved by administering a minimum of 4 L of high quality colostrum, defined as a colostrum containing more than 50 g of IgG/L, within the first 24 h of life, when the closure of the intestinal barrier against antibodies occurs (Godden, 2008). In our study, calves received a volume of colostrum ranging from 2 to 6 L within 24 h from birth, indicating a proper timing of administration but a lack of quantity in some cases. In particular, the first administration was performed at 4.4 h of life while the second administration occurred on average 18 h after birth.

No significant differences were observed between the two cow breeds for the investigated traits: the least squares means of Holstein and Brown Swiss cows were 93.5 and 95.0 g IgG/l colostrum, 347.8 and 323.8 g IgG fed to calf, and 265.7 and 285.8 g DM/kg colostrum, respectively.

Cows were well distributed across parities, with a minimum of 9 cows in third lactation and a maximum of 16 cows in second lactation (Table 1). Moreover, each parity group comprised about 1/3 of Brown Swiss and 2/3 of Holstein cows. The IgG colostrum concentration varied significantly ($P < 0.05$) among parities with the main differences occurring between first- and fourth- parity cows, and first- and \geq fifth-parity cows (Table 1). Concerning the amount of IgG offered to the calves, no differences were detected comparing the colostrum of first-, second- and third-parity cows with colostrum of fifth- or more parity cows, while higher levels ($P < 0.05$) were observed in colostrum of fourth-parity cows (Table 1). However, the amount of IgG offered to calves is partially affected by the volume of colostrum administered to the calves and thus needs further investigation. The concentration of IgG appeared to increase almost linearly with parity. This is consistent with results of Connelly et al. (2013) who reported greater IgG content in colostrum of older cows likely due to a wider exposure to antigens during their lifetime. The statistical analysis revealed the absence of significant differences between parities for dry matter content, differently to findings of Zarei et al. (2017) who highlighted the possible role of Ca and P (not measured in the present study) as main elements affecting colostrum dry matter. It is worth noting that first- and \geq fifth-parity cows seemed to involve a greater risk of FPT, i.e. the prevalence of FPT was 50% and 60%, respectively (Table 1). However, this finding should be studied also in relation to the volume of colostrum offered or assumed by the calves and to the timing of administration on a larger sample size.

Table 1 Colostrum quality, total amount of IgG fed to calf, and percentage of Failure of Passive Transfer (FPT) for different parities

Item	Parity					SEM	P-value	
	1	2	3	4	≥ 5			
	n	14	16	9	11	10		
Colostrum quality	IgG concentration (g/L)	75.4 ^a	79.4 ^{ab}	101.7 ^{ab}	106.5 ^b	106.2 ^b	3.65	0.010
	Colostrum dry matter (g/kg)	280.7	239.4	284.5	269.0	280.2	5.94	0.085
IgG fed to calf ¹ (g)		285.3 _a	295.5 ^a	359.9 ^{ab}	446.0 ^b	322.1 ^{ab}	19.03	0.048
FPT, n (%)		7 (50)	3 (19)	2 (22)	3 (27)	6 (60)	-	0.140 ²

¹The amount of IgG fed to calf was calculated as the product of the IgG concentration of the colostrum and the volume of colostrum administered to the calf; ²From chi-square test; SEM – standard error of the mean

The strengthening of the calf's immune system should begin in the colostrum phase which usually lasts from 2 to 4 days. However, the absorption of IgG decreases starting from 12 h after calf birth and ends around 24 h after birth (Rogers and Capucille, 2004). Therefore, during this period, action must be taken to prevent the cases of FPT occurrence. Two blood tests (turbidity test and spectrophotometer analyses) were carried out on the calves serum to investigate the quantity of absorbed IgG and identify any cases of FPT. The serum turbidity test showed that 23 out of 60 calves tested had FPT, meaning about 38% of incidence. Results showed that the Brown Swiss calves studied were similar to the Holstein calves for the FPT occurrence (45% for Brown Swiss and 35% for Holstein; Table 2). This indicates a good management of the animals in the considered farm since usually newborned Brown Swiss calves are less reactive and lively than those of other specialized dairy breeds showing weak sucking ability in the early days of life (Maltecca et al., 2007) with delayed and reduced first colostrum meal and impaired absorption of Ig (Godden, 2008). The calf gender did not influence the occurrence of FPT, while when the time interval from calf birth to first colostrum meal is considered, it appears that a colostrum administration performed in the first 4 h of life induces a greater risk of FPT (50%) compared with administration from 5 to 24 h after birth (17%; Table 2). We could speculate that this result might be due to the dilution effect exerted by the amniotic fluid still present in the gastrointestinal tract of the newborn (and gradually absorbed in the first hours of life) on the colostrum assumed early after birth (Parrish and Fountaine, 1952), potentially compromising stomachal curd formation and IgG absorption (Miyazaky et al., 2017).

Based on the differences in the obtained absorbance, the spectrophotometric measurements showed the possibility to discriminate between serum IgG concentrations lower or higher than 10 mg/ml in solutions at 14% of sodium sulfite and between IgG concentration lower or higher than 5 mg/ml in solutions at 16% of sodium sulfite. No significant differences were found in the absorbances between

the IgG concentration categories when the turbidity was evaluated in solutions at 18% of sodium sulfite (Table 3). On the results obtained through the spectrophotometric evaluation, a PCA was performed and two components were extracted, accounting for 91% of the total variance, and they are graphically presented in Figure 1, which shows a clustering of samples according to FPT occurrence.

The red group was declared positive to FPT by the turbidity test previously performed and the green group was declared negative to FPT by the same test.

Table 2 Failure of Passive Transfer (FPT) incidence according to breed and gender of the calf, and time from calf birth to the administration of the first colostrum meal

Breed	Holstein Brown Swiss	FPT (%)	P-value
		35 45	0.453
Gender	Male Female	41 34	0.575
	Timing of 1 st colostrum meal (h)	1 to 4 5 to 24	46 17

Table 3 Spectrophotometric measurements obtained for the three immunoglobulin levels determined by visual turbidity test within each sodium sulphite dilution. Values are expressed as absorbance at 590 nm and reported as means calculated through the one-way ANOVA procedure

IgG range visual measurement test	< 5 mg IgG/ml	< 10 mg IgG/ml	> 15 mg IgG/ml	SEM	P-value
Absorbance					
Sodium sulphate dilution	14%	0.021 ^a	0.020 ^a	0.012	<0.001
	16%	0.019 ^a	0.113 ^b	0.014	<0.001
	18%	0.131	0.128	0.172	0.233

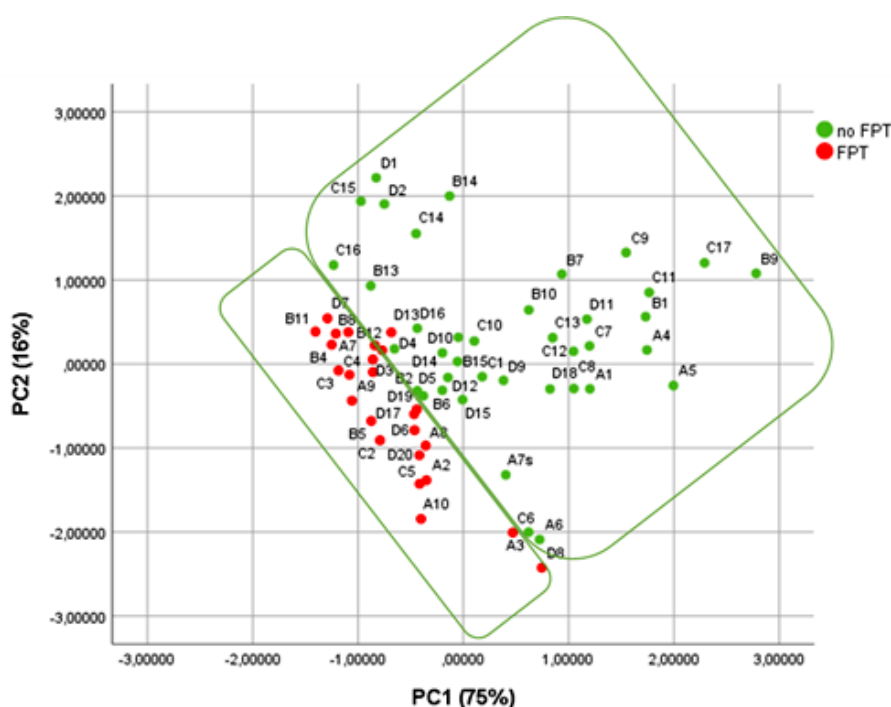


Figure 1 2D-score plot from principal component analysis (PCA) on the matrix of the spectrophotometric measurements of the 3 sodium sulphite solutions (14%, 16%, 18%) for 60 serum samples on PC1 and PC2. The red group was declared positive to Failure of Passive Transfer (FPT) and the green group was declared negative to FPT

Considering the promising results obtained, it would be interesting in future studies to expand the dataset and to validate the spectrophotometric method to evaluate the turbidity generated by the precipitation of the IgG in sodium sulfite solutions. It appears in fact that the spectrophotometer allows for a good discrimination of FPT cases, similarly to the visual method, potentially providing at the same time a quantitative description of the IgG content of serum, expressed by the value of the absorbance. The lack of a gold standard method for the calibration of the spectrophotometric measurement adopted in the present study represent a limiting factor for further discussion. However, based on the results of Sedlinska et al. (2005) on foals serum is conceivable that the calibration process could be possible.

4 Conclusions

In the present study the breed of the dam did not affect colostrum quality in terms of IgG concentration and supply and dry matter content, whereas only increasing levels of immunoglobulins across parity were detected, with no effect on FPT occurrence in calves. Dam/calf breed and calf gender did not affect the occurrence of FPT while concerning time at first colostrum meal better results were observed when colostrum was fed after 4 hours from parturition. However, further studies on larger samples size are needed. The spectrophotometer allows for the diagnosis of FPT cases potentially providing at the same time a quantification, through the absorbance values of the IgG content of serum; further studies for the calibration and validation of this method are needed.

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