Determination of immunoglobulin concentrations and genetic parameters for colostrum and calf serum in Charolais animals

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ABSTRACT

Colostrum samples from 366 Charolais primiparous cows, as well as serum from their calves at 24 to 48 h of age, were collected to gain an overview of the situation regarding passive immune transfer in beef cattle, from both the phenotypic and genetic points of view. All samples were analyzed to quantify their G₁ immunoglobulins by radial immunodiffusion (RID) and their IgG, IgA, and IgM using ELISA. The average concentrations obtained in colostrum were 84 mg/mL for RID-IgG₁, and 158 mg/mL, 4.5 mg/mL and 10.8 mg/mL for ELISA-IgG, -IgA, and -IgM, respectively. The corresponding values in calf serum were 19.9, 30.6, 1.0, and 1.9 mg/mL. Apart from the general environmental effect (farm-year combination and laboratory conditions), the characteristics of the dams tested did not reveal any influence on colostrum immunoglobulin concentrations. Calving difficulty, as well as the birth weight and sex of calves, were found to be associated with serum concentrations in some cases. Heritability estimates were low to moderate, with the highest being for RID-IgG₁ in colostrum ($h^2 = 0.28$, standard error = 0.14) and serum ($h^2 = 0.36$, standard error = 0.18). Phenotypic correlations among the different immunoglobulins were generally positive or null, and none of the genetic correlations were significant due to large standard errors. The phenotypic correlation between dam colostrum and calf serum values was 0.2 for RID-IgG₁ and null for the 3 ELISA measurements. The correlation between RID-IgG₁ and ELISA-IgG was, unexpectedly, null for colostrum and 0.4 for serum. Increased RID-IgG₁ levels in calf serum were associated with improved survival, as well as better early growth and fewer health problems. These results thus showed that despite generally higher concentrations in beef than in dairy cattle, passive transfer was unsuccessful

in a considerable number of calves. This should be brought to the attention of breeders to avoid negative effects on survival and subsequent performance. The heritability estimates were encouraging; however, obtaining phenotypes on a large scale constitutes a real limitation regarding these traits.

Key words: passive immune transfer, radial immunodiffusion, immunoglobulin

INTRODUCTION

The immunity of newborn calves is not assured at birth. Indeed, the placental barrier in ruminants prevents the in utero transfer of immunoglobulins (Borghesi et al., 2014), and the functional but naive immune system of the newborn does not permit an effective immune response for the first 3 wk of life (Franklin et al., 2003). It is therefore crucial for newborns to acquire immunoglobulins through the intake of maternal colostrum. The first mammary secretions after birth are defined as colostrum. Because it rapidly but progressively turns into milk within a few days, only the secretions that occur during the first 24 h are generally considered as colostrum (Jaster, 2005). Bovine colostrum contains 3 major classes of immunoglobulins: IgG, IgM, and IgA (Korhonen et al., 2000). The most abundant immunoglobulins, IgG, play the main role in the immune response for most infections, IgA are more involved in protecting the mucous membranes (including intestinal), and IgM are the first line of defense against several infections and infestations (Butler, 1969; Muller and Ellinger, 1981; Godden, 2008). According to Larson et al. (1980), IgG, IgA, and IgM account for approximately 85 to 90%, 5%, and 7%, respectively, of all the total immunoglobulins in colostrum, with the main isotype, IgG₁, accounting for about 80 to 90% of IgG. Most recent studies have focused on IgG, or even IgG₁, because of their abundance and important roles.

The acquisition of maternal immunoglobulins through colostrum to ensure immunity is known as the transfer of passive immunity or passive transfer. Levels

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<10 mg/mL of IgG in calf serum are considered as a failure of passive transfer when sampled between 24 and 48 h of age (Jaster, 2005). Animals that have failed passive transfer have mortality and morbidity risks up to 6 times higher than those which have succeeded (McGuirk and Collins, 2004). In addition, successful passive transfer has also been associated with better growth performance as well as superior milk production in adulthood (Faber et al., 2005). Recent literature in the dairy field has insisted on the importance of offering sufficient quantities of colostrum containing high levels of IgG (50 mg/mL or more) soon after birth to maximize the chances of successful passive transfer (Jaster, 2005; Le Cozler et al., 2016). However, it has also been shown that not all cows are able to produce colostrum of this quality (Gulliksen et al., 2008; Quigley et al., 2013; Le Cozler et al., 2016). The importance of passive transfer is the same in beef and dairy cattle. However, females from beef breeds are not usually machine milked, and thus it is much more problematic to obtain colostrum samples from these animals. The rare studies to have analyzed colostrum from beef breeds reported that immunoglobulin concentrations seemed to be higher than in dairy breeds (Guy et al., 1994; Murphy et al., 2005; Allemand, 2008), but this subject is still largely unknown and requires further exploration. In both dairy and beef cattle, studies have generally focused on phenotypic aspects of the phenomenon, and only a few studies in the past have estimated heritability for immunoglobulins in colostrum; these studies suggested that it could be high (Kruse, 1970; Dardillat et al., 1978; Gilbert et al., 1988), although a more recent study found heritability of 0.2 for IgG in calf serum (Maltecca et al., 2009). Nevertheless, the literature remains scarce regarding the genetic determinism of immunoglobulin concentrations in colostrum and serum.

During our study, we were able to obtain colostrum samples from 366 Charolais cows on experimental farms, and sera from their calves between 24 and 48 h of age. The IgG₁ levels were quantified in each sample using radial immunodiffusion (RID), and IgG, IgA, and IgM were quantified by ELISA. Using this extremely rare data set, our aim was to generate new knowledge on immunoglobulin levels in beef cattle colostrum, their genetic parameters, the success of passive immune transfer, and its relationship with subsequent performance.

MATERIALS AND METHODS

Ethics Statement

During this experiment, all animals were handled with care in line with the Institute for Agriculture, Food and Environment's ethics policy and in compliance with

the guidelines on animal research issued by the French Ministry of Agriculture (<https://www.legifrance.gouv.fr/eli/decret/2013/2/1/2013-118/jo/texte>).

Animals and Feed Management

The experiment was performed at 2 experimental farms belonging to the French National Research Institute for Agriculture, Food and Environment (INRAE, formerly INRA): Le Pin au Haras (Exmes, France; farm 1) and Bourges-La Sapinière (Osmoy, France; farm 2). The calvings considered for the analysis occurred in winter between December 2013 and January 2018. All dams were primiparous Charolais cows because of the design of another experiment that was run simultaneously on the same animals.

At farm 1, the heifers were out at grazing until 3 wk before the first expected calving. They were then brought inside on straw in penned groups of 10 to 14. When a heifer showed signs of calving, she was isolated in an individual pen and equipped with a calving detector that alerted staff when the cow started parturition. Twenty-four hours after birth, the cows joined the group of new mothers and their calves, which were all turned out to grass again as soon as weather conditions permitted (usually April, when the calves were 3–4 mo old) and remained until weaning in September, when the male calves were sold. The process was similar at farm 2, except that animals never went outside, but fresh silage was brought in to them. The feeding plan consisted of ad libitum low energy hay (farm 1) or grass silage (farm 2) with 100 g of mineral and vitamin supplementation before calving, and ad libitum high energy grass silage after birth together with 1 kg commercial pellets.

Sampling

Colostrum samples were collected immediately after calving before the first suckling by the calf. Secretions from all functional teats were pulled to obtain a volume of 15 mL, and the first spurt from each teat was discarded. The samples were then frozen at -20°C . Approximately 10 mL of blood was sampled from the calves between 24 and 48 h after calving. The blood was left to coagulate for 4 to 16 h and then centrifuged at $1,700 \times g$ for 10 min. Two milliliters of serum was then extracted and stored at -20°C . Once all the samples had been collected, they were transferred to the laboratory at the Joint Research Unit for Animal Genetics and Integrative Biology (GABI, INRAE; Jouy-en-Josas, France), where aliquots were produced and ELISA tests performed. One aliquot was sent to Labocéa (Fougères, France), which performed the RID test.

Measurements of IgG₁, IgG, IgM, and IgA in Calf Serum and Colostrum

Bovine IgG₁ were assayed in colostrum and serum samples using RID by Labocéa (Fougères, France). The samples were diluted using the Microlab 625 diluter (Hamilton) at 1:222 or 1:1,000 for serum or colostrum, respectively. The RID bovine IgG₁ test was performed using IDRing Viewer and IDRing Meter software (all from IDBiotech, Issoire, France), according to the manufacturer's instructions.

The IgA, IgG, and IgM levels were evaluated in colostrum and serum samples using the Bovine IgA, IgG, and IgM ELISA Quantitation Sets and the ELISA Starter Accessory Kit (Bethyl Laboratories, Montgomery, TX), following the manufacturer's instructions. Seven points of 2 by 2 serial dilutions were performed for standard curves, starting at 500 ng/mL for IgA and 250 ng/mL for IgG and IgM. The serum samples were diluted within ranges of 10^{-2} and 10^{-4} for IgA, 10^{-4} and 10^{-6} for IgG, and 10^{-3} and 10^{-5} for IgM. Colostrum samples were diluted within ranges of 5.10^{-3} and 5.10^{-5} for IgA, 2.10^{-5} and 2.10^{-7} for IgG, and 10^{-4} and 2.10^{-5} for IgM. All assays were performed in duplicate. If the differences in optical densities obtained were greater than 20%, the sample was re-assayed. Quality controls at the plate level were also performed, and the samples were re-assayed if negative controls on the plates were higher than 0.15 AU (arbitrary units).

Other Traits

To test for other effects that might influence or be influenced by immunoglobulin levels, other phenotypes were collected and new traits were computed. With respect to environmental conditions, the farm and year of calving were combined in farm-year contemporary group effects. The conditions regarding quantitative measurements (sets of samples analyzed at the same time) were also referred to as "laboratory conditions."

Dam characteristics were also recorded as follows: weight at calving (mean of 2 measurements between d 1 and 6), BCS at calving (scale of 1 = thin to 5 = extra fat; scored at weighing), age at calving (in months), age at the onset of cyclicity (in months), maternal instincts (scored on a scale of 1 = refuse the calf to 5 = complete acceptance), and calving difficulty (scored on a scale of 1 = no help needed to 4 = caesarian section). No cows were recorded as experiencing any health events during the week before calving.

Calf characteristics were also obtained as follows: the age of the calf in hours at the time of serum collection; sex of the calf; and weights at birth, 10 d, and then every month. Their weights at 30, 120, and 210 d were

estimated from a regression on the weights available within 1-mo intervals. In addition, health events were recorded up to 7 mo of age, corresponding to the age at which the youngest calf was sold. Several binary variables were defined using 0 = no health event and 1 = at least 1 record including the following: early digestive problems (up to 7 d of age), later digestive problems (starting at 8 d of age), digestive problems (any age), pulmonary problems, others (every record not of a digestive or pulmonary nature), and sick (at least 1 health event, whatever its nature). The durations of episodes were also recorded and used to compute the following 3 variables: duration of digestive problems, duration of pulmonary problems, and total duration of health events. Finally, 3 survival traits were defined at 7 d, 3 mo, and 6 mo, with 0 = still alive and 1 = dead. Descriptive statistics concerning all these variables are available in Supplemental Table S1 (<https://doi.org/10.3168/jds.2020-19423>).

Statistical Analyses

Eight traits related to immunoglobulins were analyzed: RID-IgG₁, ELISA-IgG, ELISA-IgA, and ELISA-IgM from the dam colostrum, and the same 4 traits from the calf serum. Descriptive statistics were calculated for each trait using Proc Means under SAS STAT software (SAS Institute Inc. Cary, NC), and tests of significant effects were calculated for each trait using Proc GLM under SAS STAT software. The following effects were tested for both colostrum traits and the calf serum traits: farm-year contemporary group effect, laboratory conditions, dam weight at calving, dam BCS at calving, age of the dam at calving, age of the dam at the onset of cyclicity, maternal instincts, calving difficulty, sex of the calf, and calf birthweight. In addition, the age of the calf (in hours) at the time of serum collection was tested for the calf serum traits only.

Genetic parameters were estimated using the restricted estimation of maximum likelihood method under WOMBAT software (Meyer, 2007). Correlations between immunoglobulin traits were estimated using bivariate linear animal models. The model considered for all traits individually taken can be expressed in a matrix notation as

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{e},$$

where $\mathbf{y}$ is the vector of observations for the trait, $\mathbf{b}$ is the vector containing fixed effects, $\mathbf{a}$ is the vector of animal additive genetic effects, $\mathbf{e}$ is the vector of residuals, and $\mathbf{X}$ and $\mathbf{Z}$ are the respective incidence matrices assigning observations to effects. For colostrum traits,

the fixed effects were the quantitative measurement effect (combination of date and plate effect) and the contemporary group effect. For calf serum traits, calving difficulty was added to these 2 fixed effects. Random effects were assumed to be normally distributed with means equal to zero and a covariance structure equal to the following equation:

$$\text{Var} \begin{pmatrix} \mathbf{a} \\ \mathbf{e} \end{pmatrix} = \begin{pmatrix} \mathbf{G} \otimes \mathbf{A} & 0 \\ 0 & \mathbf{I}_r \otimes \mathbf{R} \end{pmatrix},$$

where $\mathbf{G}$ is a (co)variance matrix of random direct additive genetic effects, $\mathbf{R}$ is the residual (co)variance matrix, $\mathbf{A}$ matrix represents the additive genetic relationships between animals, $\mathbf{I}_r$ is the identity matrix, which has an order equal to the levels of the appropriate residual's effect, and $\otimes$ is the Kronecker product. For calf serum traits, maternal genetic effects may exist; however, considering the number of animals, the absence of direct performance of the dams, and the fact that there is only 1 calving per dam, it would have been potentially confusing to include it in the model. Not considering it may have slightly inflated the direct genetic heritability.

The pedigree file contained 5 generations including 1,024 animals. Trait heritability was estimated from the ratio between the animal variance component and the sum of the animal variance component and the residual variance. The possible link between immunoglobulin concentrations and later performance of the calf was also tested using trivariate linear animal models including colostrum measurements, calf serum measurements, and the given tested non-immunoglobulin trait. The model was the same as before, with the same fixed effect for the colostrum and serum immunoglobulin traits and with the farm-year contemporary group as fixed effect for the non-immunoglobulin trait. The following traits were tested: weights at 30, 120 and 210 d, early digestive problems, late digestive problems, digestive problems, pulmonary problems, other health problems,

all health problems, duration of digestive problems, duration of pulmonary problems, duration of all health problems, dead at 7 d, dead at 3 mo, and dead at 6 mo.

RESULTS

Descriptive Statistics

Descriptive statistics regarding immunoglobulin concentrations in colostrum and calf serum are presented in Table 1. For colostrum, the proportions of the 3 main immunoglobulins were 91, 3, and 6% for IgG, IgA, and IgM, respectively. The corresponding proportions in serum were 87, 1, and 11%. As expected, the IgG concentrations determined using ELISA were higher than the IgG₁ concentrations measured by RID. Each immunoglobulin displayed considerable variability between animals, with some individuals producing extreme values. For example, there were a few calves with almost no immunoglobulins in their blood, and 6 had such low IgG₁ levels that they were below the threshold of detection of the RID device and were not quantified. Based on their RID and ELISA results, about 22 and 10% of calves, respectively, did not reach the IgG level of 10 mg/mL deemed to be the threshold for successful passive transfer. Similarly, based on RID and ELISA results, about 16 and 6% of the cows, respectively, produced colostrum containing IgG levels lower than 50 mg/mL, and were therefore considered as poor-quality producers.

Factors Influencing Immunoglobulin Concentrations

The significant factors that influenced immunoglobulin concentrations and the R^2 of general linear model (GLM) models are presented in Table 2. Laboratory conditions were extremely important and generally responsible for most of the R^2 . The contemporary group effect was also significant in half of the models. Variations between years were not similar at the 2 farms, justifying the use of an interaction effect. For calf

Table 1. Number of samples (N), mean, SD, minimum, and maximum for each immunoglobulin analyzed

Type of sample	Immunoglobulin and analysis ¹	N	Mean (mg/mL)	SD	Minimum	Maximum
Colostrum	RID-IgG ₁	366	84.07	45.20	21.0	462.5
	ELISA-IgG	360	158.44	88.61	4.03	701.0
	ELISA-IgA	360	4.52	3.74	0.13	29.7
	ELISA-IgM	360	10.79	6.87	0.63	58.6
Calf serum	RID-IgG ₁	346	19.91	10.78	4.0	67.5
	ELISA-IgG	352	30.57	24.68	0.01	174.5
	ELISA-IgA	352	0.96	1.15	0.002	8.4
	ELISA-IgM	352	1.86	1.62	0.01	12.7

¹RID = radial immunodiffusion.

Table 2. Effects significantly ($P < 0.05$) affecting one of the immunoglobulins analyzed and the R^2 in the models

Type of sample	Immunoglobulin ¹	Laboratory conditions	Farm-year combination	Calving difficulty	Time between birth and sampling	Calf sex	Birth weight	R^2
Colostrum	RID-IgG ₁	X	X					0.12
	ELISA-IgG	X	X					0.36
	ELISA-IgA	X	X					0.06
	ELISA-IgM	X						0.15
Calf serum	RID-IgG ₁		X	X	X		X	0.16
	ELISA-IgG	X		X				0.39
	ELISA-IgA	X						0.06
	ELISA-IgM	X				X	X	0.18

¹RID = radial immunodiffusion.

serum, calving difficulty was significant ($P < 0.0001$ and $P = 0.03$ for IgG₁ and IgG, respectively) for the 2 IgG measurements, with calves experiencing difficulties displaying the lowest IgG levels. For example, the corrected mean for IgG₁ was 23.5 mg/mL among calves born without assistance, 20.9 mg/mL in calves born with some assistance, 14.8 mg/mL in calves born with considerable assistance, and 7.5 mg/mL in calves born following a cesarean section. The same trend was observed in the IgA and IgM calf models, but it was not significant. In the calf IgG₁ model, birth weight ($P = 0.03$) and the time elapsing before sampling ($P = 0.02$) were also significant, although they were only responsible for a very small proportion of the R^2 (3 points for both traits taken together). However, the influence of these 2 traits was weak, as the correlations between each of them and IgG₁ levels were not significant. The IgM model also revealed an effect of birth weight ($P = 0.04$), with heavier calves tending on average to have slightly lower IgM levels than lighter calves (correlation of -0.11). Under this last model only, calf sex was also significant ($P = 0.02$), with male calves having higher IgM levels than female calves (2.1 mg/mL versus 1.7 mg/mL). For reasons of homogenization, only shared and strong effects were retained as fixed effects in the corrective models (i.e., laboratory conditions and contemporary group, as well as calving difficulty for calf serum).

Heritability and Correlations Between Immunoglobulins

The heritability coefficients and phenotypic and genetic correlations between the immunoglobulins are presented in Table 3 for colostrum and Table 4 for calf serum. The heritability estimates were low (0.002 for calf IgA) to moderate (0.36 for calf IgG₁), with the highest values in both cases obtained for IgG₁. As for genetic correlations, almost all correlations with IgA did not converge. In addition, because the number of animals studied was a bit low for this type of analysis, the standard errors of the estimates were broad, and thus none of the correlations were significant. We nevertheless decided to present them, but not discuss them any further. In terms of phenotypes, the correlations between immunoglobulins were generally positive or null. Surprisingly, the correlation between IgG₁ and IgG was almost null for colostrum (-0.03), but strongest for calf serum (0.41). The correlation between IgA and IgM was high in both types of sample.

Correlations Between Dam and Offspring Immunoglobulin Concentrations

The phenotypic and genetic correlations between immunoglobulin values in dams and their offspring are shown in Table 5. Once again, the broad standard er-

Table 3. Heritability (on the diagonal), genetic correlations (above the diagonal), and phenotypic correlations (below the diagonal) for the 4 types of immunoglobulins (and type of analysis) measured in colostrum, with SE in parentheses

Item	RID ¹ -IgG ₁	ELISA-IgG	ELISA-IgA	ELISA-IgM
RID-IgG ₁	0.28 (0.14)	0.12 (0.65)	Failed	-0.27 (0.40)
ELISA-IgG	-0.03	0.08 (0.12)	Failed	0.17 (0.62)
ELISA-IgA	0.23	0.16	0.05 (0.11)	0.39 (0.73)
ELISA-IgM	0.10	0.21	0.49	0.22 (0.13)

¹RID = radial immunodiffusion.

Table 4. Heritability (on the diagonal), genetic correlations (above the diagonal), and phenotypic correlations (below the diagonal) for the 4 types of immunoglobulins (and type of analysis) measured in calf serum, with SE in parentheses

Item	RID ¹ -IgG ₁	ELISA-IgG	ELISA-IgA	ELISA-IgM
RID-IgG ₁	0.36 (0.18)	0.29 (0.41)	Failed	0.23 (0.61)
ELISA-IgG	0.41	0.20 (0.14)	Failed	0.65 (0.59)
ELISA-IgA	-0.10	-0.02	0.002 (0.08)	Failed
ELISA-IgM	-0.003	0.12	0.40	0.06 (0.09)

¹RID = radial immunodiffusion.

rors did not enable any significant genetic correlations. However, it is worth noting that the genetic correlation for IgG₁ was high and almost reached significance. In terms of phenotypic results, there was a moderately positive correlation for IgG₁, but no correlations were observed for IgA, IgG, and IgM samples.

Correlations Between Immunoglobulin Concentrations and Calf Health, Growth, and Survival

The results of correlations between immunoglobulin levels in colostrum or calf serum and the 15 traits considered with respect to calf health, growth, and survival are presented in Supplemental Table S2 (<https://doi.org/10.3168/jds.2020-19423>). No correlations with any of the immunoglobulins from colostrum were significant. However, some correlations were significant for calf serum. For calf IgG₁, calves with the highest levels were also those which were heaviest at 30 d of age [phenotypic correlation (**rp**) = 0.11]. They also tended to be healthier, with fewer days sick (**rp** = -0.17), fewer records of health events (**rp** = -0.14), and fewer records of health events other than digestive or pulmonary problems (**rp** = -0.19). Finally, they were also more likely to survive as there were fewer deaths during the first 7 d, the first 3 mo, and the first 6 mo (**rp** = -0.17, -0.25, and -0.24, respectively). Comparing the 76 calves with IgG levels lower than 10 mg/mL of IgG with the 160 calves with levels higher than 20 mg/mL of IgG, the 7 dead calves observed at 7 d had all failed in terms of passive transfer; the same applied to 90% of the 18 and 19 calves dead at 3 and 6 mo respectively, with extremely significant chi-squared values ($P < 0.001$).

Calves with the highest IgG levels were associated with a heavier weight at 120 d (**rp** = 0.13), but also more pulmonary problems (**rp** = 0.15). The animals with the highest IgA levels were more likely to display early digestive problems (**rp** = 0.11), health problems other than digestive or pulmonary (**rp** = 0.16), and have overall more days sick (**rp** = 0.20). Finally, it appeared that animals with high IgM levels tended to grow slightly more slowly than others (**rp** = -0.15 at 30 d, and -0.12 at 120 d).

DISCUSSION

Composition of Colostrum and Serum Immunoglobulins and Factors Influencing Composition

The proportions of the different immunoglobulins found in colostrum were in line with those reported in the literature, with more than 90% IgG (Elfstrand et al., 2002; Kehoe et al., 2007; Godden, 2008). The raw means for immunoglobulin levels in colostrum were within the same range as those already reported in beef cattle (Guy et al., 1994; Murphy et al., 2005; Allemand, 2008), but higher than most values reported in dairy cattle studies (e.g., Kehoe et al., 2007; Bartier et al., 2015; Morrill et al., 2015; Le Cozler et al., 2016). This influence of breed on colostrum immunoglobulin levels has been widely demonstrated (Muller and Ellinger, 1981; Weaver et al., 2000), and some authors have suggested that the difference between dairy and beef breeds might be due to a dilution effect (Guy et al., 1994; Godden, 2008), a view supported by the results reported by Silva-Del-Río et al. (2017), who found cows with the highest immunoglobulin levels were those producing

Table 5. Genetic (with SE in parentheses) and phenotypic correlations between the dam and her offspring for the 4 types of immunoglobulins (and type of analysis)

Item	RID ¹ -IgG ₁	ELISA-IgG	ELISA-IgA	ELISA-IgM
Genetic correlation	0.56 (0.30)	-0.13 (0.68)	Failed	0.30 (0.85)
Phenotypic correlation	0.19	-0.02	-0.04	0.03

¹RID = radial immunodiffusion.

the least colostrum. Consequently, the proportion of cows not reaching the 50 mg/mL threshold was lower than that usually reported in dairy studies (e.g., Kehoe et al., 2007; Le Cozler et al., 2016). As for calf serum, the levels were also within the same range as that found in the literature (Murphy et al., 2005; Allemand, 2008; Dunn et al., 2018), and the 22% and 10% of calves found to have failed regarding passive transfer as determined by RID and ELISA, respectively, were below the levels of 35% or 41% reported in dairy calves (Weaver et al., 2000; Godden, 2008). However, it is worth noting that the cut-off values of 10 mg/mL and 50 mg/mL for serum and colostrum, respectively, were established from RID measurements (Jaster, 2005; Godden, 2008), and different cut-off points may be more appropriate for ELISA testing. Moreover, these thresholds were established for total IgG, and not IgG₁ alone, despite 1 experiment that reported a similar threshold for IgG₁ (Tyler et al., 1996).

Numerous effects on immunoglobulin levels in colostrum have been reported previously, such as breed, dam age, length of dry period, or calving season (Weaver et al., 2000; Conneely et al., 2013). Most of these factors did not vary during our experiment, as all the cows were primiparous Charolais animals that calved in winter. Age at calving has been 1 of the tested effects, but the range of variation (a mean of 34.9 mo and a SD of 2.2 mo) was much smaller than that found in studies which determined this effect to be significant in animals from different parities (Gulliksen et al., 2008; Bartier et al., 2015). Older cows may produce colostrum that is richer in immunoglobulins, possibly because they have been exposed longer to farm-specific pathogens (Godden, 2008).

Regarding the effects that influence calf serum IgG levels, the time elapsing before the first colostrum feed is very important because of newborn gut permeability to immunoglobulins, which decreases rapidly during the first 24 h after birth (Weaver et al., 2000; Godden, 2008). During our experiment, the livestock technicians were asked to ensure that the calf suckled immediately after the colostrum sample was collected; therefore, this effect was not considered because all calves were expected to take their first feed during the first hours after birth. The significance of the period between birth and blood sampling in 1 of our models could have been linked to this gut closure effect. The influence of calving difficulty observed here appeared to be logical and has already been reported (Waldner and Rosengren, 2009; Furman-Fratczak et al., 2011): calves born following a difficult labor are less vigorous and therefore less likely to ingest sufficient colostrum to ensure their immunity (Homerovsky et al., 2017). As far as we know, no influence of birth weight or calf sex could be found in

the literature, although this was tested by some teams (Lomba et al., 1978; Barry et al., 2019).

Heritability and Dam to Offspring Transmission

Heritability estimates for immunoglobulin levels in colostrum are still scarce in the literature and cover a broad range of values. Conneely et al. (2013) reported heritability of 0.10 (standard error 0.07), Puppel et al. (2019) found heritability of 0.5 in their review, and Gilbert et al. (1988) estimated it at 0.41; all these values concerned IgG only. Our own estimates were in line with this broad range and suggested, once again, that selection could be performed on IgG levels in colostrum, especially considering the genetic standard deviations of 22.8 mg/mL and 21.5 mg/mL for RID-IgG₁ and ELISA-IgG, respectively. However, the cost and time required to perform laboratory measurements are too high to develop large-scale phenotyping on commercial farms. Indirect measurements, such as use of a Brix refractometer, might be an option, and the heritability of 0.27 recently published using these measurements was encouraging (Soufleri et al., 2019). However, with suckling cattle, it is still necessary to milk the dam, which can be dangerous and cannot be performed routinely on commercial farms.

Regarding the heritability estimates for IgG in calf serum, values in the literature were slightly higher than for colostrum, which was precisely in line with our findings. Maltecca et al. (2009) estimated heritability to be 0.18, and Norman et al. (1981) and Gilbert et al. (1988) found higher values of 0.52 (± 0.28) and 0.69 (± 0.30), and 0.56 (± 0.25), respectively. Once again, our estimates were closer to the recent values and slightly lower than historic values. It is worth noting that all of these estimates involved large standard errors. Norman et al. (1981) also estimated the heritability of IgM levels in calf serum and found it to be 0.30 (± 0.26) and 0.35 (± 0.26) at 24 and 36 h, respectively, which are higher than our own estimates. Once again, the IgG traits could be considered for selection based on their moderate heritability and reasonable genetic variability (genetic SD of 6.1 and 8.9 mg/mL for RID-IgG₁ and ELISA-IgG, respectively).

The correlations between values in dam colostrum and calf serum differed markedly, depending on the type of immunoglobulin analysis performed: no correlation was found at all for the 3 immunoglobulin types measured using ELISA, and there was an observable phenotypic correlation and a high (and almost significant, despite the large standard error) genetic correlation for IgG₁. Findings in the literature also vary considerably in this respect. For example, Genc and Coban (2017) reported a phenotypic correlation of 0.43, and Murphy et al.

(2005) found a correlation of 0.09. Norman et al. (1981) described a weak correlation for IgG, but a positive association for IgM. These variations are not very surprising, as the immunoglobulin levels in calves are not solely dependent on the quality of the dam's colostrum, but also on their ability to suckle and the quantity they absorb, as well as the moment of suckling, as already mentioned (Faber et al., 2005; Homerosky et al., 2017). Therefore, when the amount absorbed and the timing of feeding cannot be considered (as was the case here), the relationship between immunoglobulin levels in colostrum and calf serum is affected. In addition, it appears that there may be a physiologic limit to the amount of immunoglobulins that a calf can take in from a given volume of colostrum (Besser et al., 1985), which further complicates the link between the 2 levels when the colostrum is very dense, which is not rare in beef cattle.

No Correlation Between RID and ELISA Findings for Colostrum IgG Levels?

Despite the fact that 2 different methods (RID and ELISA) were used to quantify IgG₁ and IgG during our study, the absence of any correlation between IgG₁ and IgG levels in colostrum was very surprising. To our knowledge, 2 groups have already published results comparing RID and ELISA to determine bovine IgG (Gelsinger et al., 2015; Dunn et al., 2018), the first involving 58 colostrum samples and 104 calf serum samples, and the second studying 20 samples of each. For RID, both studies measured total IgG (and not just IgG₁) levels; this option was not available to us. Both studies reported that the raw values obtained on the same sample tested using both RID and ELISA were not directly comparable. However, Gelsinger et al. (2015) reported a correlation of 0.36 for colostrum and 0.59 for calf serum, and Dunn et al. (2018) found correlations of 0.83 for colostrum and 0.97 for serum.

Two principal hypotheses can be advanced regarding the difference between our results and those reported in the literature. The first is that there was indeed no correlation between RID-IgG₁ and ELISA-IgG in colostrum from our Charolais cows. Although IgG₁ is considered to be the main isotype in bovine dairy colostrum (Larson et al., 1980; Elfstrand et al., 2002; Kehoe et al., 2007), to our knowledge, the relative proportions of IgG₁ and IgG2 in colostrum and calf serum have not been widely reported, particularly in beef breeds. In addition, individual variations in the proportion of IgG₁ among total IgG have not been studied. This difference in the type of measurement (IgG₁ vs. IgG) would definitely lower the expected correlation between

the 2 methods, but the degree of this reduction remains unclear.

The second possibility is a lack of accuracy regarding quantification by one or both of the methods or some deterioration of the sample during storage or processing that may have differently affected the assessment by the two methods. It was noted that some samples were particularly viscous after thawing and it was very difficult to dilute them before ELISA testing. This may have affected the accuracy of the dilution, especially for IgG, when the dilution factor is more important. Dilution problems were not reported by the laboratory, which performed the IgG₁ test using RID under automated conditions. In addition, this viscosity was not observed when the sample was collected from the cow, even though some samples were already very dense. This led us to hypothesize an influence of freezing. A few studies have already investigated the possible effects on IgG levels of freezing and freeze-thaw cycles. Abd El-Fattah et al. (2014) reported that the storage of colostrum at -20°C for 3 mo did not significantly affect IgG and IgM concentrations. In human colostrum, about 40% of IgA disappeared after 1 yr of the frozen storage, but no significant changes were observed after 6 mo. Argüello et al. (2003) addressed the issue of freeze-thaw cycles and performed 7 cycles on goat colostrum, concluding that IgG levels tended to fall (by about one-third), but the effect was not significant in view of the number of samples. Finally, Morrill et al. (2015) observed a slight decrease in IgG levels following 3 freeze-thaw cycles over a 1-yr period. We did not find any study that had investigated the conservation of immunoglobulin concentrations for more than 1 yr. In our case, some samples had been frozen for about 5 yr; in addition, they were thawed and frozen again at least once for aliquoting. It is therefore likely that not all of our samples aged well. Nevertheless, there was no direct effect of the duration of freezing, as the year effect observed at each of the farms appeared to be random and was not linear over time. In view of the correlation of 0.41 for calf serum, which was lower but closer to previous values in the literature, it is very likely that the conditions of conservation of some samples, in addition to dilution difficulties, had an effect on the accuracy of our results.

Influence of Calf Serum Immunoglobulin Levels on Subsequent Calf Development

The effects of adequate passive transfer on calf mortality have already been demonstrated in the literature, even over the long-term (Weaver et al., 2000). For example, Gilbert et al. (1988) reported that calves with unsuccessful passive transfer are 2 to 4 times more

likely to die before weaning. The correlations we found were significant and moderate and the chi-squared results extremely significant. One can hypothesize that because the correlation was only moderate, and the chi-squared results displayed spectacular differences, this might reveal a threshold effect. It is indeed possible that the protective effect of IgG no longer increases above a given concentration. For example, Lopez et al. (2020) put calves on different diets, and they displayed different levels of IgG (all higher than 10 mg/mL), but there were no subsequent differences in their health or growth.

As for other traits, an effect on growth was mentioned by Faber et al. (2005) and Quigley et al. (2013), which was in line with our findings: calves with higher serum IgG levels were heavier at a given age. Similarly, Furman-Fratczak et al. (2011) reported a positive influence of IgG levels on health performance, as we observed for RID-IgG₁. However, to our knowledge, no association between IgA and IgM levels and subsequent performance has been studied in the recent literature, so it is impossible to conclude whether the counter-intuitive results obtained here for these 2 immunoglobulins were artifacts or a possible sign of an immune response.

CONCLUSIONS

The immunoglobulin levels in colostrum and calf serum for beef cattle were updated, and despite a higher averaged IgG level in colostrum than the classic values for dairy cattle, considerable variability was observed, and some animals did not produce colostrum containing sufficient IgG to protect their calves. Colostrum quality is not the only factor that influences the ability of calves to obtain sufficient immunity, and the importance of early suckling and the amount absorbed needs to be better explained to farmers, even in beef cattle, as passive transfer was unsuccessful in almost a quarter of calves. In addition, calf serum IgG₁ levels are associated with growth and lower mortality, which is of fundamental economic and welfare importance. The IgG levels in both colostrum and calf serum are genetically variable and moderately heritable, but the implementation of selection would require the acquisition of biological samples (colostrum or serum) that are not easy to obtain on commercial beef farms.

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