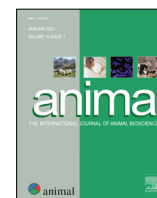




# Animal

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### Variation of immunoglobulins G, A, and M and bovine serum albumin concentration in Holstein cow colostrum

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#### ABSTRACT

Immunoglobulins G (**IgG**), A (**IgA**), and M (**IgM**) represent 70–80% of total proteins in cattle colostrum and are essential for the passive transfer of antibodies from the dam to the calf. Considering the practical difficulties of colostrum sample collection and the high cost of analysis, non-genetic sources of variation of the three immunoglobulins fractions have been scarcely studied together on a large scale in dairy cows. In the present study, IgG, IgA, IgM, and bovine serum albumin (**BSA**) were determined in colostrum samples of Holstein cows through bovine-specific radial immunodiffusion kits; such phenotypes allowed to investigate the effects of parity, herd, and calving season, and interactions. Only the first colostrum was considered in the present study, as the calf was separated from the dam immediately after birth and was not allowed to suckle. The average of IgG ( $n = 676$ ), IgA ( $n = 573$ ), IgM ( $n = 658$ ), total immunoglobulins ( $n = 525$ ), and BSA ( $n = 614$ ) was 91.31, 4.20, 105.99, 5.05, and 2.47 g/L, respectively, and all traits positively correlated to each other. Overall, the immunoglobulins were less concentrated in colostrum of first- and second-parity cows than later-parity cows. These findings suggest that colostrum quality, based on Ig, is overall greater in cows that experienced more than two lactations, likely due to a greater experience of the immune system and to a wider immune heritage compared to younger cows. As regards the effect of calving season, the concentration of all Ig tended to be generally greater in colostrum sampled from August to November. Moreover, there were differences in IgG, IgA, and IgM concentration among the nine herds involved. Future studies will investigate the relationships of these traits with yield, and gross and detailed composition of bovine colostrum and will consider their genetic background to evaluate potential selection strategies to improve colostrum quality.

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#### Implications

Immunoglobulins G, A, and M are important for the passive transfer of antibodies from the dam to the calf. In this study, immunoglobulins and bovine serum albumin (g/L) were measured in colostrum samples collected in nine Holstein farms. Colostrum traits varied according to herd, cow parity, and calving season. In particular, immunoglobulins concentration was greater in cows that experienced more than 2 lactations and in cows that calved from August to November. Part of the variability of the traits was unexplained, suggesting that further information can be considered for future investigations, like colostrum yield, dry period management, and additive genetic effect.

#### Introduction

More than in other mammals, the immunoglobulin (**Ig**) concentration of colostrum in bovines is important for the newborns. In fact, cows are characterized by a cotyledonary synepitheliochorial placenta which does not allow direct transfer of Ig from the dam to the fetus. This explains why calves are agammaglobulinemic at birth and the acquisition of antibodies occurs only by an appropriate intake of good quality colostrum. Immunoglobulins G (**IgG**), A (**IgA**), and M (**IgM**) represent 70–80% of total proteins of bovine colostrum, but the most important for the achievement of the transfer of passive immunity in calves is IgG (McGrath et al., 2016). Ideally, colostrum administered to calves should present  $\geq 50$  g/L of IgG (Buczinski and Vandeweerdt, 2016; McGrath et al., 2016) and an optimal administration has to provide not less than 4 L of colostrum in the first 12 h of life (Jaster, 2005; Godden et al., 2019). Essentially, the '3Q' rule (quickness, quality and quantity) should be followed.

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Serum and colostrum contain both IgG1 and IgG2, with the former being the predominant in colostrum. Transcytosis processes are in charge of Ig passage from blood to colostrum (Samarütel et al., 2016). Among serum proteins, bovine serum albumin (BSA) is involved in the transport of several molecules and its presence in colostrum is useful to monitor the blood-milk barrier permeability and the transition from colostrum to mature milk (Carter and Ho, 1994; Samarütel et al., 2016).

Several tools, like colostrometers and refractometers, are currently available for low cost, in field, and indirect determination of colostrum quality (Quigley et al., 2013; Soufleri et al., 2019). Nevertheless, only the gold standard, radial immunodiffusion (RID), provides very accurate and repeatable results despite expensive (on average >100 US \$ per plate, i.e., per 21 samples), difficult to manage, and time-consuming (24 h of incubation). For these reasons, RID is not suitable for day-to-day on-farm application.

To our knowledge, very few studies have been carried out on the three Ig fractions measured with RID in a large number of cow colostrum samples. In fact, published papers have focused either on IgG fraction only (Gulliksen et al., 2008; Morrill et al., 2012; Kessler et al., 2020) or on indirect measures of IgG (Bielmann et al., 2010; Quigley et al., 2013; Soufleri et al., 2019). In addition, often the colostrum collection time was not standardized. As reviewed by McGee and Earley (2019), some studies used the first colostrum, i.e., colostrum of cows whose calf was moved immediately after birth, whereas in other papers calves were allowed to suckle before colostrum collection and/or the time window dedicated to sampling was very wide.

To investigate genetic aspects of colostrum traits and develop prediction models using infrared spectroscopy (De Marchi et al., 2018), both collection of phenotypes with reference analysis and a preliminary phenotypic study on a large scale are needed. Therefore, the aims of the present study were to characterize colostrum IgG, IgA, IgM, their sum, and BSA (g/L) in a large number of Holstein cows and investigate their correlations and phenotypic variation according to parity, season, and herd.

## Material and methods

### Experimental design

Ethical approval was not required for the present study as per institutional guidelines/local legislation, as only farmers had interactions with their cows. The owner of the animals involved in the study signed a written informed consent.

The collection of samples of colostrum took place in nine commercial farms of northern Italy covering the seasonal variability. The herd size ranged from 60 to 190 lactating cows, with an average of 103. Vaccination before calving against Rotavirus, Coronavirus, and *E. coli* (triple-vaccine) was not performed in the farms of the present study to avoid biased results (Dunn et al., 2017). All cows were farmed under intensive system with total mixed ration administration, free stall barns, twice-a-day milking, and no access to pasture.

Before the trial, the sampling protocol was provided and explained to each farmer, highlighting aims and methodology of the study. In particular, only colostrum produced within 6 h after calving was collected and stored in plastic sterile tubes (120 mL) without preservative (SMIPA srl, Vicenza, Italy). According to the experimental protocol, colostrum was not sampled for this study if the calf was born during the night or without the supervision of the farm personnel. Therefore, only the first colostrum was considered in the current study, as the calf was separated from the dam immediately after birth and was not allowed to suckle.

Farmers were in charge of colostrum collection and were instructed to annotate the cow ID on the tube and to freeze

(−20 °C) the samples as soon as possible. Periodically, colostrum samples were retrieved from the farms, transferred at refrigeration temperature to the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy) and stored at −20 °C until analysis. Information on date of birth, date of calving, and parity of sampled cows were also retrieved.

### Radial immunodiffusion

#### Analysis

Samples were thawed overnight at 4 °C in water and then RID analyses were carried out using bovine-specific assays according to the manufacturer's instructions. In particular, for this study, 34 'Bovine IgG RID Kit', 34 'Bovine IgM RID Kit', 30 'Bovine IgA RID Kit', and 30 'Bovine Ultra Low Level Albumin RID Kit' were purchased in advance from Triple J Farms (Bellingham, WA, US). Each kit enclosed a plate with 24 wells and three reference sera. In order to fall within the detection range of the assay, each colostrum sample was diluted 1:5 (v/v) for IgG and BSA and 1:3 (v/v) for IgA and IgM in pure deionized water (Millipore Corporation, Burlington, MA, USA). Subsequently, 5 µL of diluted colostrum were inserted in the wells of the RID plates (IgG, IgA, IgM, and BSA). After incubation at 20 °C for 24 h, plates were scanned at high resolution to measure diameters of precipitated rings using the image processing program ImageJ (Laboratory for Optical and Computational

**Table 1**

Concentrations of the reference sera used for the determination of immunoglobulins and bovine serum albumin in cow colostrum through the radial immunodiffusion assay.

Trait <sup>1</sup>	Reference sera, g/L		
	1	2	3
IgG	1.80	14.72	28.03
IgA	0.53	1.94	3.87
IgM	0.62	2.00	3.81
BSA	0.25	1.00	2.00

<sup>1</sup> IgG = immunoglobulins G; IgA = immunoglobulins A; IgM = immunoglobulins M; BSA = bovine serum albumin.

**Table 2**

Frequency of cow colostrum samples (*n* = 678) according to herd, parity, and bimonthly classes of calving season.

Effect	Frequency, %
Herd	
A	22.27
B	20.21
C	12.98
D	10.91
E	9.00
F	7.52
G	6.93
H	5.90
I	4.28
Parity	
1	28.91
2	29.65
3	18.44
4	12.09
≥5	10.91
Calving season	
December–January	15.49
February–March	7.96
April–May	13.72
June–July	14.01
August–September	23.75
October–November	25.07

Instrumentation, University of Wisconsin-Madison, WI). All RID analyses were performed by the same operator.

For each well, the precipitated ring was measured in duplicate to calculate the final diameter (mm) as the average of the two measurements. The final diameter was used to derive the concentration (g/L) of the target component (IgG, IgA, IgM, or BSA) through a standard curve developed specifically for each single plate by using both known concentrations and diameters of the three reference sera (Table 1). Not readable or non-circular RID rings due to issues during colostrum dilution or pipetting were treated as missing values, as well as concentration values out of the kit range. The total Ig content (IgTOT) was calculated as the sum of IgG, IgA, and IgM for those samples with the three fractions available.

### Validation

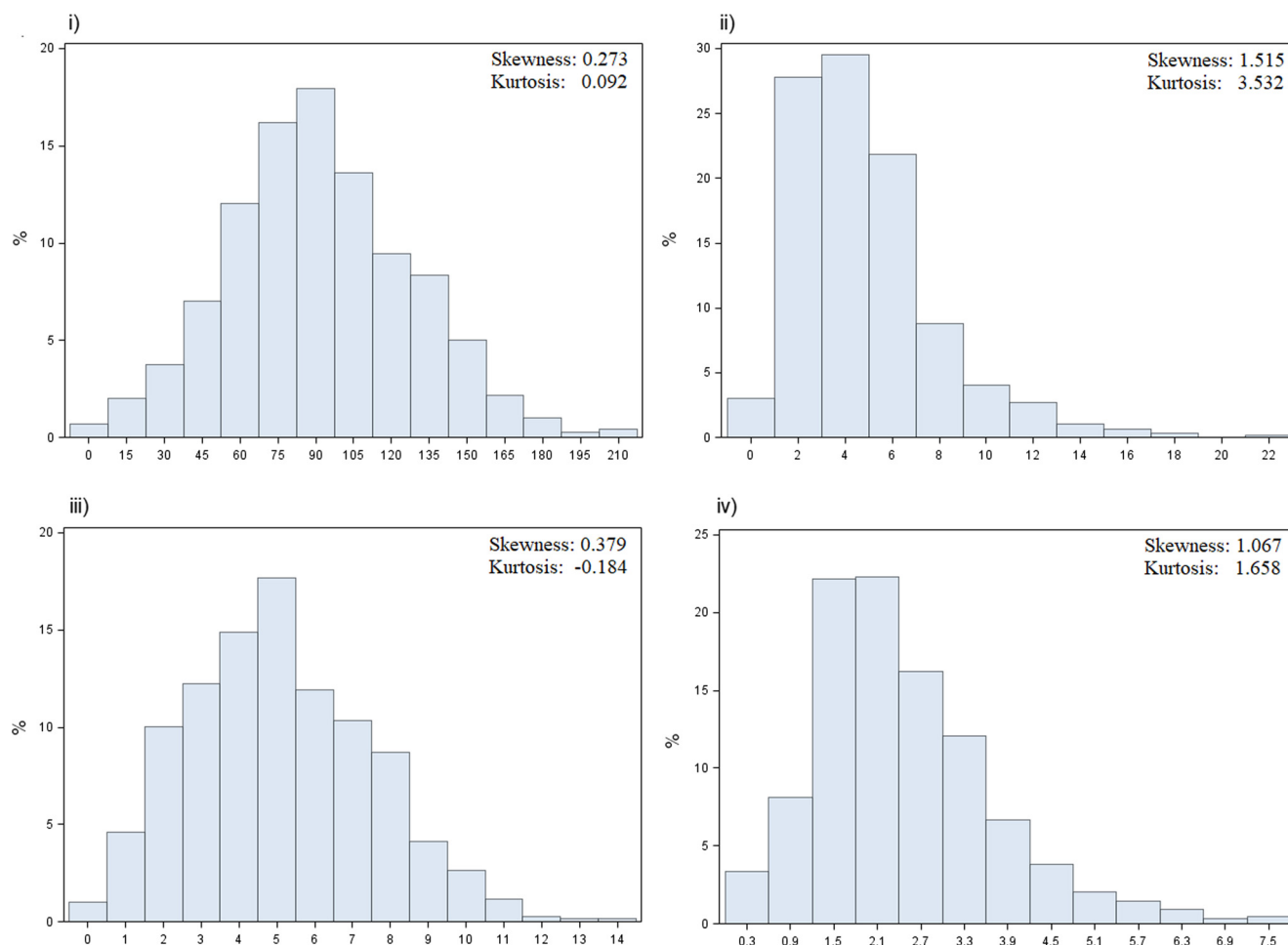
A preliminary trial was carried out before the analyses to assess RID repeatability. The latter was calculated from the intra-assay CV ( $CV_{RID}$ , %) of samples tested in quintuplicate by a single operator. Briefly, four colostrum samples were diluted in pure water (1:5 v/v and 1:3 v/v) as described previously and inserted in five wells of each RID plate (IgG, IgA, IgM, and BSA), where the three reference sera were added as well. Plates were then left at room temperature (20 °C) for 24 h. After incubation, diameter of the rings was measured to calculate the concentration of the target component. Separately for IgG, IgA, IgM, and BSA plate, the  $CV_{RID}$  was calculated as the average of the individual CV of the four samples measured in quintuplicate, as:

$$CV_{RID} = \frac{\left[ \left( s_1 / \bar{x}_1 \right) + \left( s_2 / \bar{x}_2 \right) + \left( s_3 / \bar{x}_3 \right) + \left( s_4 / \bar{x}_4 \right) \right]}{4} \cdot 100$$

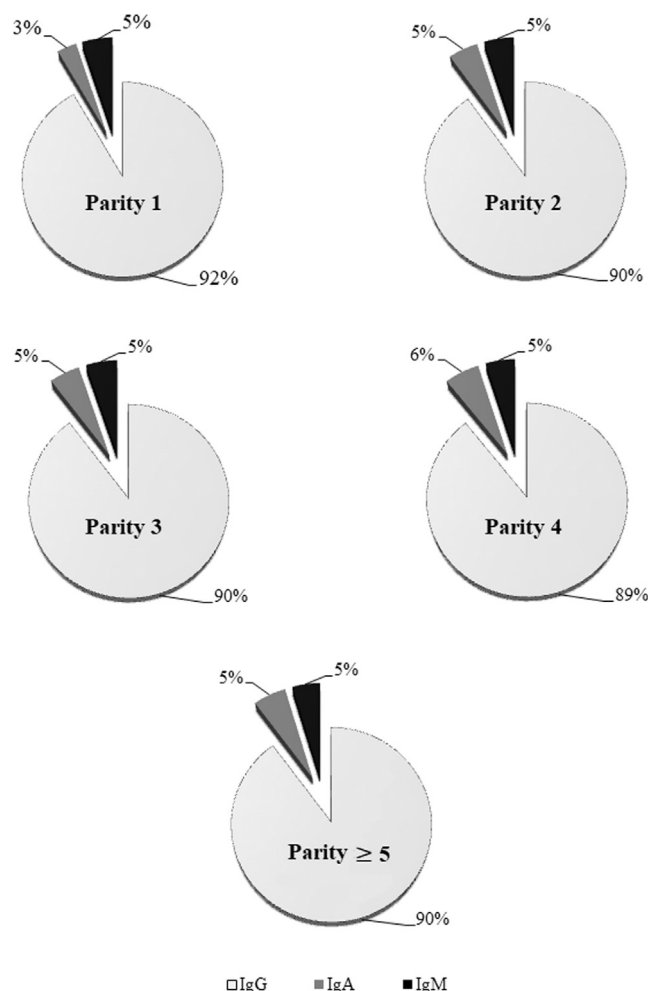
where  $\bar{x}_i$  and  $s_i$  are the mean and the SD of the five concentrations available for the same sample. The intra-assay  $CV_{RID}$  was 7.56, 2.46, 3.03, and 3.53% for IgG, IgA, IgM, and BSA. Based on [Homburger and Singh \(2008\)](#) and considering the guidelines of the [US Department of Health and Human Services, Food and Drug Administration \(2001\)](#), coefficients <10% are considered precise enough and, in the present study, allowed using a single well per each sample for the determination of colostrum Ig and BSA. Thus, each plate could host 21 colostrum samples, as three wells hosted the reference sera.

### Analysis of variance

Samples of cows different than Holstein were also present in the original dataset, but they were removed prior to statistical analysis as they represented only few cases. The same was done for a limited number of colostrum samples ( $n = 18$ ) that were collected between June and August 2020. After editing, data consisted of 678 samples of purebred Holsteins (one sample per cow). Calvings occurred from April 2019 to May 2020 and thus covered the whole seasonal variability. In particular, six bimonthly classes of calving season were identified: (1) December–January, (2) February–March, (3) April–May, (4) June–July, (5) August–September, and (6) October–November. Parities were classified as 1, 2, 3, 4, and  $\geq 5$ , with the latter including cows with 5–8 calvings; all parity



**Fig. 1.** Distribution of immunoglobulins (i) G, (ii) A, and (iii) M, and (iv) bovine serum albumin (BSA) concentration (g/L) in cow colostrum. The number of records was 676, 573, 658, and 614 for immunoglobulins G, A, and M, and BSA, respectively.



**Fig. 2.** Partition of immunoglobulins G, A, and M in colostrum of cows of different parities.

classes were represented ( $\geq 3$  cows) in all the nine farms. Frequency of observations for each herd, seasonal class, and parity is reported in Table 2.

Data were analyzed using the GLM procedure of SAS v.9.4 (SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + S_i + P_j + Y_k + H_l + (S \times P)_{ij} + (P \times H)_{jl} + e_{ijkl},$$

where  $y$  is the phenotypic observation of IgG, IgA, IgM, IgTOT, or BSA;  $\mu$  is the intercept of the model;  $S_i$  is the fixed effect of the  $i$ th bimonthly calving season (six levels);  $P_j$  is the fixed effect of the  $j$ th parity (five classes);  $Y_k$  is the fixed effect of the  $k$ th year of calving (2019 and 2020);  $H_l$  is the fixed effect of the  $l$ th herd (nine levels);  $(S \times P)_{ij}$  is the fixed interaction effect between season of calving and parity class;  $(P \times H)_{jl}$  is the fixed interaction effect

between parity class and herd; and  $e_{ijkl}$  is the random residual  $\sim N(0, \sigma_e^2)$ , where  $\sigma_e^2$  is the residual variance. The year of calving was included to account for the fact that April and May were present in both 2019 and 2020. In all the nine herds, at least three calvings took place in each bimonthly seasonal class.

Differences between least squares means (LSM) were tested using the Bonferroni multiple comparison *posthoc* test ( $P < 0.05$ ). Pearson's correlations between traits and data distributions were obtained using the CORR and the UNIVARIATE procedure of SAS software v. 9.4 (SAS Institute Inc., Cary, NC), respectively.

## Results

The diameter of the RID rings averaged 78.58, 59.69, 57.79, and 53.21 mm for IgG, IgA, IgM, and BSA. The distributions of the traits are reported in Fig. 1 and their proportions in colostrum of cows of different parities are depicted in Fig. 2. All the investigated traits followed a normal distribution (Fig. 1). Descriptive statistics of the three Ig, IgTOT, and BSA are shown in Table 3; the most variable trait was IgA with CV equal to 63.42% while the least variable feature was IgTOT (34.72%).

Overall, the three Ig fractions were positively correlated to each other in all parities (Table 4). In particular, IgTOT showed a correlation close to unity with IgG in all parity orders and in general the weakest (0.388) and the strongest (0.648) correlations were estimated between IgG and IgA in parity 1, and between IgG and IgM in parity 2, respectively. Associations of BSA with Ig were moderate to low, with the strongest correlation (0.555) estimated with IgTOT in parity 4.

The coefficients of determination for IgG, IgA, IgM, IgTOT and BSA were 0.29, 0.36, 0.21, 0.33, and 0.19, respectively, meaning that a large part of variation of these traits remained unexplained. The analysis of variance revealed a significant effect of parity ( $P < 0.001$ ) and herd ( $P < 0.001$ ) on all the traits, while month of sampling significantly ( $P < 0.05$ ) affected IgG, IgM, IgTOT, and BSA, but not IgA ( $P = 0.103$ ). In particular, IgG was less concentrated in colostrum of first- and second-parity cows compared with later parities. The IgA was the lowest in primiparous cows and intermediate in cows of parity 2 (Fig. 3). In the case of IgM, the concentration was the greatest in cows of parity 3, the lowest in parities 1 and 2, and intermediate in parities 4 and  $\geq 5$  (Fig. 3). In general, the LSM of IgTOT for parity 1 was the lowest and differed significantly from those of later parities (Fig. 3).

In general, Ig and BSA peaked in August–September, but comparisons with other bimonthly calving seasons highlighted some significance only in the case of IgG, IgM, and BSA (Fig. 4). The interaction between parity and month of sampling affected only IgTOT and BSA ( $P < 0.05$ ).

In colostrum sampled in the first 6 h after calving, Ig and BSA concentrations varied among the nine herds (Fig. 5). For example, IgTOT ranged from 91.61 g/L (herd B) to 120.32 g/L (herd E), and the LSM of herd B differed from those of herd E and F at  $P < 0.10$ . The LSM and IgA of herd H (3.53 g/L) differed significantly from IgA of herds 1, 3, 5, 6, and 9 ( $P < 0.01$ ; Fig. 5). The interaction between fixed effects of parity

**Table 3**  
Descriptive statistics of the cow colostrum traits.

Trait <sup>1</sup> , g/L	N	Mean	CV, %	Minimum	Maximum
IgG	676	91.31	39.56	0.68	216.70
IgA	573	4.20	63.42	0.13	22.14
IgM	658	5.05	48.52	0.18	14.01
IgTOT	525	105.99	34.72	20.37	228.59
BSA	614	2.47	50.73	0.18	7.73

<sup>1</sup> IgG = immunoglobulins G; IgA = immunoglobulins A; IgM = immunoglobulins M; IgTOT = sum of the immunoglobulin fractions (G, A, and M); BSA = bovine serum albumin.

**Table 4**Pearson's correlations ( $P < 0.05$ ; ns = not significant) between colostrum traits<sup>1</sup> in the whole dataset and within different cow parities.

Parity	Trait, g/L	IgA	IgM	IgTOT	BSA
All	IgG	0.537	0.607	0.994	0.321
	IgA		0.508	0.607	0.230
	IgM			0.670	0.294
	IgTOT				0.328
1	IgG	0.388	0.581	0.995	0.342
	IgA		0.409	0.444	0.276
	IgM			0.642	0.214
	IgTOT				0.324
2	IgG	0.579	0.648	0.995	0.353
	IgA		0.518	0.634	0.262
	IgM			0.698	0.320
	IgTOT				0.330
3	IgG	0.446	0.630	0.994	0.157 <sup>ns</sup>
	IgA		0.442	0.516	-0.026 <sup>ns</sup>
	IgM			0.640	0.240
	IgTOT				0.114 <sup>ns</sup>
4	IgG	0.552	0.564	0.991	0.389
	IgA		0.595	0.625	0.350
	IgM			0.664	0.323
	IgTOT				0.555
≥5	IgG	0.598	0.456	0.992	0.320
	IgA		0.558	0.669	0.319
	IgM			0.534	0.395
	IgTOT				0.459

<sup>1</sup> IgG = immunoglobulins G; IgA = immunoglobulins A; IgM = immunoglobulins M; IgTOT = sum of the immunoglobulin fractions (G, A, and M); BSA = bovine serum albumin.

and herd (Fig. 6) was significant only for IgA ( $P < 0.001$ ) and approached significance for IgG ( $P = 0.056$ ).

## Discussion

In this study, samples of first colostrum were collected within 6 h from calving, following Strekozov et al. (2008). Concentrations of Ig were characterized by high variability (Table 3), due to the progressive and fast change in colostrum composition in the first hours after calving (Schuenemann, 2015). In fact, IgG in bovine colostrum is maximum immediately after calving and then linearly decreases for physiological reasons together with the ability of calf's gut to absorb molecules. The permeability of the intestinal tract in calves is maximum in the first 6 h after birth to maximize the passive transfer of Ig from the dam colostrum to the calf blood. Serum IgG < 10 mg/mL in the first 48 h of life indicates a failure of the passive transfer, a condition that exposes newborn calves to several diseases (Weaver et al., 2000; Godden, 2008) and impairs the survival rate (Gomez and Chamorro, 2017). Other than the '3Q', the presence of a dedicated person in charge of colostrum feeding and calf care in the first hours after birth and the availability of specific facilities for feeding could make the difference in transfer of passive immunity. Unfortunately, there are no indirect and rapid tools able to accurately determine blood IgG in calves in field conditions, while the refractometer is commonly used for indirect and precise determination of colostrum IgG level (Soufli et al., 2019).

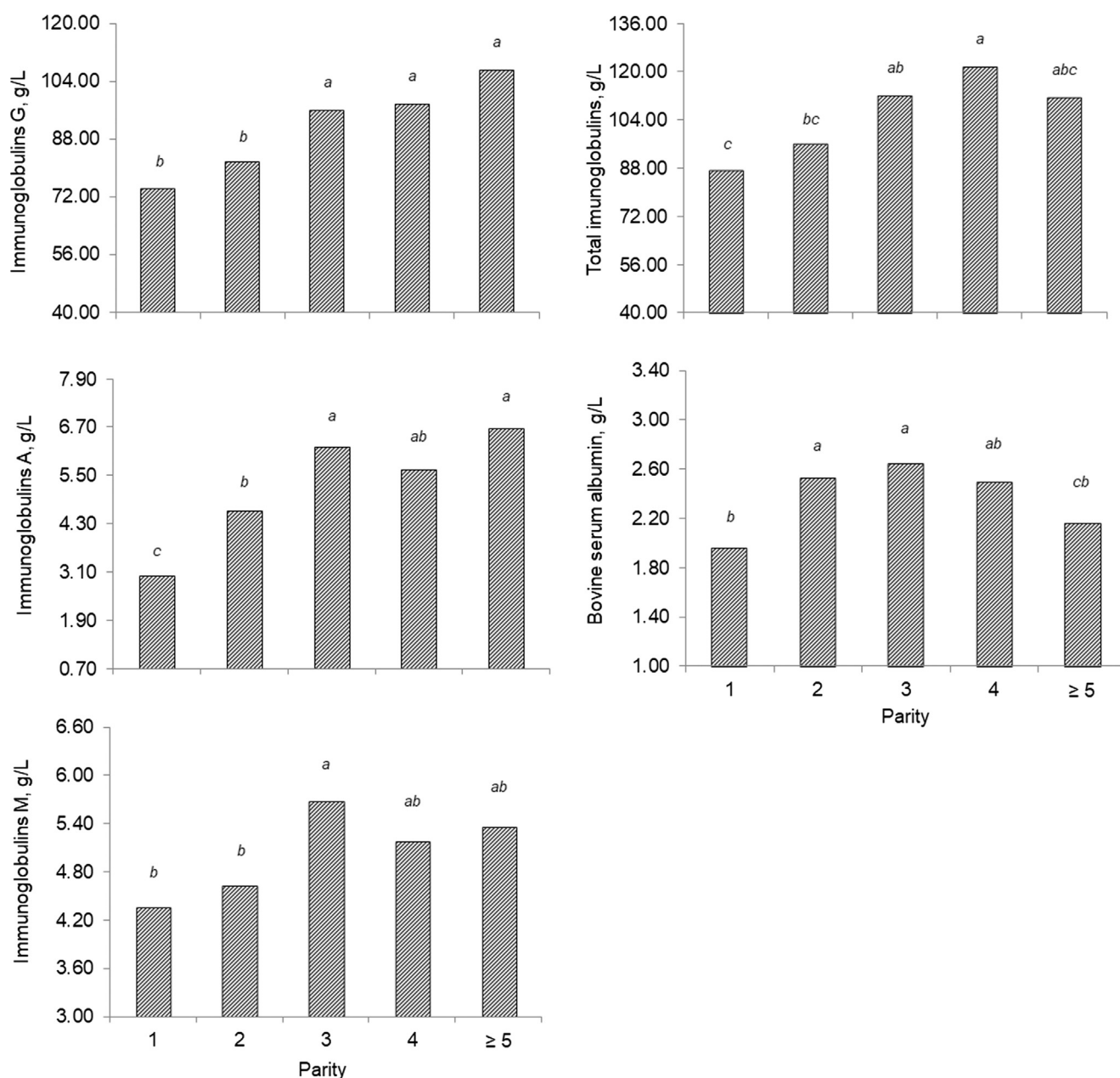
McGrath et al. (2016) reviewed studies on colostrum quality and reported that IgTOT varies from 30 to 200 g/L. The average IgG concentration of the present study is similar to that (98.36 g/L) reported by Ciniti et al. (2019) in Simmental cows sampled within 6 h from calving. Dunn et al. (2017) used the ELISA method to measure the IgG concentration in colostrum of 1 239 cows from commercial dairy farms in Northern Ireland and they reported an average of 55.00 g/L; in that study, sampling and quantification

method differed compared to those of the present study and only 7.23% of samples had IgG > 90 g/L. Cabral et al. (2016) reported RID IgG to vary from 21.4 to 141.4 g/L in 111 multiparous Holsteins, but the collection time was wide, ranging from 1 to 14.5 h after calving. In US, Morrill et al. (2012) observed an average IgG of 68.8 mg/mL in 827 colostrum samples from different farms. In that study, samples were stored under different conditions and belonged to different breeds, explaining why authors found a very large SD for IgG (32.9 mg/mL). Also, in the paper of Bartier et al. (2015), the average IgG determined through RID in 460 samples (63.7 mg/mL) was lower compared to the present study. The average IgG concentration of the current study (Table 3) was comparable to the results of Le Cozler et al. (2016) who reported average IgG1 concentration of 54.1 g/L in colostrum samples of Holstein cows ( $n = 77$ ). In this regard, it is important to highlight that cow colostrum shows IgG1 and IgG2 in a concentration of 80% and 20% of total IgG, respectively. In the present study, the adopted RID assay was able to detect the overall IgG, without discriminating between the two forms.

According to the literature, there is variability in terms of IgG across determination methods commercially available (Gapper et al., 2007). Some authors investigated colostrum Ig with ELISA assay; among these, Taniguchi et al. (2016) found average concentration (SD) of IgG, IgA, and IgM of 138.5 (32.6), 7.5 (8.9), and 9.3 (4.9) mg/mL in 24 samples of Japanese black multiparous cows.

According to the few studies available in the literature, there is apparently no effect of storage condition on Ig concentration. Cummins et al. (2017) observed a similar IgG concentration in colostrum stored under different conditions and Morrill et al. (2012) reported similar IgG concentration in fresh, refrigerated and frozen colostrum. Despite this, in both papers, colostrum kept at warmer temperatures was characterized by greater bacteria concentration, but Cummins et al. (2017) observed that there was no difference in the absorption of IgG by calves in fresh colostrum and colostrum stored at 4 °C for 2 d. As suggested by





**Fig. 3.** Least squares means of cow colostrum immunoglobulins G, A, and M, their sum, and bovine serum albumin concentration for the fixed effect of parity. Letters indicate significantly different means ( $P < 0.05$ ).

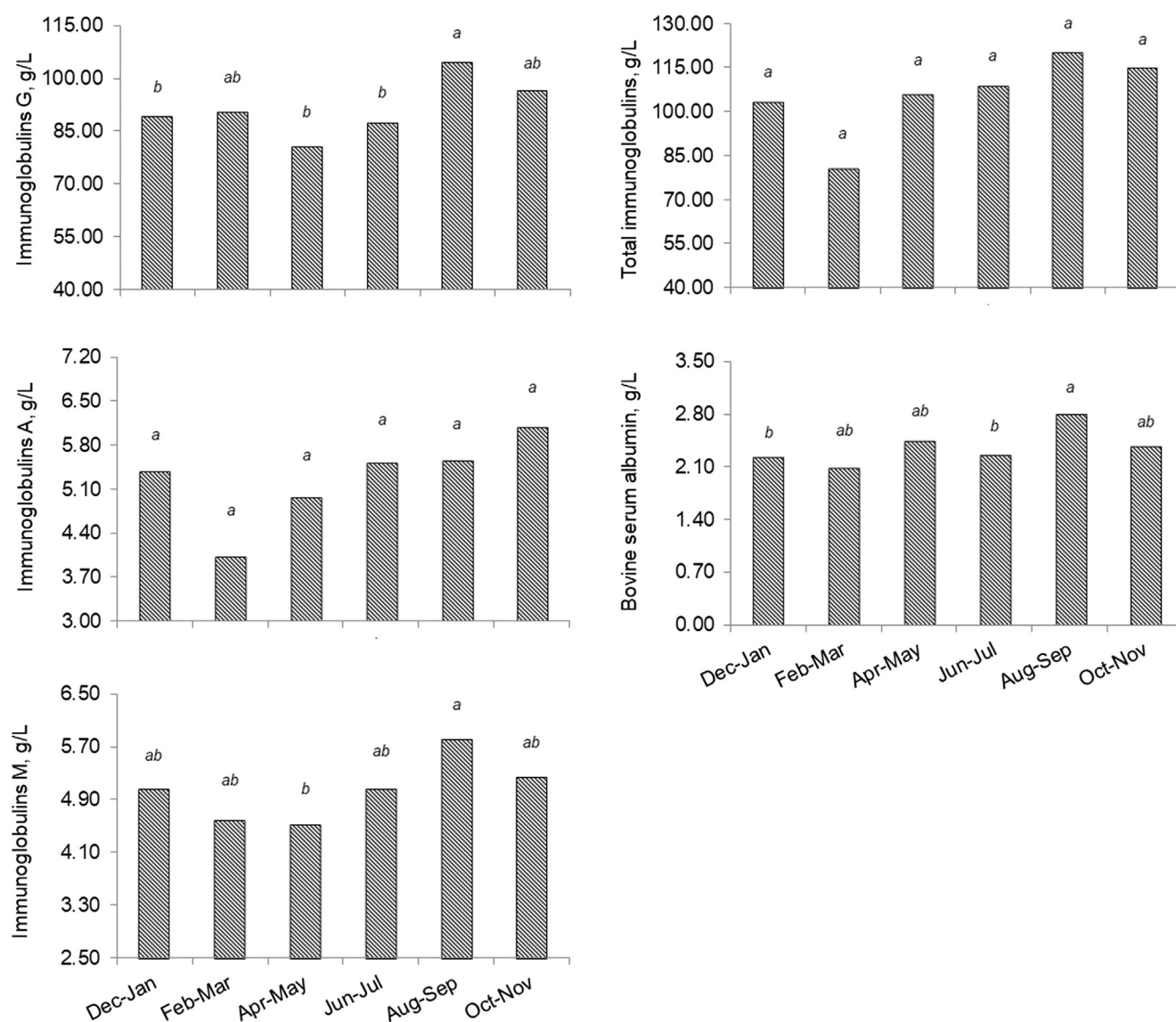
Balthazar et al. (2015), the thawing method can affect colostrum IgG and, regardless of the method used, a loss of around 20% in terms of IgG concentration is expected after thawing. In fact, authors demonstrated that bain-marie at 40 °C was at the same time the most laborious and the most conservative method (IgG). Authors concluded that microwaving for 30 min (200 W) is recommended for frozen samples characterized by a good initial quality level, since the final concentration of IgG after thawing was still greater than 50 g/L. Moreover, Balthazar et al. (2015) reported the presence of clots with bain-marie temperatures >60 °C.

Saldana et al. (2019) investigated the effect of heating on several colostrum traits, including IgG determined by RID. They applied different heating treatments on three different colostrum pools (low, medium, and high quality) and findings revealed that, regardless of the quality level, the IgG concentration was the great-

est in unheated colostrum and the lowest in colostrum heated to 60 °C for 60 min. However, unheated colostrum showed the greatest amount of bacterial population compared to colostrum treated at 60 °C for 30 and 60 min.

Colostrum BSA is an indicator of permeability of milk-blood barrier and this is the first study providing information on this feature on a large sample size. The average BSA concentration ( $2.47 \pm 1.25$  g/L; Table 3) was twice the one ( $1.20 \pm 0.50$  g/L) reported by Samarütel et al. (2016). This difference is likely due to the limited sample size (31 cows), a different window allowed for colostrum sampling, and the different method used to determine BSA in the study of Samarütel et al. (2016).

Based on Fig. 2, the contribution of the three Ig fractions was almost stable across the different lactations, with IgG being the major Ig. A similar pie chart for bovine colostrum has been

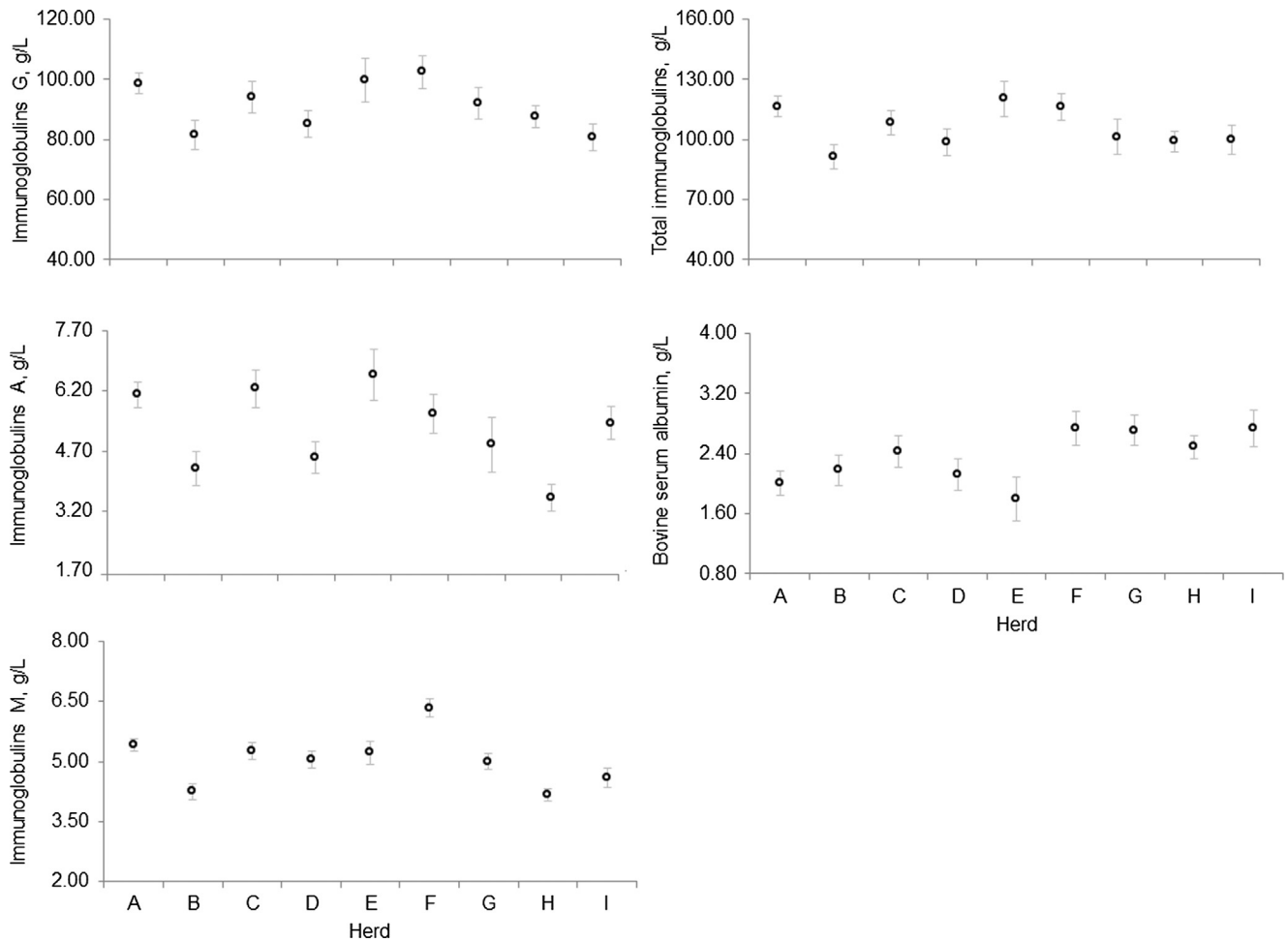


**Fig. 4.** Least squares means of cow colostrum immunoglobulins G, A, and M, their sum, and bovine serum albumin concentration for the fixed effect of month of sampling. Letters indicate significantly different means ( $P < 0.05$ ).

reported by Hurley and Theil (2011), but not within parity. Basically, Fig. 2 suggested that there is no change in the colostrum Ig fractions across parities and that phenotypic and genetic strategies aimed at improving quality of colostrum in all lactations could focus on IgG only. This is also supported by the associations between the traits (Table 4). As regards correlations, a comparison with the literature was difficult due to lack of papers dealing with IgA and IgM other than IgG. The three fractions were moderately correlated, suggesting that quality of colostrum can be likely pursued by indirect strategies focused on a single fraction, like IgG. Segura et al. (2020) assessed correlations between IgG, IgA, and IgM determined with ELISA kit in colostrum of 56 sows collected within the first 24 h postpartum; those authors reported a significant correlation only between IgG and IgM (0.995). Colostrum BSA was weakly correlated with Ig; this was expected since Ig and BSA have different synthesis pathways, physiological roles, and transport mechanisms. In a smaller number of cows, Samarütel et al. (2016) estimated a significant correlation (0.38) between colostrum BSA and IgG2, but the association between BSA and IgG1 was not significant. The positive correlation estimated between

BSA and Ig was expected, since both are affected by the blood-milk barrier permeability level.

The differences in Ig observed between cows of the first two parities and those in later parities might be the result of both a greater experience of immune system and a wider serum Ig heritage in old than young cows. The effect of parity on bovine colostrum quality has been reported in several studies and there is an overall consensus on the inferiority of first-parity cows for the Ig concentration compared with cows in later lactations. For instance, Biemann et al. (2010) reported better colostrum quality in multiparous cows and observed that primiparous had the greatest number of samples with IgG below the ideal quality cut-off (50 g/L). In general, literature shows that the colostrum of third- and fourth-parity cows tend to have greater density (1.068 vs. 1.059 kg/m<sup>3</sup>) and greater concentration of solids than colostrum of heifers (Strekozov et al., 2008; McGrath et al., 2016). Some farmers tend to discount and discard colostrum of heifers as it is often considered of low quality and not adequate for the farm colostrum bank (Kehoe et al., 2007; 2008). According to the present study, all colostrum should be tested with rapid tools, like refractometers,



**Fig. 5.** Least squares means of cow colostrum immunoglobulins G, A, and M, their sum, and bovine serum albumin concentration for the fixed effect of herd. Standard errors range from 3.46 to 7.32, 0.31 to 0.69, 0.25 to 0.54, 5.00 to 8.89, and 0.16 to 0.29 g/L, respectively.

regardless of the cow parity in order to avoid suboptimal choices for colostrum bank and failure of passive transfer. However, similarly to the current study, [Shivley et al. \(2018\)](#) did not observe differences between first- and second-lactation cows in terms of IgG measured with RID (72.3 vs. 72.0 g/L, respectively). Nevertheless, supporting findings of the present study, the same authors reported that IgG concentration in colostrum of cows in parities 1 and 2 was significantly lower than that in later parities. This is the first study aimed at investigating variability of IgA and IgM measured with RID across parities in cattle.

As regards month of calving, the trends of all traits were similar, i.e., they tend to peak in late summer-early autumn. However, the high standard errors of LSM did not allow to detect significant differences for IgA and IgTOT ([Fig. 4](#)). Supporting these findings, [Strekozov et al. \(2008\)](#) observed that the density of colostrum was maximum in autumn for both primiparous and multiparous cows and that the greatest proportion of 'very good' colostrum samples, i.e., those with IgG > 100 g/L was collected in autumn. A potential good explanation for this result is that colostrum quality tends to be greater in autumn and winter due to a dilution effect caused by a reduction in colostrum yield. In fact, the volume of colostrum excreted by the mammary gland is reported to be usually lower in such a period ([Conneely et al., 2013; Godden et al., 2019](#)). These findings agree with the results of [Gavin et al. \(2018\)](#), who observed that colostrum yield is strongly correlated with the photoperiod before and around calving in Jersey cattle.

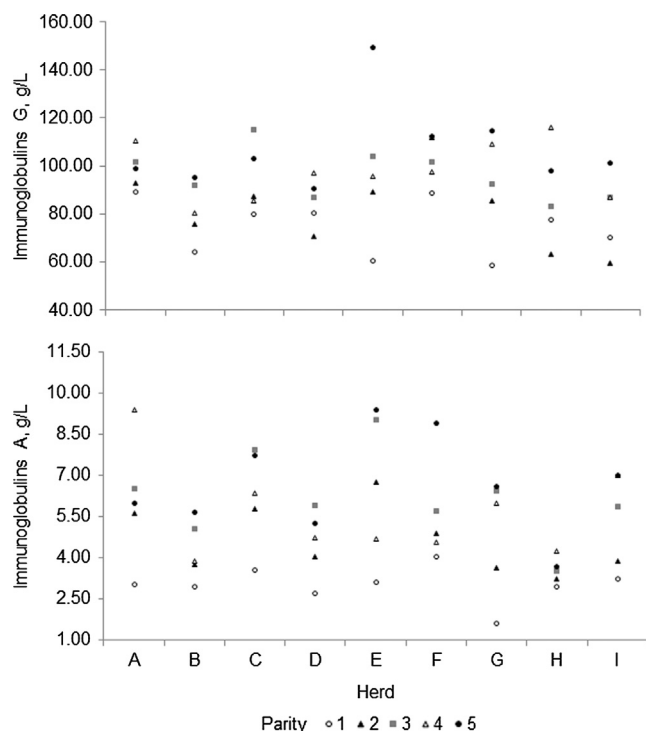
Those authors concluded that the amount of colostrum produced by the cow depends on physiological and hormonal regulations in cattle; in particular, the yield is greater when the photoperiod increases, i.e., when the hours of light increase at the expense of hours of darkness (spring to summer in the northern hemisphere).

The presence of a dilution effect could not be verified in the present study, since the volume of colostrum yielded by each cow was not recorded. However, it is important to highlight that recording the first colostrum yield with good accuracy and in a standardized manner in field conditions is quite a challenge and may require trained personnel. In fact, [Cabral et al. \(2016\)](#) reported a very large variability in colostrum yield (from 0.5 to 39.7 kg) recorded by nine farmers and in the collection time (from 1 to 14.5 h after calving).

The fixed interaction effect between parity and season of calving was not significant for the three Ig fractions G, A, and M, suggesting no relevant differences in terms of concentration among seasons of calving within parity. On the other hand, the interaction was significant for IgTOT and BSA ( $P < 0.05$ ). In particular, in all parities, the concentration of IgTOT tended to increase from summer to autumn and in all seasons, cows in first two parities clustered from the others.

In general, colostrum quality differed across the herds involved ([Fig. 5](#)), with slight differences in the rankings among the three fractions. The IgTOT was the greatest in herd E, which was in the 'top 2' for IgG and IgA, and in fourth position for IgM ([Fig. 5](#)). Finally, for IgG and IgA, there were differences across herds among





**Fig. 6.** Least squares means of cow colostrum immunoglobulins G and A concentration for the interaction effect between parity and herd. Standard errors range from 5.20 to 19.43 and 0.47 to 2.68 g/L, respectively.

cows of same parity (Fig. 6), suggesting that the 'baseline' of colostrum quality of a farm depends on its specific management, environmental factors, and also genetics of the herd. Related to this aspect, the herd effect included in the statistical model of the current paper accounted for, among others, the effects of dry period diet and management. In fact, within herd, the composition of the diet is the same for all cows close to calving. Literature shows that colostrum Ig levels can be considered independent from the cow energy balance at calving and are apparently not affected by dietary supplementation of conjugated linoleic acids (Eger et al., 2017). Nevertheless, using data of 16 calves, Żarczyńska et al. (2019) demonstrated that selenium supplementation in late gestation enhances passive transfer from dam to calf and colostrum IgG. However, further research is needed to extend such finding to other cattle breeds, feeding systems, and environments.

## Conclusions

In the present study, sources of variation of the three Ig fractions and BSA concentrations of bovine colostrum determined using RID were investigated on a large number of samples. Overall, the Ig concentration was lower in colostrum of first- and second-lactation cows compared with subsequent parities, likely due to a greater experience of the immune system and a wider Ig heritage of older than younger cows. Both Ig and BSA tended to be greater in autumn and there were differences in terms of colostrum quality among the nine herds involved, suggesting that management and environment play a key role in the determination of the Ig level. Considering that the model explained only part of the variability of the studied traits, further potentially useful information might be recorded for future investigations, like colostrum yield, milk productivity level in previous lactation, temperature-humidity index, dry period length and diet, and genetic merit of the cow.

## Ethics approval

Not applicable.

## Data and model availability statement

None of the data were deposited in an official repository. The data that support the findings presented in this study are available from the first author upon reasonable request.

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## Declaration of interests

None.

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