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REGULAR ARTICLES

Calf's sex, parity and the hour of harvest after calving affect colostrum quality of dairy cows grazing under high tropical conditions

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Abstract High-quality colostrum is an important factor influencing neonatal calf health, and quality assessment is essential to obtain good health results. This research evaluated the effects of the calf's sex, the parity of the cow and the hour of colostrum harvest after parity on the fat, nonfat solids, protein and Ig contents in Holstein colostrum for cows under high grazing conditions in the tropics. The effects of the calf's sex and parity on somatic cell count (SCC) at the first milking postpartum were determined. A comparison was made between a laboratory method and a farm method for the estimation of the fat and protein content of colostrum. Thirty-three cows were sampled in the study. The calf's sex was shown to have an effect on the amount of colostrum, on the concentration of fat, and on the amount of milk produced by lactating Holstein cows; all were higher in cows that gave birth to a female calf. Colostrum protein decreased after the first hour postpartum, and the Ig concentration had a tendency to decrease after 4 h. The cows that had parity 1–2 had lower Ig concentrations and total production of Igs, and higher SCC at the first milking postpartum. Ekomilk was a reliable method to measure the colostrum fat on the farm.

Keywords Cows · Fat · Holstein · Immunoglobulins · Protein

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Introduction

The colostrum is the first lacteal secretion obtained from mammals postpartum and is of special importance for ruminants because they do not have an exchange of immune factors in utero; the colostrum provides protection with a high amount of immunoglobulins, without which the ruminant would not survive (Stelwagen et al. 2009). Previous studies have demonstrated that the optimal passive transfer of immunoglobulins to calves through the colostrum occurs within the first 4-h postpartum, gradually declines until hour 24 when it stops and the maximum absorption in the calf's gut occurred in the first 6 h of life (Godden et al. 2009). In addition to the importance of colostrum for immune protection, it is recognised for its important nutritional contribution to the newborn. The fat and protein content of colostrum are important to neonatal calves for their adaptation, development and growth. Several studies demonstrated that calves cannot make fat from carbohydrates very effectively and any increase in adiposity must be from dietary fat intake (Joost et al. 2007). Thus, under cold stress conditions or situations where feed intake is compromised because of illness, the only way to provide greater calories and energy reserves is through the increased intake of dietary fat. Similarly, the protein in colostrum not only consists of the immunoglobulins (Ig) but also of bioactive components such as major milk proteins, hormones, growth factors and cytokines Sobczuk-Szul et al. (2013). Unfortunately, many producers continue to incur significant losses associated with the poor management of colostrum use. In addition, there are different reports of colostrum quality from cows under indoor and temperate environments, and there is a lack of information about colostrum quality under tropical grazing conditions. Additionally, farmers need a method to easily measure colostrum quality (nutritional composition) on farms because the current commercial options for

colostrum measurement have not had their validity amply demonstrated under high tropical grazing conditions.

This study evaluated the following: (a) the effects of the calf's sex, the parity of the cow, and hour of colostrum harvest after parity on the fat, nonfat solid (NFS), protein and Ig contents in Holstein cow colostrum under high tropical grazing conditions; (b) the effects of the calf's sex and parity on the somatic cell count (SCC) at the first milking postpartum and (c) a comparison between a laboratory method and a farm method for estimating the fat and protein contents of colostrum.

Materials and methods

Facilities

All experiments were conducted on the research farm property of the Solla S.A. Company, which is located in Santa Rosa de Osos at 2600 m above sea level and close to Medellin (Colombia). The average temperature is 18 °C, which varies between 4 and 27 °C, the relative humidity is 70 %, and the annual precipitation is 2400 mm. All characteristics belong to the Forest Very Humid Mountain Low category according to the Holdridge score (1967).

All experimental procedures have been carried out in accordance with the EU Directive 2010/63/EU for animal experiments and have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Analyses

Two analyses were conducted. The first one evaluated the effects of the calf's sex, parity of the cow and hour of colostrum sampling after parity on the fat, NFS, protein and Ig contents in Holstein cow colostrum under high grazing conditions in the tropics. In addition, the commercial methodology used by farmers, known as Ekomilk, to evaluate the colostrum quality on the farm was evaluated. Furthermore, the effects of the calf's sex and parity on the SCC were determined in the laboratory for the first milking postpartum only. The second analysis compared the results for protein and fat contents measured with a portable, reliable and easy method commonly used on farms known as Ekomilk with the results measured with a laboratory method known as Milkoscan. Colostrum is very thick, and not all samples could be analysed in the laboratory. Thirty-three samples were measured at the first milking postpartum by Ekomilk, but only 19 samples could be measured with Milkoscan. Therefore, for the second analysis, the 19 samples that could be determined by both methods were analysed.

Animals—management and feeding

The data were obtained from 33 Holstein cows that were selected based on parity and age (17 with parity 1–2, 36.3± 8.7 months of age and 16 with parity ≥3, 68.4±8.1 months of age). All of them were from rainy season (maximum 2 months between parity). The average weight was 530± 58 kg, and the average body condition score was 3.5±0.2 (1–5 scale according to Ferguson et al. 1994). There were 33 calves: 17 males and 16 females.

For the experiment, all the animals were handled similarly, with grazing everyday in *Pennisetum clandestinum* Hochst ex Chior (kikuyo), mineral supplementation, 2 kg of concentrate and ad libitum water. In the last month of pregnancy, all cows received supplementation with prepartum concentrate under the following scheme: (1) between the fourth and third antepartum week, 1 kg of concentrate was given to the cows; (2) between the third and second antepartum week, 2 kg of concentrate was given to the cows; (3) between the second and first antepartum week, 3 kg of concentrate was given to the cows and (4) 4 kg of concentrate was given to the cows during the last week. Concentrate was provided once daily in plastic feeders in the paddock. The cows were in the same grazing paddock during 1 day and night, there were 43 grazing paddock, and the cows come back to the same paddock after approximately 42 days. The pre-grazing herbage mass was approximately 3.25 kg/m^2 with 20 % of post-grazing mass. The herbage mass (cut to 12–15 cm above the ground) was estimated by the double sampling method (Haydock and Shaw 1975) during the same day of grazing, before and after the cows come into the new paddock. Herbage samples for composition analysis were taken before the cows come into the new paddock. The herbage and concentrate compositions are shown in Table 1.

Colostrum and milk sampling

Almost immediately after parity, all the cows were milked thoroughly to obtain the colostrum to supply their calves and

Table 1 Grass and concentrate composition (%)

	Grass	Concentrate
Crude protein (CP)	23.13	14.00
Crude fibre		7.00
Neutral detergent fibre (NDF)	58.47	24.80
Acid detergent fibre (ADF)	25.57	10.20
Lignin	2.06	
Ether extract (EE)	3.23	5.50
Ashes	9.06	5.18
Net energy of lactation (NEL)(Mcal/kg)	1.15	1.50
In vitro dry matter digestibility	73.11	

to obtain samples for analyses. Colostrum was measured in litres. Samples were collected at 0, 1, 2, 3, 4, 5 and 6 h postpartum. The first sample of colostrum corresponded to the complete first milking of the cows. The next milkings were obtained by collecting approximately 250 mL of colostrum every hour until 6 h after parity. During lactation, the cows were milked twice per day in the milking parlour and the milk yield was measured by using an automatic recording system in kilogrammes (MM27BC, DeLaval International AB, Sweden).

Measurement of total immunoglobulin concentration

The colostrum was brought to a temperature of 21 °C for the determination of Ig (0 to 6 h after parity) according to the scale marked in a colostrometer (TM, Biogenics 1980).

Colostrum quality

The nutritional quality of the colostrum was determined by measuring the % fat, % protein and nonfat solids (NFS) at 0, 1, 2, 3, 4, 5 and 6 h after parity, using an Ekomilk analyser (Milk Analizer KAM98-2A, Bulteh 2000 Ltd). For comparative analysis of the methods (Ekomilk vs. Milkoscan), the same samples were transported from the farm to the reference laboratory in tubes cooled at 4 °C and the milking postpartum was analysed using Milkoscan equipment. Milkoscan was also used to measure SCC.

Analyses of data

The PROC MIXED option of the Statistical Analysis System software package (Institute SAS) was used to test the significance of the fixed effects of the calf's sex, parity, hour of colostrum sampling and interaction between calf's sex and parity on colostrum fat, protein, NFS and Ig, in a model with cow considered to be a random effect. The SCC were analysed for the same effects within an hour of sampling. The degree of substitutability between methodologies for determination of fat and protein in the colostrum was assessed using the test of Bland–Altman (Bland and Altman 1986). For the means, $P \leq 0.05$ was statistically significant, whereas $P \leq 0.1$ was considered to be a tendency.

Results

Considering the effects of sampling time, the animal's sex, parity and using an Ekomilk analyser, the colostrum had an average fat content of 5.82±0.21 (g/100 mL), a NFS content of 18.23±0.28 (g/100 mL), a protein content of 6.65±0.11 (g/100 mL) and Ig of 65.52±1.93. SCC (cell/mL) was only measured at the first milking postpartum and had an average of 1936.8±731.62 cells/mL.

The results of the different effects are presented in Table 2. There were significant effects of sex and parity on colostrum fat but not for protein or NFS. The fat concentration was higher in the colostrum of cows with a female calf and in the younger cows, but was stable over time. The cows with a female calf produced higher amounts of colostrum and milk ($P<0.05$) during lactation than cows with a male calf (colostrum, 6.75 and 5.87 L and milk 6805 and 6486 kg, respectively, $P<0.05$). The cows with parity 1–2 produced lower amounts of colostrum than the cows with parity ≥3 (6.1 vs. 7.3 l, respectively, $P<0.05$). The protein and NFS concentrations decreased significantly after the first hour of sampling. The Ig concentration changed significantly with the sex of calf and was higher for cows with a male calf, but there were no differences for total Ig (mg). Parity also affected Ig concentration and total Ig (mg) and was higher for cows with parity ≥3. There was a tendency for Ig to decrease in concentration after 4-h postpartum. There was a tendency for an increase in the RCS in the colostrum of younger cows. Interaction between calf's sex and parity was not significant $P>0.05$.

With the interchangeability test of Bland–Altman, the regression slopes (β) were not significant for fat ($P>0.05$), but there was a tendency for protein. These results suggested that the laboratory method (Milkoscan) is interchangeable with the farm method (Ekomilk) for fat but perhaps not for protein. The results are shown in Table 3. A linear relationship between Milkoscan and Ekomilk methodologies was established to estimate fat (Fig. 1). A high coefficient of determination was obtained (97.9 %).

Table 2 Effect of calf's sex, parity and harvest time on colostrum composition of dairy cows grazing under high tropical conditions

Effects	Fat (%)	NFS (%)	Protein (%)	Ig (mg/mL)	Ig (mg)	SCC
Sex of calf						
Females	6.61	18.27	6.68	61.12	407	2849
Males	5.02	18.25	6.66	69.26	412	1300
P value	0.00	0.98	0.95	0.01	0.35	0.32
Parity						
1–2	6.63	17.97	6.55	61.98	397	3883
≥3	5.10	18.55	6.78	68.98	421	266
P value	0.00	0.30	0.32	0.03	0.05	0.07
Harvest time (h)						
0–1	5.92a	19.83a	7.31a	72.75a		
2–3–4	5.80a	18.23b	6.66b	72.98a		
5–6	5.74a	16.72c	6.04c	62.08b		
P value	0.94	<.00	<.00	0.06		

Averages with different letters in the same column for the same parameter represent significant differences ($P \leq 0.05$)

NFS notfat solids, *SCC* somatic cell count

Table 3 Comparison of fat and protein contents of colostrum using the Milkoscan or the Ekomilk method

	Colostrum fat (%) (first milking postpartum)	Colostrum protein (%) (first milking postpartum)
N	19.00	19.00
Milkoscan	4.73	15.09
Ekomilk	5.21	7.30
SE	0.46	0.73
P value β	0.20	0.09

Discussion

Colostrum—major nutrients

The values for colostrum fat in this study were similar to those reported by Morrill et al. (2012) (n=531, fat=5.6 %) and by Sobczuk-szul et al. (2013) (n=256, fat=5.7 %) for the first milking postpartum. The cows with a female calf produced higher amounts of colostrum with a higher concentration of fat than cows with a male calf. Considering the importance of this nutrient for calves, these results could indicate a survival mechanism that favours females. Calves use fat from colostrum for heat production to maintain a constant body temperature, which is particularly important in cold weather (Joost et al. 2007). Parity also affected colostrum fat concentrations, which were higher in the younger cows with lower production of colostrum so that the fat was more concentrated.

Protein and NFS concentrations began to decrease after the first hour of sampling and were reduced by 17 % at 5–6 h postpartum. Because the Ig only changed after 4-h postpartum, an early decrease in the percentage of total protein could be related to changes in casein as a primary protein, but this study did not measure caseins independently to confirm this hypothesis. Sobczuk-szul et al. (2013) found reduced k-casein concentrations in the colostrum of Jersey and Polish Holstein–Friesian cows after the first milking postpartum. These authors cited this most likely resulted from a decrease in casein glycopeptides in the κ-casein structure because this component is only presented immediately postpartum. Among the components of casein, κ-casein is the only glycosylated protein that was identified by Inagaki et al. (2014); these authors found that glycans were responsible for the anti-rotavirus activity of casein and that the mode of action was identified as a direct binding of glycans to the rotavirus. Bovine rotavirus is the major cause of neonatal calf diarrhoea worldwide (Parreño et al. 2010). We hypothesised that the special composition of colostrum casein until the first hour postpartum could influence positively the health of the calf, and we proposed that this should be investigated in depth.

Previous experience from other studies (unpublished) has shown that the measurement of milk fat with portable equipment on farms could be better and more reliable if the equipment was calibrated with accuracy. For protein, the values obtained by Ekomilk were almost half of those obtained by Milkoscan, and according to the Bland–Altman method, these methods were not interchangeable for protein measurements. However, it is important to consider that the effects of parity and the sex of the calf on protein were shown to be significant with both Ekomilk and Milkoscan methods. This indicated that protein data from both methods could be used for evaluating the effects of various factors, but that the protein contents would be underestimated with Ekomilk.

Colostrum Ig

Colostrum Ig can be measured with a colostrometer to estimate colostrum total Ig concentration with sufficient accuracy to separate good-quality colostrum (>50 mg/mL of IgG) and poor-quality colostrum (<50 mg/mL of IgG) (Heinrichs and Jones 2011). The average Ig concentration in all cases of this

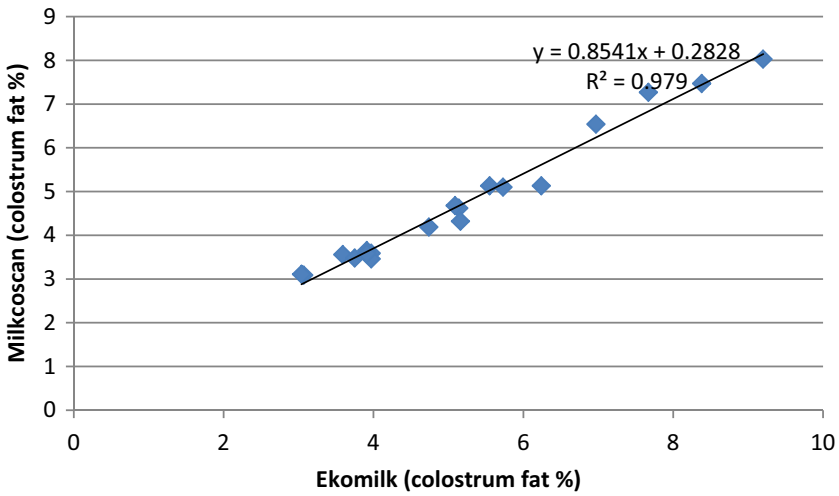

Fig. 1 Relationship between Milkoscan and Ekomilk methodologies for measurement of colostrum fat

study was well above the 50 mg/mL threshold, which indicated high-quality colostrum (Godden et al. 2009).

The colostrum from younger cows (parity 1–2) was lower in Ig concentration than the colostrum of older cows (parity≥ 3) ($P<0.05$), and a similar result was found for the total amount of Ig produced in the colostrum (mg) ($P>0.05$). Age is a major factor affecting immunity, and younger cows would have limited time on the farm for exposure to pathogens and vaccinations. Therefore, less exposure and fewer vaccinations would decrease the concentration of circulating antibodies, which would decrease the antibody content transferred into colostrum Similar to our results, Gomes et al. (2011) reported Ig was lower in the first and second lactations of cows than later lactations, and Morrill et al. (2012) reported that colostral immune quality continued to increase with parity after the second calving and that older cows generally have the best colostrum in terms of immune quality. However, it is worth noting that similar to Morrill et al. (2012), in this study, some younger cows were found to produce very good nutritional and immunological quality colostrum, and for this reason, colostrum quality should always be evaluated before giving it to calves to avoid rejecting the colostrum of young cows with sufficient quality.

The total Ig concentration was also affected by the sex of the calf and was higher for males than for females. These results contrasted with those reported by Silper et al. (2012), who did not find an effect of sex on the colostrum Ig of crossbred Holstein–Zebu cows. A dilution effect could have been possible because the cows with a female calf produced higher amounts of colostrum than cows with a male calf, but there was no difference in the total amount of Ig produced (relating Ig concentration with volume of produced colostrum). Kehoe et al. (2011) found that concentration of IgG decreased as colostrum volume increased.

The effect of sex on the volume of colostrum found in the present study is very important if it is analysed for future effects. However, it should be considered as preliminary results that need to be investigated in depth. The experimental cows with a female calf produced almost 5 % more milk during lactation than cows with a male calf. Our results support those recently published as novel by Hinde et al. (2014). These authors demonstrated that in Holstein cows, there were effects exerted by the foetus in utero that allowed for programmed milk production. According to these authors, hormones from the foetus and placenta may differ between foetal males and females, which subsequently enter the maternal bloodstream and affect the milk producing cells in the mammary glands. Our results and those published by Hinde et al. (2014) suggest that the programme is not only for production in lactation but also for the production of colostrum. In this way, the Holstein cows would favour females, not males and produce more colostrum with higher fat concentrations and then more milk during lactation. Thus, we hypothesise a novel relationship between the foetal sex and the amount of colostrum produced and the amount of milk produced during the lactation. New questions arise from this work, and further studies are needed to examine whether the amount of colostrum produced by a cow could be used as a predictor for the amount of milk produced during its lactation.

The concentration of Ig was stable in the colostrum until 4-h postpartum. The decrease was clear, but there was only a statistically significant trend. Moore et al. (2005) sampled the colostrum at 2, 6, 10 and 14-h postpartum to evaluate the effects of time on the colostrum IgG concentration. They found a decrease in the IgG content of the colostrum corresponding to increased time post calving; the IgG concentration 2-h post calving was 113 g/L and significantly decreased to 94, 82 and 76 g/L at 6, 10 and 14 h, respectively. Nardone et al. (1997) reported similar findings when analysing colostrum at 1, 12, 24 and 36-h post calving. Based on the capacity of calves to absorb Ig, the recommendation should be to give calves colostrum during the first 6-h postpartum (Godden et al. 2009). However, the high immune quality of colostrum found in this study indicates that colostrum should be harvested until 4-h postpartum. However, our results focused on the importance of giving colostrum to the calves until the first hour postpartum in order to impact its nutritional and immunological composition at the same time.

SCC

On average, the SCC was high in colostrum compared with the maximum quantity found in normal milk (400.000/mL, Hillerton and Berry 2004); this result could be attributed to the high content found in younger cows. The SCC in the colostrum of younger cows was almost 15 times the amount found in the older cows. These results agree with other reports (Ferdowsi et al. 2010; Schutz et al. 1990). The causes for a higher amount of SCC in the colostrum from younger cows (particularly from the first lactation) compared with multiparous cows have been reported as a mastitis problem (Ferdowsi et al. 2010), as a dilution effect because there is a lower amount of colostrum in primiparous cows or as a natural expected condition because of the first lactation (Schutz et al. 1990). A mastitis problem would not explain our results because there were minimal reports of clinical or subclinical mastitis in the experimental cows. The dilution effect could have partly affected our results because the younger cows produced lower amounts of colostrum than older cows, but it could not be the full explanation because the differences in SCC were very high and did not correspond to the differences found in the amount of colostrum produced. However, the natural physiological condition of younger cows could have had a significant effect on the high amount of SCC. Schutz et al. (1990) suggested that the elevated SCC might be associated more with the oedema and physiological changes from

the beginning of milk secretion for the first lactation than with mastitis infection. According to Olejnik (1994), SCC in non-infected cows at the first and second lactation were apparently time-dependent and could not be regarded as characteristic features of individual animals. After calving, primiparous cows adapt to a new social and physical environment as they continue to modify their anatomical and physiological characteristics (Zucali et al. 2009). Regardless of the cause of the high content of SCC in colostrum, it is not advisable to use this type of colostrum for the calf because it can affect the ability to absorb immunoglobulins. The increased colostrum SCC will interfere with gut health and functionality and IgG absorption capacity, and thus, the SCC will depress early passive immune transfer despite adequate colostrum intake (Ferdowsi et al. 2010). Ferdowsi et al. (2010) found a positive association between increased colostral SCC and faecal looseness. Therefore, the colostrum SCC could be helpful on farms for screening the colostrum to determine whether it is suitable for feeding to calves. Unfortunately, this will not be possible until an easy test could be used on the farm. Colostrum SCC can be measured very accurately in the laboratory, but these tests would not be able to provide results as quickly as the farms require. In conclusion, farmers should use not only the Ig concentration but also the nutritional composition and SCC composition to determine immediately postpartum whether the colostrum is suitable for feeding to calves.

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Conflict of interest The authors declare that they have no conflict of interest.

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