



ORIGINAL ARTICLE

Increased colostrum somatic cell counts reduce pre-weaning calf immunity, health and growth

E. Ferdowsi Nia¹, A. Nikkhah^{1,2}, H. R. Rahmani¹, M. Alikhani¹, M. Mohammad Alipour¹ and G. R. Ghorbani¹¹ Department of Animal Science, Isfahan University of Technology, Isfahan, Iran, and² Department of Animal Sciences, University of Zanjan, Zanjan, 313-45195 Iran**Keywords**

colostrum, diarrhoea, growth, immunoglobulins, somatic cell, weaning

Correspondence

A. Nikkhah, Department of Animal Science, Isfahan University of Technology, Isfahan 84156, Iran. Tel: +0098 912 7891124; Fax: +0098 311 3913501; E-mail: anikha@yahoo.com

Received: 22 December 2008;

accepted: 14 May 2009

Summary

Our objective was to study the relationships between colostrum somatic cell counts (SCC, a criterion for mastitis severity at parturition) and early calf growth, blood indicators of immunity, and pre-weaning faecal and health states. Sixty-nine Holstein cows were assigned to three groups of greater ($n = 21$, 5051×10^3), medium ($n = 38$, 2138×10^3) and lower ($n = 10$, 960×10^3) colostrum SCC (per ml) in a completely randomized design. Calves received 2 l of colostrum on day 1, and jugular blood was sampled at birth, at 3 h after the first colostrum feeding and at 42 days of age for immunoglobulin G (IgG) measurements. Calves were fed transition milk from their dams until 3 days of age and whole milk from 4 to 60 days of age twice daily at 10% of body weight. Health status and faecal physical scores were recorded daily for 42 days. Increased colostrum SCC was associated with increased serum IgG at parturition. Colostrum pH increased and fat percentage decreased linearly with the rising SCC. Feeding colostrum with greater SCC was associated with reduced serum IgG concentrations at 3 h after first colostrum feeding, greater incidences of diarrhoea and compromised health status during the first 42 days of age, and reduced weaning weight gain, but had no effects on calf body length and withers height. Colostrum volume and percentages of protein, lactose, solids-non-fat, total solids and IgG were comparable among groups. Results suggest a role for SCC, as an indicator of mastitis and colostrum health quality, in affecting calf health. As a result of the novelty of calf health dependence on colostrum SCC found, future studies to further characterize such relationships and to uncover or rule out possible mediators are required before colostrum SCC could be recommended for routine on-farm use in managing dry cow and calf production.

Introduction

Adequate feeding of the first colostrum immediately after birth is vital for calf passive protection against environmental pathogens, while the calf develops its own immune system (Wittum and Perino, 1995; Davis and Drackley, 1998; Quigley and Drewry,

1998). Pre-weaning calf health and morbidity and mortality rates depend on colostrum quality and its immediate small intestinal IgG assimilation (Nocek et al., 1984). According to Maunsell et al. (1998), >40% of dairy calves in the US had inadequate passive Ig transfer over the few first days after birth. Depressed passive immunity transfer could

partly be due to colostrum contamination with micro-organisms, which can impair antibody absorption and reduce the number of receptors for Ig uptake (James et al., 1981). However, only half of the calf mortality was due to inadequate IgG intake (Wells et al., 1996). It has been our intuition that other major colostrum factors exist, which affects neonatal calf immunity. Mastitis has been shown to influence raw milk and the quality of dairy products (Auld and Hubble, 1998). Pre-parturient mastitic glands have reduced colostrum volume and total IgG mass (Maunsell et al., 1998). However, limited information exists on how pre-parturient mastitis and colostrum cell counts affect calf immunity, health and growth. We hypothesized that increased colostrum SCC, representing dam's compromised systemic and local immune functions, will interfere with gut health and functionality, IgG absorption capacity, and thus will depress early passive immune transfer despite adequate colostrum intake. As the first colostrum is the major source of early Ig uptake by the not resistant calf, the above cascade will compromise the health of the highly sensitive and neonate small intestine, thereby depressing nutrient assimilation and causing diarrhoea. The impaired gut function will depress appetite, affect reticulorumen expansion and postpone calf's own immune system development and competitive tissue growth. Delayed weaning and prolonged dependence on pricy milk will increase feed and labour costs, thus making calf raising less profitable (Davis and Drackley, 1998; NRC, 2001; Ghorbani et al., 2007). In addition, recently, a mastitis case (*Mycoplasma bovis*) was reported in a 7-week old calf, and its possible link to colostrum quality was suggested (Roy et al., 2008). Thus, it was our conjecture that colostrum hygienic properties and factors other than Ig, such as SCC affecting pre-weaning growth and health, will be likely to have longer-lasting effects, e.g. an enhanced later sensitivity to mastitis in heifers. Our objective was to determine the effects of dam mastitis severity

measured as colostrum SCC on pre-weaning calf health, immunity and growth.

Materials and methods

Experimental design, calves and treatments

Sixty-nine Holstein cows (44 multiparous and 25 primiparous) were assigned based on their colostrum somatic cell counts to three groups of (1) greater, $5051 \pm 1608 \times 10^3$, (2) medium, $2138 \pm 560 \times 10^3$ and (3) lower, $960 \pm 238 \times 10^3$, somatic cell counts (SCC, mean \pm SD) per ml, respectively, representing varying degrees of dam mastitis (Table 1). Cows were selected for the experiment if they produced at least 2 l of colostrum in the first milking to ensure that colostrum and Ig adequacy was not a problem for successful passive immunity transfer and that SCC was the major determinant of calf performance. The average parity was 1.71 ± 0.46 for the greater SCC cows, 1.66 ± 0.48 for the medium SCC cows and 1.40 ± 0.52 (mean \pm SD) for the lower SCC cows. All cows were fed a close-up diet with 61:39 forage to concentrate ratio (24% alfalfa hay, 26% corn silage, 11% straw and 38% concentrate), 1.53 Mcal NE_L per kg of dietary DM and 16.8% CP. Cows were transferred to and cared for in a disinfected maternity box upon noticing calving signs. The calving box was fully bedded with clean straw to provide a comfortable and hygienic place for lying and calving. Newborn calves (body weight, BW = 41.6 ± 1.6 kg; mean \pm SE) were allowed to be treated by dams, and a commercial iodine solution was used to sanitize the navel of the calves upon parturition. Shortly afterwards (i.e. 1 h) and before suckling could occur, calves were separated from the cows and received 2 l of colostrum. After 12 h of birth, another 2 l of colostrum was fed. On day 2 and 3, calves received transition milk at 10% of birth BW twice daily at 08:00 and 17:00 hours. From day 4 to 60 of age, calves were fed whole milk at 10% of BW twice daily at 08:00 and 17:00 hours.

Table 1 Treatment cows, calf numbers and colostrum somatic cell counts (SCC)*

Item	Somatic cell counts group (treatment)		
	Lower	Medium	Greater
Number of cows with viable calves (n)	10	38	21
Average dry period (DP) length, days	61.1 (± 9.2)	62.1 (± 11.2)	62.4 (± 15.3)
Maximum and minimum DP length, days	73 and 42	85 and 35	101 and 42
Average SCC/ml	$960 (\pm 239) \times 10^3$	$2138 (\pm 560) \times 10^3$	$5051 (\pm 1608) \times 10^3$
Maximum and minimum SCC ($\times 10^3$)/ml	1355 and 641	3158 and 1381	9620 and 3459
Number of calves finishing the study (n)	3 males & 5 females	15 males & 16 females	9 males & 9 females

*Values within parentheses are standard errors.

The milk containers were washed after each feeding. Colostrum was fed using a sanitized plastic bottle by keeping the head in an upward position to mimic natural suckling and stimulate the formation of oesophageal groove. All calves received colostrums and transition milk from their own dam to determine the associations of colostrum SCC, blood IgG concentrations and pre-weaning calf health and growth for all dam-calf pairs. Calves were kept in a half-open salon until 7 days of age, in individual hutches (1.5 × 1.12 × 1.2 m) bedded with clean straw until 42 days of age and in group-based yards later on. The outdoor individual hutches were made of metal and cement with an exercise area of 1.2 × 2.1 m. The bedding was renewed daily, and when necessary, was cleaned and disinfected (sovlorin cetrinide-C with 1.5% w/v chlorhexidine gluconate and 15% w/v cetrinide; Damloran, Boroojerd, Iran). Calves had free access to a starter concentrate (Table 2) from day 3 on to promote early and adequate solid fermentable feed intake, and thus, to support early reticulorumen development. The water and starter containers were washed once a day. Fresh water was provided 1 h after milk delivery. The calves' horns were born at week 3 of age. The animals were cared for according to the guidelines of the Iranian Council of Animal Care (1995). This study was conducted at the calf raising facilities of Golshahr in south-eastern Najafabad in the central Iranian province of Isfahan from June to November 2006.

Table 2 Feed ingredients and chemical composition of the calf starter concentrate (DM basis)

Ingredients	g/kg dietary DM
Ground barley grain	250
Ground corn grain	260
Solvent Soybean meal	280
Wheat bran	160
Calcium carbonate	10
Sodium chloride	5
Mineral and vitamin supplement*	15
Dicalcium phosphate	20
Chemical composition	
Dry matter, g/kg	897
NE _m , Mcal/kg	2.0
NE _g , Mcal/kg	1.6
Crude protein, g/kg	213
Acid detergent fibre, g/kg	70
Neutral detergent fibre, g/kg	173

*Contained 250 000 IU vit A, 50000 IU vit D, 1500 IU vit E, 2.25 g Mn, 120 g Ca, 7.7 g Zn, 20 g P, 20.5 g Mg, 186 g Na, 1.25 F, 3 g S, 14 mg Co, 1.25 g Cu, 56 mg I, and 10 mg Se per kg supplement.

Farm and laboratory measurements

Calves were weighed at birth and at 30 and 60 days of age. Body length and wither height were measured at birth and at 60 days of age. Faecal materials were scored daily by the same three individuals throughout the study for shape and consistency, with the scores of 1 = firm, 2 = slightly loose, 3 = loose and 4 = watery faeces (Larson *et al.*, 1977). The scorers were unaware of the treatment groups at all times. A looser or more watery faeces would suggest a less efficient nutrient assimilation across the neonate and pre-weaning calf gastrointestinal tract. Health status was scored based on standing and suckling abilities (0 = unable to stand, 1 = able to stand; 0 = suckling absent, 1 = suckling present; Jones *et al.*, 2004). Faecal score data, when considered with physically visible calf status signs, would provide a more compelling evidence for overall calf well-being. Feed was sampled and analysed for DM, ash (at 500 °C overnight), neutral detergent fibre (NDF; Van Soest *et al.*, 1991; using heat-stable alpha-amylase) and ADF (AOAC, 1997). Colostrum samples were obtained from the first milking within 1–2 h after parturition for composition, SCC and IgG analyses. Colostrum pH and density were measured immediately and a sub-sample was kept at –18 °C until nutrient and IgG analysis. The pH was measured using a portable pH-metre (model HI8314, Hanna Instruments, Cluj- Napoca, Romania) equipped with an electrode and an automatic temperature sensor to adjust for temperature changes. Before each set of measurements, the pH-metre was calibrated with two commercial buffers with pH values of 4 and 7. The electrode and the sensor were kept in the sample solution without touching the container, and the pH was recorded once the value was fixed. An average of three pH measurements was used for each sample. Colostrum SCC was measured using Fossomatic method (Maunsell *et al.*, 1998). After thawing, colostrum samples were first diluted (1:1, vol:vol) using phosphate buffer solution (pH 7.4) to reduce sample viscosity and prevent technical difficulties usually encountered with undiluted and highly viscous samples (Maunsell *et al.*, 1998). Of the diluted sample, 30 ml was aliquoted and fixed with 0.2% potassium dichromate before testing was performed. Diluted colostrum was also analysed for protein, fat, lactose, solids-non-fat and total solids using an infrared analyzer (Milk-O-Scan 133 B infra-analyzer, FØSS Electric, Denmark). Jugular blood was sampled at birth, 3 h after the first colostrum feeding and at 42 days of age. Blood was

sampled to non-treated vacutainers and placed on ice immediately after bleeding. Blood was shortly transferred to the laboratory and allowed to clot at room temperature before centrifugation for 10 min at $2300 \times g$ to harvest serum. Serum was kept at -18°C until analysed for IgG. The frozen serum and colostrum samples were thawed at room temperature and mixed well for measuring IgG concentrations by ELISA (ELISA Starter Access. Package E103, Bethyl Laboratories, Montgomery, TX, USA).

Statistical analysis

Data were analysed as a mixed model using PROC MIXED of SAS Institute (SAS, 2003). The final models consisted of fixed effects of treatment (colostrum SCC group), cow parity, and their interaction plus the random effect of cow within parity. The method of estimating least square mean values was Restricted Maximum Likelihood, and the method of calculating denominator degrees of freedom was Kenward-Roger (SAS Institute, 2003). Orthogonal contrast coefficients for the increasing numbers of colostrum somatic cells were acquired using Proc IML of the SAS program (2003). Pearson correlation coefficients for serum Ig were obtained using the Proc Corr of SAS program. The significant treatment differences were declared at $p < 0.05$ and trends were set at $p \leq 0.10$. Results are presented as estimated least square mean \pm standard errors of mean values.

Results

A linear increase in colostrum pH ($p = 0.06$) and a decrease in its fat percentage ($p = 0.04$) existed as SCC increased (Table 3). Colostrum volume and density and percentages of protein, lactose, solids-non-fat and total solids, and colostrum IgG concentrations and mass were not significantly different among treatments. Increased SCC was associated with elevated serum IgG concentrations in cows at parturition ($p < 0.01$). There was a linear tendency for reduced calf serum IgG concentrations at 3-h after the first colostrum feeding as colostrum SCC increased ($p = 0.10$). Calf serum IgG concentrations at birth and at 42-days of age did not differ among groups (Table 3).

Calf body weight gain through the first 30 days of life decreased linearly ($p < 0.01$) as colostrum SCC increased (Table 4). The weight gain from 30 to 60 days of age was not affected by treatments. There was a linear decline in BW gain through 60 days of age with the increasing colostrum SCC ($p = 0.05$, Table 4). Calves born from multiparous cows tended to have greater weight gain up to 30 days of age (5.2 vs. 3.5 kg, $p = 0.04$) and had greater weight gain through 60 days of age (28.6 vs. 24.3 kg, $p = 0.03$), compared with calves born from primiparous cows. The number of days with faecal scores of 3 and 4 (loose and watery) over the first 42 days increased linearly with colostrum SCC ($p < 0.01$, Table 4). During the same period, a numerical

Table 3 Colostrum nutritional properties and serum IgG concentrations of dams and calves

Item	Colostrum somatic cell counts*			SEM	p-value	
	Lower	Medium	Greater		Linear	Quadratic
Volume, kg	5.3	5.9	4.7	0.7	0.45	0.34
Density, g/ml	1.07	1.06	1.07	0.03	0.72	0.45
pH	6.28	6.36	6.40	0.03	0.06	0.32
Fat %	5.9	6.0	4.5	0.5	0.04	0.42
Protein %	16.2	17.4	17.2	0.7	0.51	0.27
Lactose %	2.7	2.4	1.9	0.3	0.17	0.74
Solids-non-fat %	18.9	20.7	21.3	1.0	0.23	0.28
Total solids %	25.4	26.0	26.1	0.7	0.65	0.64
IgG, mg/ml	73.0	79.8	82.1	8.0	0.55	0.68
Total IgG mass†, g	428.2	443.2	365.0	43.0	0.48	0.65
Dam serum IgG at parturition, mg/ml	17.8	22.9	30.1	2.8	<0.01	0.63
Calf serum IgG at birth, mg/ml	0.016	0.020	0.012	0.03	0.39	0.29
Calf serum IgG at h-3, mg/ml	16.2	16.6	11.4	2.0	0.10	0.43
Calf serum IgG at 42 days of age, mg/ml	29.3	28.8	25.1	3.6	0.46	0.86

*The lower, medium and greater cell counts correspond to 960×10^3 , 2138×10^3 and 5051×10^3 per ml respectively. Calves received 2 l of the first colostrum shortly after birth and received transition milk and whole milk twice daily at 10% of BW.

†Total IgG mass produced by the dam (the first-milking colostrum volume \times IgG concentration).

Table 4 Calf health and performance*

Item	Colostrum somatic cell counts†			SEM	p-value	
	Lower	Medium	Greater		Linear	Quadratic
Birth BW, kg	40.7	41.8	41.5	1.4	0.84	0.64
BW at 30 days of age, kg	46.4	46.3	43.9	1.2	0.17	0.69
BW at 60 days of age, kg	70.1	67.8	65.4	1.9	0.12	0.67
BW gain through 30 days of age, kg	5.7	4.9	2.5	0.7	<0.01	0.88
BW gain during 30–60 days of age, kg	23.8	21.1	21.4	1.5	0.44	0.27
BW gain through 60 days of age, kg	29.4	26.0	23.9	1.6	0.05	0.35
Faecal score‡	5.6	6.2	11.2	1.3	<0.01	0.45
Health status§	5.9	7.3	9.3	1.3	0.11	0.75
Wither height at birth, cm	71.9	73.1	72.8	1.1	0.69	0.48
Wither height at 60 days of age, cm	81.2	82.2	81.8	0.9	0.79	0.44
Body length at birth, cm	34.2	34.9	35.1	0.8	0.56	0.74
Body length at 60 days of age, cm	45.2	44.9	43.8	1.0	0.37	0.91

*The data belong to the calves that finished the experiment, i.e. $n = 8, 31,$ and 18 for the lower, medium and greater colostrum SCC groups respectively. Calves received 2 l of the first colostrum shortly after birth and received transition milk and whole milk daily at 10% of BW.

†The lower, medium and greater counts correspond to $960 \times 10^3, 2138 \times 10^3$ and 5051×10^3 per ml respectively.

‡Faeces was evaluated and scored daily until weaning based on physical shape of faeces: 1 = firm, 2 = slightly loose, 3 = loose and 4 = watery. Values represent the number of days with faecal scores of 3 and 4.

§Health status was scored based on standing and suckling abilities (0 = unable to stand, 1 = able to stand; 0 = suckling absent, 1 = suckling present; Jones et al., 2004). Values represent the number of days with a health score of zero.

increase existed in the number of days with a health status score of zero (unable to stand and suckling absent) as colostrum SCC increased ($p = 0.11$). Wither height and body length were comparable among treatment calves (Table 4).

Colostrum pH was correlated ($p < 0.01$) negatively with colostrum IgG concentrations ($r = -0.42$) and positively with dam's blood IgG concentrations at calving ($r = 0.88$). Colostrum pH and density (g/ml) were highly correlated ($r = 0.99, p < 0.01$). Serum IgG concentrations of the cows at parturition were negatively correlated with colostrum IgG concentrations ($r = -0.52, p = 0.02$). The calves born from dams with greater serum IgG concentrations at calving tended to have lower serum IgG concentrations at 42 days of age ($r = -0.42, p = 0.08$). Colostrum SCC were negatively correlated with BW gain up to 30 days ($r = -0.47, p < 0.001$) and 60 days ($r = -0.31, p = 0.02$) of age.

Discussion

This study provides novel relationships between colostrum SCC or indicators of pre-partal mastitis, with neonatal calf blood Ig concentrations, faecal physical form, health status and pre-weaning growth. However, whether such relations were essentially direct or mediated by other metabolic and immune factors remain unknown, thus requiring further research. It is known that calf mortality can

be doubled if passive immunity transfer during the first few days after birth is depressed (e.g. blood IgG of <10 mg/ml; Wells et al., 1996), with only half of the calf mortality being due to inadequate IgG intake. This data offer evidence that colostrum effects on calf immunity and health can partly depend on other factors, such as SCC, than only sufficient IgG uptake. However, upcoming studies are needed to identify and characterize numbers and types of micro-organisms that contribute to the elevated colostrum SCC. Given the similar colostrum Ig concentration and total mass among the three colostrums in our study, the linear reduction in serum IgG with the increasing colostrum SCC at 3 h after the first colostrum delivery suggests that the intestinal IgG assimilation may have been affected by high colostrum SCC. This decrement in blood IgG was biologically meaningful, as blood was sampled shortly after the first colostrum feeding when the greatest Ig levels were ingested and the small intestine possessed the highest Ig absorption capacity (Balfour and Comline, 1962; Davis and Drackley, 1998). It may be suggested that colostrum SCC might have interfered with normal intestinal Ig absorption. Decreased colostrum fat concentrations with greater SCC suggest that *denovo* fatty acids synthesis and acylation with glycerol of long-chain fatty acids and possibly long-chain fatty acids uptake may be impaired in infected mammary cells. Mastitis influences milk secretions and dairy products quality

(Auld and Hubble, 1998). Neutrophils are the major somatic cells and ingest milk fat and casein (Kehrli and Shuster, 1994), which could contribute to varying colostrum fat and protein composition when varying SCC. A reduced colostrum fat content with mastitic and involutary mammary glands has been reported (Sordillo *et al.*, 1987).

The unchanged colostrum IgG concentrations were in accordance with the similar colostrum volume. The inverse relationship between colostrum IgG and dam serum IgG concentrations at parturition alongside the linearly increased dam serum IgG with colostrum SCC suggests that the dam immune system was challenged by the mammary infections leading to elevated IgG synthesis. These data put forward the notion that colostrum IgG concentrations and volume may be insufficient to well defining colostrum quality, and thus, to accurately predict colostrum effects on calf health. Nonetheless, these findings require further and more detailed substantiation before colostrum SCC can be recommended for on-farm use. Such complementary experiments are essential particularly as the increasing colostrum SCC and reduced calf serum Ig concentrations were associated with depressed calf health and pre-weaning weight gain. The colostrum ranges of fat, protein and IgG concentrations as well as SCC agree with previous reports (Jensen and Eberhart, 1981; Maunsell *et al.*, 1998). For instance, Maunsell *et al.* (1998) reported average colostrum SCC of $4413 \times 10^3/\text{ml}$ and $891 \times 10^3/\text{ml}$ for infected and uninfected glands, respectively, comparable to the greater and lower SCC groups in this study. The pH values of <6.5 represent the expected pH range of mammary secretions soon around calving (Hurley, 1987). The linear increase in colostrum pH with the rising SCC and the unaltered IgG and protein concentrations concur with the results of Maunsell *et al.* (1998). Authors collected mammary secretion samples at -14, -7 days pre-partum and within 3 days of calving and found that pre-partal mastitis reduced colostrum volume and IgG mass. Maunsell *et al.* (1998) concluded that as calves usually receive a fixed amount of colostrum, factors such as non-lactating period and lactation number may affect calf immune transfer. In view of the relationships among colostrum SCC, calf health and growth found in our study, a role for colostrum SCC in affecting early calf health, immunity and growth is suggested. As discussed earlier, determining the nature and uncovering the possible mediators of such associations demand future studies. The greater weight gain of calves born from multiparous cows compared with calves born from primiparous cows

could be expected because primiparous cows have smaller abdominal capacity and partition more nutrients toward their own tissue growth (NRC, 2001). Consequently, more nutrients would be available for calf growth in multiparous cows.

To guarantee early passive immunity transfer, alternatives to low quality colostrums, namely spray-dried bovine serum have been used (Arthington *et al.*, 2000; Jones *et al.*, 2004; Quigley *et al.*, 1998). By acknowledging the extensive published evidence for the importance of sufficient IgG absorption, this data suggest that colostrum SCC could be one of the key on-farm management criteria in choosing the amount and type of such supplements, or whether using these products can be economically justifiable. Leucocytes and mainly neutrophils are the major somatic cells recruited by the mammary cells to combat infections by adhesion to pathogens (Kehrli and Shuster, 1994; Kimura *et al.*, 1999). Given the similar blood IgG levels at 42 days of age among different groups despite the reduced weight gain with the rising colostrum SCC, it may be suggested that restoring normal pre-weaning immune status was prioritized over aggressive tissue accretion. Prospective works are projected to specify calf response to various types and numbers of colostrum leucocytes and epithelial cells at both gastrointestinal and systemic levels. Such knowledge will allow furthering the current data and may permit setting local and global colostrum SCC categories as quality control criteria for optimal calf health. Monitoring colostrum SCC might also aid in refining present nutritional guidelines for dry cows and pre-weaning calf management.

In summary, novel positive associations between increased colostrum SCC with dam serum IgG concentrations at parturition, calf serum IgG concentrations at 3 h after the first colostrums ingestion and faecal looseness were found. Depressed body weight gain through 60 days of age and a tendency for inferior calf health score during the first 42 days of life were associated with increased colostrum SCC. It cannot be explored if these associations truly indicated cause and effect relations or were mediated by other possible colostrum metabolic and immune factors. No relationships existed between colostrum SCC with colostrum volume, protein and IgG concentrations. The findings suggest a role for SCC in affecting colostrum quality and pre-weaning calf performance. However, future studies to further characterize the above associations are needed before colostrum SCC could be recommended as a routine calf management criterion. It is warranted to investigate if

pre-partal mastitis and colostrum SCC affect later mammary sensitivity to infections in milking animals.

Acknowledgements

The authors thankfully acknowledge Isfahan University of Technology (IUT, Isfahan, Iran) for financial support, and staff at Lavark Research Station (Isfahan University of Technology, Isfahan) and Golshahr Corp. (Najafabad, Isfahan) for calf and cow care and Walter Hurley of Department of Animal Sciences, University of Illinois in Urbana for thoughtful discussions.

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