‘Effects of continuous milking on bovine colostrum quality’

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Abstract

This study reports the influence of continuous milking on bovine colostrum quality. Continuous milking has been described to reduce health problems during the dry period and parturition, but may affect colostrum quality which is essential for calf health. In this study colostrum immunoglobulin concentrations of 227 cows was quantified with a Bethyl Bovine Quantitation ELISA for IgG, IgG1, IgG2, IgA and IgM. Colostrum samples of continuous milked (CM) cows (n=38) were compared with colostrum samples of cows (n=189) after a traditional dry period (DP) of at least 42 days. Colostrum immunoglobulin concentration was significant lower (average IgG=24,9) after continuous milking for IgG, IgG1, IgG2, IgA and IgM (p<0,001) compared with DP-controls (average IgG= 41,5 mg/ml) for all parities. Results indicate at least 1,6 times more colostrum supply is required for providing adequate immunity to calves after continuous milking. A survey among farmers indicated presence of more health problems with calves associated with failure of passive transfer (FPT) after continuous milking. Parity differences in colostrum quality (DP-controls; n=189) was only significant for IgA for parity 1 and 2 compared to 5. Trends for increased colostrum quality for higher parities were visible, but heifers produced average quality. Colostrum samples with antibodies against Mycobacterium avium subspecies paratuberculosis (MAP) contain higher antibody concentrations (p=0,001). Colostrum volume, udder health, milking time and other factors influencing colostrum quality were discussed.

Abbreviations

CM: continuous milked -- DP: dry period -- IMI: intramammary infection -- SCC: somatic cell count -- IM: intramuscular --SC: subcutaneous -- ID: intradermal -- Ig: immunoglobulin -- FcRN: neonatal FC-receptor -- AEA: apparent efficiency of absorption -- TBV: total blood volume -- PCV: packed cell volume -- MAP: *mycobacterium avium* subspecies *paratuberculosis* -- pIgR: polymeric immunoglobulin receptor -- Ht: hematocrit -- RIA: radioimmunoassay -- ELISA: enzyme-linked immunosorbent assay

Content

1. Introduction

Background

Continuous milking (CM) means skipping the dry period of presumably dairy cows. CM increases in popularity, because of the increasing startup problems postpartum and regulations for reducing use of antibiotics in diary cattle. Feed changes around the dry period are contributing to subclinical ketosis. After a conventional dry period subclinical ketosis has been reported in 6,9 to 43% of the cows in the first two months postpartum in European herds.. Subclinical ketosis contributes to Negative Energy Balance (NEB) related problems like metritis, mastitis, clinical ketosis and displaced abomasum. (109) The negative energy balance postpartum and correlated problems can be reduced by continuous milking. (3, 22, 59)

Reduction of the use of antibiotics in animals is needed. Systematic drying off with antibiotics contributes largely to the use of antibiotics in dairy cows and is therefore criticized. Drying off without antibiotics is not popular in Dutch farms and increases the risk of mastitis. (13) Continuous milked cows skip the dry period, which allows to stop the use of dry-off antibiotics.

Effects of continuous milking on milk production and cow health were described by several studies. Although production effects were presumably negative, general health and metabolic effects were positive. (23, 58, 92) CM cows skip the mammary gland involution at the beginning of a dry period and therefore produce less milk in the subsequent lactation. (1, 4, 29, 92, 99, 106)

Direct effects of skipped mammary gland involution on colostrogenesis and immunoglobulin transfer are still unclear. The effect of CM on colostrum quality is not studied in realistic conditions with realistic cow numbers. Good quality colostrum is essential for the calf health, growth and performance as calf and cow. (70, 75, 81) This study investigates the effects of CM on colostrum quality.

Colostrogenesis

Colostrogenesis is the change in composition of the udder secretion due to mammary developments in the last weeks before parturition. This change starts slowly and increases to a peak around parturition. Only the first milked secretion postpartum is colostrum because of rapid change to normal milk composition. The colostrum immunoglobulin concentration decreases rapid while the calf gut closes within 24-36 hours for antibodies. Colostrogenesis is influenced by several hormones like prolactin, estrogen and progesterone, but the exact mechanism is not completely understood. (7, 8) Indicating a starting point for colostrogenesis is difficult, because the slowly changes of the udder and milk. Studies describe changes in udder secretion composition from up to eight weeks before parturition. (7, 8, 36, 45) Milking in the last weeks before parturition could decrease the colostrum quality by disturbing the accumulation of immunglobulins.

Immunoglobulins in the mammary gland originate from systemic and local sources. Immunoglobulins of systemic sources are transported towards the alveoli, while other immunoglobulins are produced by local plasmacells. (46)

IgG1 is mainly transferred from blood to udder by receptor mediated transcytosis. (8, 18, 57) IgG1 binds to the FcRn-receptor (neonatal Fc-receptor) and is internalized via endocytosis. (8, 15, 51) After that, it is transported to the apical site where it is released in the alveolar lumen. (6, 43, 45) The [IgG1] in the alveolar lumen increases, but leaking of IgG1 back to the extracellular fluid limits colostrum [IgG1]. (46) The FcRn-receptor distribution varies from presumably basolateral (and intracellular) to luminal before parturition and regulates the quantity of IgG transport to the udder around parturition. (65) The receptor distribution can clarify the time pattern of the mass of IgG in the udder. The FcRn distribution is assumed to be regulated by hormonal influences around parturition. (7, 18, 107) Genetic haplotypes of the bovine FcRN-receptor influence the chance for FPT, although the exact mechanism is unknown. Association between FCGRT haplotype 3 and colostral IgG mass was suggested. (18, 21, 51, 76)

The IgG2-receptor interaction is not uniformly described. Cervanac et. al. described stronger binding of IgG2 to FcRN compared to IgG1, but also less transport to the alveoli and more recycling back to the extracellular fluid. (18) However, other authors describe that IgG2 has a lower affinity to the FcRn-receptor, and therefore less present in colostrum. (76, 98) Anyway, the [IgG1] is normally much higher as [IgG2] bovine in colostrum. (45, 56, 62, 107)

For IgM and IgA, local production by plasma cells was suggested but not thoroughly studied. (46, 52, 53, 82) Receptor-mediated transport was also described for the polymeric IgA and IgM isotypes. (107) Translocation of IgA or IgM to the mammary gland is facilitated by the polymeric immunoglobulin receptor (pIgR). Expression of the pIgR-receptor varied for different haplotypes.(10) Dimeric and pentameric immunoglobulins bind to the pIgR-receptor for transcytosis. On the apical site of the mammary epithelial cells, the pIgR is cleaved and IgA or IgM is released.(18, 39) Concentrations of dimeric IgA and pentameric IgM in bovine colostrum are inferior to the high [IgG1].

The slow increase of the immunoglobulin concentrations in colostrum supposes the importance of accumulation. CM cows do not have accumulation of milk or immunoglobulins like cows with a DP. This

study shows the effects of continuous milking on colostrum immunoglobulin content.

Composition of colostrum

The composition of colostrum versus milk is very different. (Table 1) (32, 61) Not only immunoglobulin concentrations but also many other components are higher in colostrum. Epidermal growth factors stimulate proliferation of epithelial cells and act as differentiation factor for many cells. Insulin-like growth factors promote cell differentiation and proliferation and have anabolic effects in vitro. Lactoferrine and lactoperoxidase have antimicrobial and immuunmodulatory effects. Many factors and substances present in colostrum have effect on growth and antimicrobial defense. (49, 61, 97) This study focuses on immunoglobulin isotypes IgG1, IgG2, IgA and IgM.

Factors influencing immunoglobulin concentration

High immunoglobulin concentrations in colostrum are essential for calf health but are influenced by many management and animal factors.

Milking time after parturition has significant effects on [Ig] of colostrum. Besides immunoglobulins, also other constituents are transported to the milk around parturition. This increase in volume is probably the mean cause of the decrease in immunoglobulin concentrations.(71) Waiting for the next milking time for colostrum collection after parturition happens in practice. This can take up to 12 hours, by milking twice daily. Dilution effect on immunoglobulin postpartum was described starting at 2 hours postpartum.(74, 87) Also 3,7% decrease/hour was described, which means 46% decrease in 12 hours. Direct postpartum collection of colostrum is important for highest quality colostrum.

Parity has effect on the [Ig] in colostrum. Older cows have a greater chance to produce colostrum of good quality. (48, 78, 100) However, heifers also produce good quality colostrum frequently. (8, 19) Older cows are more exposed to pathogens and therefore transfer more immunoglobulin to the udder at parturition. Lower FcRN-expression in younger cows was also supposed. (70, 78)

Also udder health has effect on the [Ig] in colostrum. Higher somatic cell counts (SCC's) were related with lower colostrum IgG. (43, 54, 64) When colostrum [IgG] was <25mg/ml, cows always had an increased SCC. (78) Good udder health was supposed to increase colostrum quality.

Season seems to have little or no effect on the [Ig] in colostrum, except of serious heat. Some studies claim lower colostrum quality in winter months.(43) No effect of season was reported by other studies. (26, 76) On the other hand, heat stress was supposed to have negative effects on colostrum quality. (80) Heat stress has negative effects on udder health and thus probably decreases [Ig] of colostrum. (102)

Colostrum volume has no significant effect on antibody mass, but significant effect to the antibody concentration. For calf health, high immunoglobulin

Parameter	Colostrum	Milk
Density	1,056	1,032
Solids (%)	23,9-27,64	12,9
Fat (%)	6,7	4,0
Total protein (%)	14,0-14,92	3,1
Caseïn (%)	4,8	2,5
Albumin (%)	6,0	0,5
Immunoglobulin (%)	6,0	0,09
IgG (mg/ml)	32	0,45
IgG1 (mg/ml)	34,96	0,35
IgG2 (mg/ml)	6,0	0,07
IgA (mg/ml)	1,66	0,1
IgM (mg/ml)	4,32	0,04
Lactose (%)	2,49-2,7	5,0
Lactoferrine (mg/ml)	0,82	-
IgF-I (ug/l)	341	15
Insuline (ug/l)	4,2-65,9	0,042-1,1
Cortisol (ng/ml)	4,4	0,35
Prolactine (ng/ml)	150	50
Plasmine (ug/ml)	0,49	0,04
A1-Antitrypsine (ug/ml)	250-800	6-20
As (%)	1,11	0,74

Table 1: Edited from Megank et. al. 2012 and Marnila et. al. 2011: Composition of bovine colostrum (1st milking) versus milk (6th milking).

concentrations are recommended above immunoglobulin mass. Increased lactose transport of high producing cows results in greater chance for lower concentrated colostrum due to osmotic dilution. (64, 86) Generally, the higher the colostrum production, the less the antibody concentration of the colostrum and the more colostrum must be ingested by the calf. (71)

Shortening dry period length has effect on the [Ig] in colostrum, but probably only in extremely short dry periods. Shortening is mostly described as a 28 day DP versus the conventional 56 day DP. Some studies described no effect of shortening DP on colostrum quality. (5, 26, 112) Negative effects on udder health and colostrum quality were also described. (42) Effects of short dry period on milk production were more thoroughly described and reviewed. (23, 59, 104) Data must be carefully interpreted, because some cows were not intended to have short dry periods. (40) Reasons for shortened DP can be abortion or twin pregnancy, which can affect the colostrum quality on itself. (23, 24) That heifers require longer dry period lengths for mammary development was also supposed. (22) Completely omitting dry period negatively effects colostrum quality and will be discussed later. (92)

Microbiological contamination

Microbiological cleanliness of colostrum is essential for the immunocompromised neonate. Bacteria break down immunoglobulins and threat animal health.(78) Pasteurization of colostrum can reduce bacterial counts, although it has to be performed well to save the immunoglobulins. (27, 34, 67) In practice, clean collection and cool preservation are the most essential points to limit bacterial contamination. (70) Colostrum can be frozen well and carefully reheating (without overheating >60 °C) does not influence antibody content. (72, 115) Microfiltration and pressure treatment was described as a good method to eliminate bacteria from colostrum recently. (38)

Immunoglobulin function

IgG is a monomeric immunoglobulin type and is supposed to be most important for calf immunity as it neutralizes viruses, bacteria and toxins. IgG1 and IgG2 have different heavy chain types and differ slightly in function. (15) IgG1 functions in opsonization and agglutination of pathogens for engulfment by phagocytes and activates the complement system. IgG2 functions comparable with IgG1 but lesser potential for agglutination and complement fixation. (62, 66, 110) Bovine IgG3 was not described as well as in humane studies and seems of minor importance for cattle.

IgA has less potent opsonization activity and is a not a potent activator of complement. It acts chiefly as a neutralizing antibody, because IgA is mainly present on epithelial surfaces where phagocytes are less present. (15, 62, 110) IgA acts

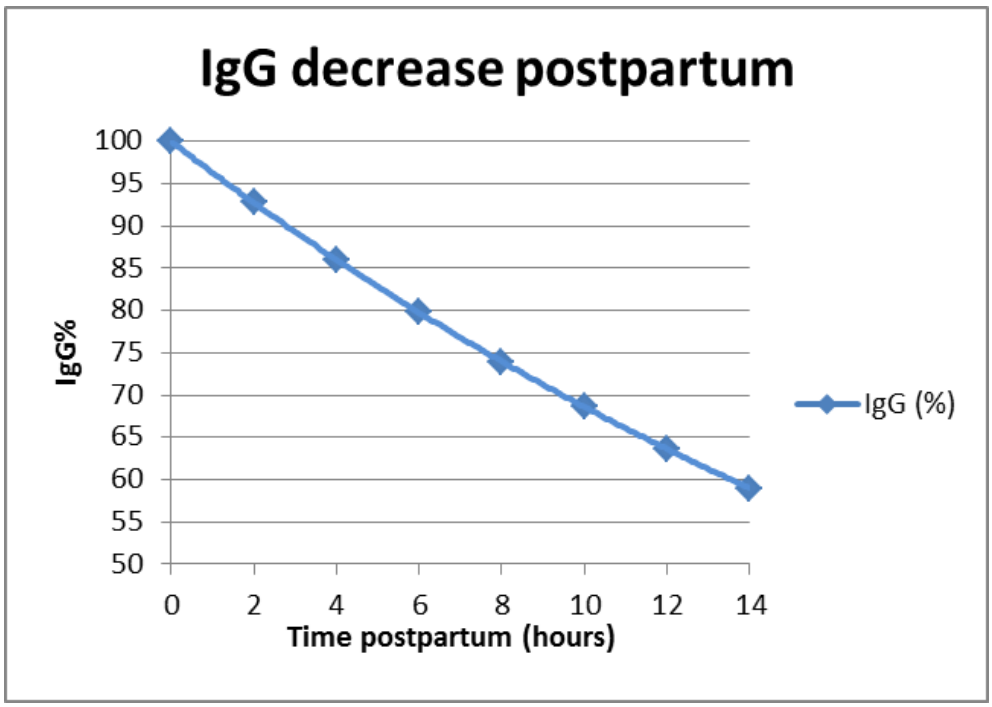

Figure 1: Postpartum colostrum [IgG] decrease visualized with data from Morin et. Al. 2010. [IgG] at parturition was set at 100%

either as monomer or as dimer.

IgM is a pentameric molecule with 10 antigen binding-sites and is mainly found in the blood or lymph because its large dimension. IgM acts as rapid responder by neutralization and complement fixation in early stages of the infection and in systemic infections. (62, 110)

Measuring colostrum quality

On farm measurement of immunoglobulins increases when calf health is suboptimal. On farm, the hydrometer (colostrometer) is used mostly because it is cheap and simple. The hydrometer measures specific gravity and converts it to colostrum IgG content. The quality is estimated by reading the color scale from green (IgG±50mg/ml) to red (IgG<20mg/ml). (70) Disadvantages of this method are the influences of the temperature and other substances on the density. (77) It is essential to measure quality consequently at 20°C to reduce this effect. (68, 69) Specificity was estimated at 0,93-0,97, so green means ±95% chance of good quality colostrum. Red/bad quality colostrum means in ±32-47% of the cases that it is truly bad colostrum. When all red/bad quality colostrum is discarded, also some good quality colostrum is wasted. Moreover, it may be possible to correct the bad quality of the colostrum by increasing the amount of colostrum fed The low sensitivity can be reduced by standardizing measure according to temperature but remains detrimental. (19, 70, 87)

The refractometer is also an acceptable on-farm indicator of colostrum quality.(11, 75) The refractometer measures deflection of light caused by the solids in colostrum. A good correlation was described between the solids and the immunoglobulin concentration of colostrum. Sensitivity (0,75-0,93) and specificity of the Brix refractometer (0,65-0,85) are pretty good. (11, 19, 90) The refractometer is less fragile and less sensitive to temperature variation as the hydrometer. (90)

Laboratories measure immunoglobulins mainly by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). Both methods are described as specific and sensitive. (30) RIA is easy and described as golden standard, but radioactive procedures are disadvantageous. (11) Save enzymatic reactions make ELISA accessible and was therefore used is this study.

Infrared spectroscopy is recently described to be a good method for determination of immunoglobulins.(96)

Quarter differences in colostrum quality

The udder is divided into quarters which are functionally segregated. Differences in quarter immunoglobulin concentration may be explained in several ways. For example, presence of the FcRn-

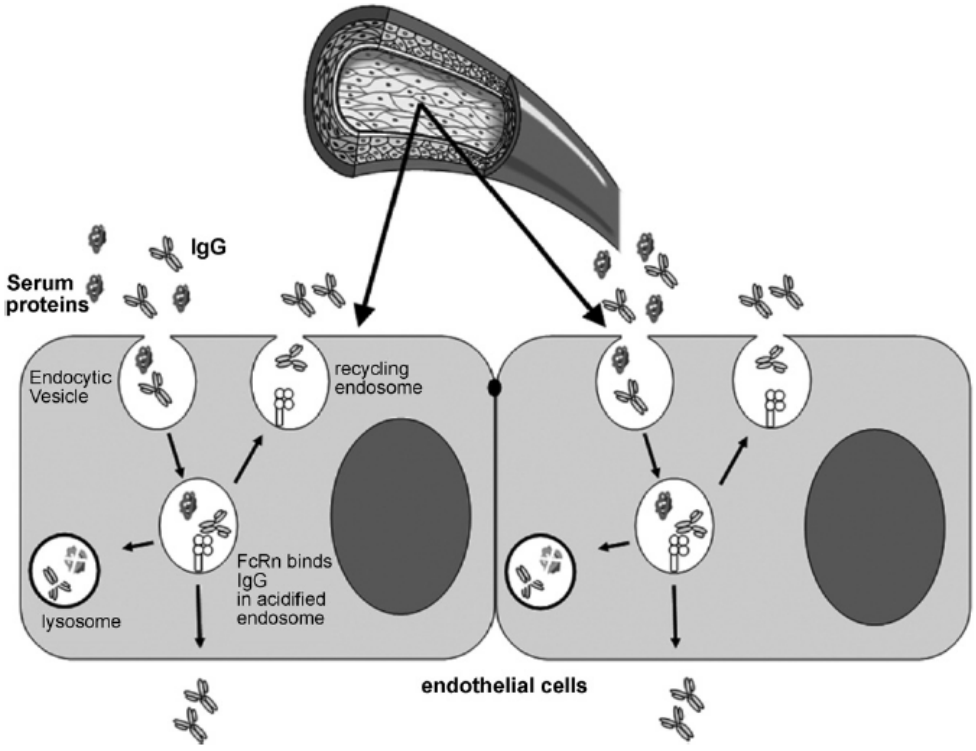

Figure 2; From Cervanac et. al. 2009: FnRn-mediated regulation of IgG catabolism in calves. IgG and other proteins are taken up by fluid-phase endocytosis. IgG reacts with FcRn and can be transported to serum or is degraded in the lysosome if it is not bound to FcRn.

receptor may be different between quarter. Also, intramammary infections (IMI) can cause differences in immunoglobulin concentration in quarters. (114) Increase of presumably IgG2 and IgG1 were reported in infected quarters and quarters with high SCC. (17, 57)

Colostrum absorbance calves

Calves are born without IgG because of the cotelydonary (syndesmochorial) type of the placenta. (9, 70, 75, 79, 113) Sufficient intake of colostrum of good quality is therefore essential for getting immune competent. Failure of passive transfer (FTP) was described in calves that were fed insufficient immunoglobulins in the 12-36 hours of life.(9) The capacity of the neonatal gut for endocytosis of immunoglobulins decreases rapidly over the first 12-36 hours after birth, because of downregulation of the neonatal FcRn-receptor and replacement of the neonatal gut epithelial cells by more mature cells. (18, 63, 70, 74, 107) The FcRn-receptor is expressed on neonatal gut endothelia and binds IgG after endocytosis and prevents degradation of IgG in the lysosome. (18) (Figure 1)

Feeding time but also feeding quantity is a key point in prevention of FPT. Different amounts of colostrum were recommended for feeding neonatal calves. Amounts of colostrum fed must be estimated with assumptions of colostrum quality, serum volume of the calves, and apparent efficiency of absorption (AEA).

Colostrum quality depends on factors described in this study like parity, dry period length, milking time, udder health and colostrum volume. Serum volume (TBV-PCV) of HF-calves was estimated between 5,3-9,9% of the body weight. (2, 44, 73, 88) Different absorption efficiencies and feeding volumes were reported like displayed in table 2. The AEA can be estimated roughly by the following formula: (89)

-AEA = plasma [IgG] (mg/ml) x plasma volume (ml) / [IgG] (mg) intake

Colostrum feeding quantity is dependent on colostrum quality and will be discussed later in this study. Frequent colostrum supply is preferable above excessive amounts. If insufficient IgG is absorbed, the blood [IgG] remains too low (<10 (87) or 13,4 (20) g/L blood), which was described as FPT. Higher mortality rates and more health problems like diarrhea and respiratory problems were associated with FPT. (81, 85)

Colostrum replacers

Awareness about the importance of immunoglobulins for the neonate is increasing. Commercial available colostrum replacers can be given to calves for providing adequate serum immunoglobulin concentrations. The efficacy of the colostrum replacers was disappointing providing FPT in 75-100% of the cases. (31, 103) Some effects were visible after duplication of the prescribed dose, but serum [Ig] was disappointing. (35) The AEA was lower for colostrum replacers compared to maternal colostrum.(103) Also, the antigen specificity of the colostrumreplacer is linked to the dams of origin, and will not completely

Year	First author	Quantity (L)/time(point)[1]	Administration	AEA (%)
2012	Russel	3/24h	oroesophageal tube	22
		4/24h	oroesophageal tube	16,8
2012	Mokhber	3/24h	oroesophageal tube	38
2012	Sato	1,5 (0h) & 2 (10h)	bottle	26
2010	Morril	2 (0h) & 2 (12h)	bottle	26,8
2009	Godden	1,5/24h	bottle	51
		1,5/24h	oroesophageal tube	40
		3/24h	bottle	41
		3/24h	oroesophageal tube	39
2007	Johnson	3,8/24h	bottle	26,1
2005	Jaster	2 (0h) & 2 (12h)	bottle	18-31
2001	Quigley &	3,9 (0h) &	bottle &	14-26
	Strohbehn	0,9-3,9 (8h)	oroesophageal tube	
1998	Quigley & Dewly	Variable	Review (unknown)	20-35

Table 2: Overview of studies about the apparent efficiency of absorption (AEA) of colostrum in neonatal calves. [1] Quantity colostrum in the first 24 hours of life. Feeding schedule described as quantity/time or as (calf age), dependent on availability of data. (6, 37, 48, 49, 77, 83, 85, 95-97, 99, 106, 109)

match farm specific bacteria and viruses. Colostrum replacers contain different amounts and specificities of antibodies, and therefore contradictory effects were reported. (33, 83, 84) Concentrated maternal colostrum containing herd specific immunoglobulins remains the best source of immunity for neonatal calves.

Effect of chronic infection on colostrum quality

Chronic infections may influence the immune response. *Mycobacterium avium* subspecies *paratuberculosis* modulates the immune system toward humoral responses and therefore increased serum antibody titer can be detected. (7, 105) Effects on colostrum quality are not described. Colostrum quality possibly decreases by enteric protein lose or chronic infection. Antibody concentration may also increase due to chronic immune stimulation and higher serum immunoglobulin concentrations. This study will test the effect of MAP on colostrum antibody concentration.

Aim of the study

This study will test colostrum of continuous milked cows and cows with a dry period for concentration of IgG, IgG1, IgG2, IgA and IgM. Differences between groups will be discussed and elucidated, as well as parity effects on colostrum quality.

2. Materials and methods

Case selection

Samples were gathered from cows of 13 Dutch farms for a period of february to august. Continuous milking farms were randomly selected from a farmers group participating in the ‘dry period shortening support group’. Control samples were collected from the teaching farm of the faculty of veterinary medicine of the University Utrecht that has a dry period length of at least 6 weeks. Also, control samples from previous studies were used. Cows with a dry period length between 2-42 days were excluded from analysis. A total of 227 samples were used in the study.

Sample collection

Farmers collected first milked colostrum as a pooled sample from all quarters in 50 ml falcon tubes as soon as possible after parturition. Every tube was linked to a questionnaire about parity, dry period length, calf colostrum supply, calf health and calf survival rates. Farmers having a colostrometer recorded also colostrum specific gravity. Samples were frozen at the farm directly after collection at -18 until analysis. Some of the samples from previous studies were quarter samples of heifers. Before pooling these heifer samples, differences in quarters were measured. Samples containing flakes or clots were discharged from the study because that was associated with infection and less IgG production. (64) Total 136 samples were previously tested for *Mycobacterium avium* subspecies *paratuberculosis* (MAP) by PCR and specific antibody ELISA. (Unpublished data)

Determination of [immunoglobulin]

Samples were centrifuged at 2500xg (Beckman Allegra X12-R Centrifuge) for 10 minutes to remove the milk fatcontent. Commercial Bovine Ig quantitation kits were used (Bethyl Laboratories, Inc, USA) according to instructions provided by the manufacturer. In short, samples were diluted 1:300.000 for IgG and IgG1 analysis and 1:30.000 for IgG2, IgM and IgA analysis after testing dilution rows. Samples were diluted for measuring absorbances within the reference row. Quantitative determination of the immunoglobulin concentrations were performed for IgG heavy chain, IgG1, IgG2, IgA and IgM. The ELISAs were performed in duplo in 96-wells plates (Costar 9018, Certified high binding-96 wells plate), with a duplo reference row on each plate. Differences between duplo’s of <10% were accepted and ELISA backgrounds were subtracted from the absorbance. Non-specific binding to the 96-wells plate was blocked by Blocking reagent for ELISA (Roche Diagnostics GmbH, Germany). Horseradish peroxidase (HRP) conjugated antibodies were used as capture antibodies and tetramethylbenzidine (TMB) for substrate and H_2SO_4 for stopping reaction. Absorbance was measured with a Thermo scientific Multiscan FC at 450 nm. The reference plot was performed by Log-transforming of the reference concentration as well as the absorbance. For accurately converting sample absorbance to immunoglobulin concentration, a linear reference plot with a $R^2 > 0,98$ was performed for each plate.

Statistical analysis

The statistical analysis was performed with IBM SPSS Statistics 20®, with a one way anova. The data was tested for normal distribution with a Shapiro-Wilkinson test. The mean [Ig] and standard deviations for all isotypes were calculated for parity groups and for the CM and the DP groups. Differences in [Ig] between groups were evaluated after post hoc Bonferroni correction, using statistical significancy at $p<0,05$..

When available, quarter differences of IgG, IgG1 and IgG2 were analyzed by a one way Anova with a post hoc Bonferroni correction.

Differences in colostrum quality between parities were analyzed within the control group by an one way anova and Bonferroni correction for all isotypes.

The effect of MAP on colostrum quality was analyzed by a one way Anova with a post hoc Bonferroni correction for the antigen and antibody positivity.

GraphPad prism® was used for creating boxplots.

Herd	# Cows	Age (av)	Production/Year Milk	Fat	Protein	Study participation CM/DP	# Samples	Average colostrum Ig mg/ml (sd) IgG	IgG1	IgG2	IgA	IgM
Z	56	4,09	6523	4,14	3,49	CM	33	30,9 (22,7)	20,0 (20,2)	2,8 (2,1)	9,8 (7,8)	4,5 (4,9)
E	96	4,02	8932	4,42	3,48	DP	32	35,9 (17,3)	31,4 (22,0)	4,5 (3,5)	11,2 (6,9)	4,3 (2,6)
G	83	4,03	6831	4,93	3,76	CM/DP	26	27,3 (16,4)	23,2 (20,0)	3,6 (1,9)	8,8 (6,3)	4,8 (3,5)
N	88	4,03	10357	4,39	3,48	DP	23	55,1 (22,2)	42,2 (24,7)	4,6 (2,8)	17,1 (10,0)	9,6 (5,3)
K	90	4,07	8701	4,62	3,57	DP	21	39,6 (19,8)	35,6 (26,5)	5,6 (3,6)	19,2 (13,2)	8,1 (5,3)
CV	-	-	-	-	-	DP	16	51,9 (18,1)	50,8 (19,7)	3,8 (2,3)	12,7 (6,3)	7,9 (2,9)
Un	114	4,01	7067	4,42	3,44	DP	16	39,8 (20,7)	36,4 (27,7)	5,6 (3,4)	17,6 (13,4)	6,3 (5,1)
T	69	5,00	9253	4,09	3,24	DP	15	56,4 (14,4)	48,5 (21,8)	4,1 (2,1)	13,7 (7,8)	7,4 (2,4)
M	128	4,05	7721	4,46	3,48	DP	13	34,0 (19,2)	26,3 (22,9)	3,9 (2,8)	13,5 (13,0)	3,7 (3,3)
Vn	91	4,00	9803	4,30	3,48	DP	12	27,5 (21,6)	27,7 (25,1)	4,2 (4,1)	9,9 (11,8)	3,8 (3,5)
D	96	4,57	8603	4,49	3,45	DP	11	48,1 (28,4)	35,6 (26,5)	4,8 (2,6)	18,5 (14,3)	8,2 (8,0)
J	114	4,02	9362	4,63	3,50	DP	9	44,2 (32,1)	35,8 (31,6)	5,3 (3,1)	20,1 (17,2)	6,5 (5,1)
V	111	5,00	6761	4,48	3,63	CM	3	31,1 (27,8)	17,7 (14,0)	3,2 (2,5)	9,5 (9,6)	2,6 (2,0)
B	67	5,08	7289	4,55	3,69	CM	3	30,8 (19,8)	23,0 (18,6)	3,1 (3,1)	7,0 (3,7)	2,8 (2,6)

Table 3; Participating herds ordered for study participation. Herd production averages and average colostrum quality.

3. Results

Effect of continuous milking on colostrum quality

Colostrum quality of CM cows (n=38) was significant lower compared to DP cows (n=186) for all immunoglobulin isotypes. (P<0,001) The immunoglobulin concentrations in the CM group versus the DP group were lower for IgG (24, 9 vs 41,5), IgG1 (14,8 vs 35,1), IgG2 (2,5 vs 4,8), IgA (8,8 vs 15,8) and IgM (6,4 vs 3,0) respectively. The lower colostrum quality within the CM cows was independent of parity for respectively IgG, IgG1, IgG2, IgA and IgM (p=0,156; p=0,614; p=0,407; p=0,102 and P=0,409). The variation between colostrum samples was quite large in both groups. Producing good quality colostrum after continuous milking was not impossible, but the chance of producing good quality colostrum is much lower compared to the cows after a dry period. (figure 3)

Results survey

Changes in milk composition of CM cows were observed by farmers beginning about two weeks before parturition. Two of the four continuous milking herds (V,G) reported problems with calf health, and one (Z) had problems a few months earlier. One farm (G) used colostrum supplements, due to lowered survival rates and experienced no improvement. Differences in survival rates between CM and DP could not been calculated because of the low numbers of deaths and lacking data corresponding to several colostrum samples. The colostrum feeding frequency was twice/day for herd V and G, and thrice/day for herd Z.

Farmers reported that CM cows had a lower production in the subsequent lactation. Easier working and reduction of startup problems were decisive advantages for implementation of CM.

Effect of parity on colostrum quality

The effect of parity on colostrum quality was tested within the dry period control group for all isotypes. No significant differences were detected for IgG (p=0,064), IgG1 (p=0,502), IgG2 (p=0,214) and IgM (p=0,257) between parities. Significant difference in [IgA] was detected only for parities 1 and 2 compared to 5 (p=0,002 and p=0,032). (figure 4)

Udder quarter [Ig] differences

No significant differences between udder quarters were detected for IgG, IgG1 and IgG2 (p=0,236, p=0,545, p=0,485 respectively). (figure 5)

Colostrometer for [IgG] estimation

In this study, one farm (G) reported specific gravity of the colostrum measured with a colostrometer on farm. Relation between ELISA [IgG] and specific gravity on farm were displayed in figure 7.

Effect of paratuberculosis on colostrum quality

A batch colostrum samples from the DP-cows were used for PCR on *mycobacterium avium* subspecies *paratuberculosis* (MAP) also. Also elisa's were performed for detecting MAP specific antibodies. Average colostrum quality was compared for different MAP test results. MAP antibody positive cows had significant higher quality colostrum, compared to MAP-antibody negative cows (p=0,001). (figure 6)

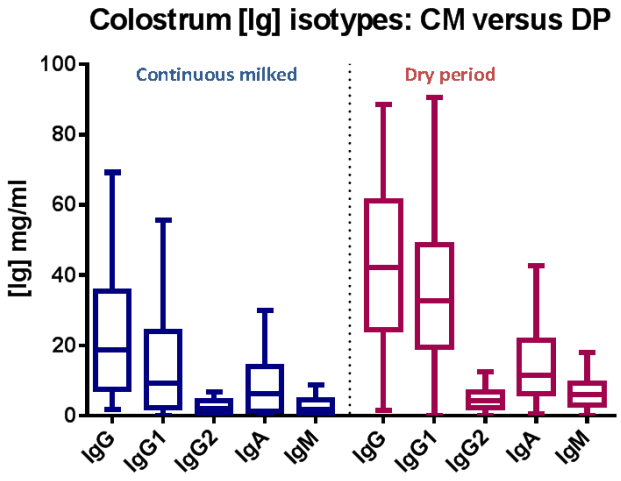

Figure 3: Difference in colostrum quality between CM (continuous milked) and DP (dry period) cows. Differences were significant for IgG, IgG1, IgG2, IgA and IgM. (p<0,001)

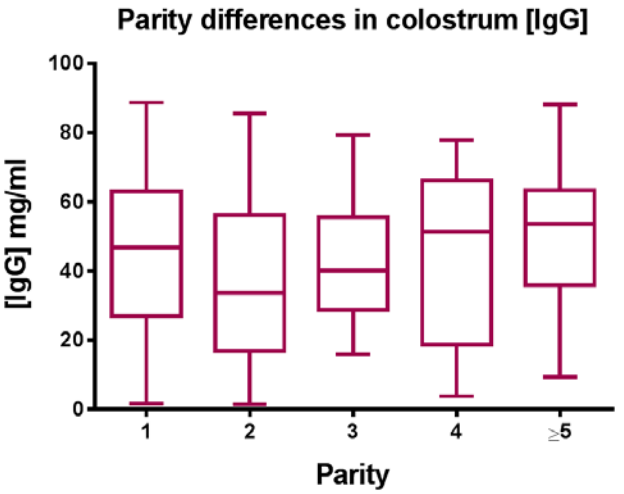

Figure 4: Parity differences in colostrum [IgG]. Parity differences were not significant (p=0,065) because the heifers produced relative good colostrum. The trend of higher colostrum quality for higher parities was visible. Pattern was comparable for other antibody isotypes.

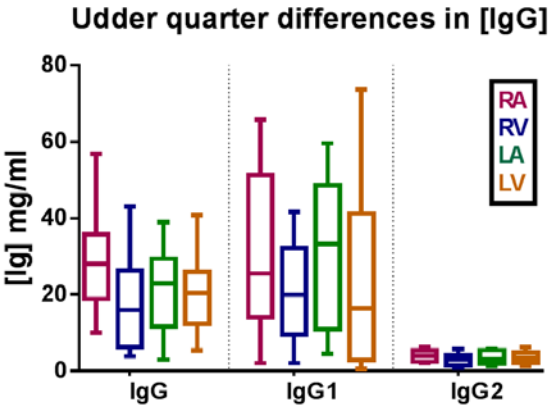

Figure 5: Udder quarter differences for IgG, IgG1 and IgG2. Comparison of first-milked colostrum between udder quarters of 10 heifers. No significant differences were detected for IgG (p=0,236), IgG1 9p=0,545) and IgG2 (p=0,485)

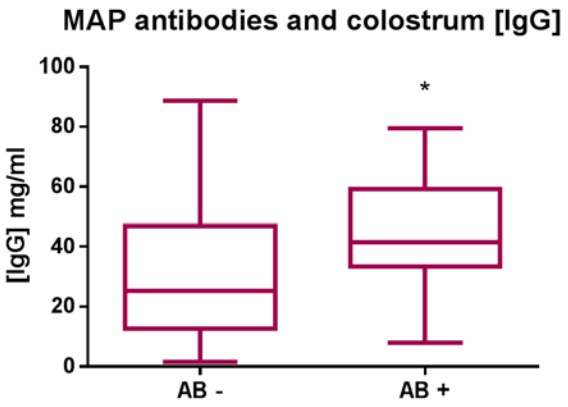

Figure 6: Significant difference (p=0,016) in colostrum [IgG] between MAP-antibody (AB) positive (n=33) and negative (n=99) cows.

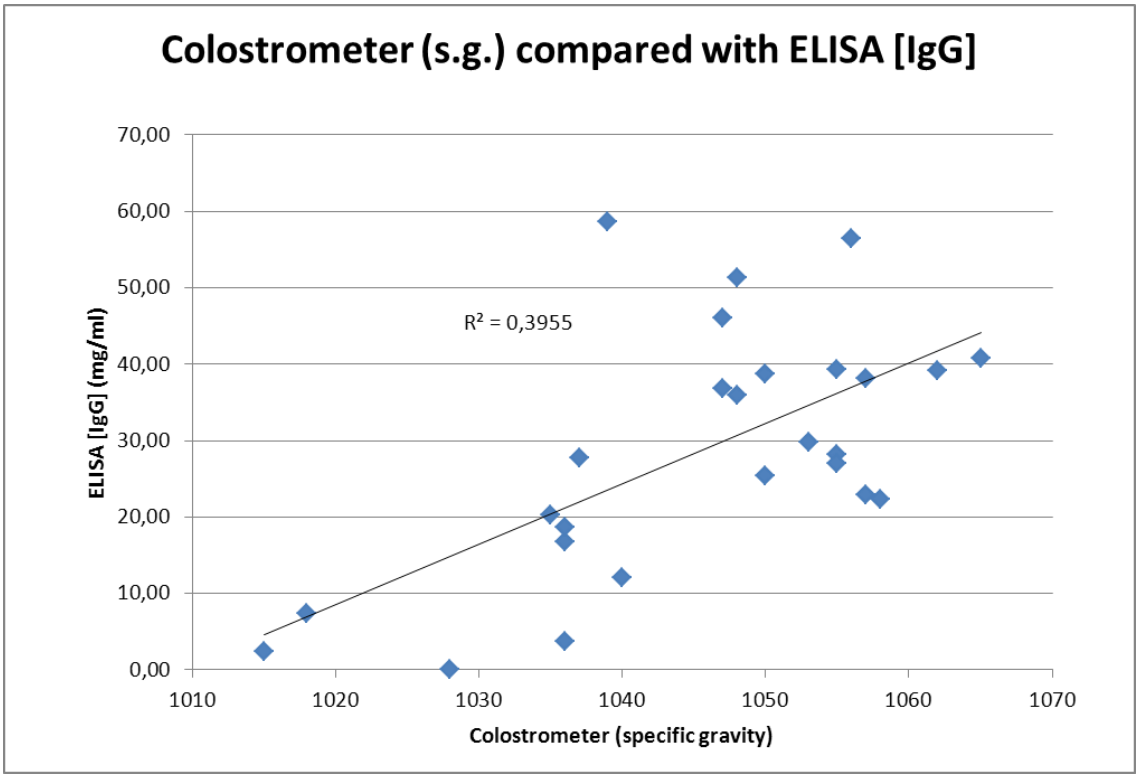

Figure 5: Colostrometer specific gravity compared with ELISA [IgG]. Colostrometer specific gravity was measured on one herd (G) by the farmer.

4. Discussion

Measure immunoglobulin

Before performing the ELISA immunoglobulin quantitation the colostrum samples were diluted to 1:300.000 which possible increased dilution errors.
A-specific binding to the 96-wells plate was blocked by Blockbuffer, a Blocking reagent for ELISA (Roche) which contained gelatin hydrolysates. The signal of the ELISA might be reduced by better blocking of the milk protein and casein in colostrum and therefore reducing background in the colostrum dilutions compared to the reference serum. (111) This might result in little underestimation of the immunoglobulin concentrations in less concentrated samples.

Generating a standard reference curve of a reference serum for comparing absorbance of colostrum samples was discussed by Li-Chan. (55) Underestimation of antibodies in milk compared to serum was possible, so using a milk-based reference next time is preferable. Therefore the concentrations of the antibody isotypes must be interpreted as approximate and not as the exact quantity. In the literature, large differences in reference concentrations for colostrum [Ig] were reported. (IgG 20-200 mg/ml) The variation between cows was increased by test-variations so comparison between different studies or methods is not recommended. Standardized test protocols in this study decreased test variation and makes results interpretable.

The relative high [IgA] in this study (11,9-21,5 mg/ml) compared to the literature (1,7-4,1mg/ml) can be caused by test variation or differences in conformation between serum and milk IgA. (62, 70, 91, 107) Different genetic haplotypes regulating PIGR-expression also influences [IgA], but simultaneous increased [IgM] was not detected.(10)

In this study, immunoglobulin concentration was determined for [IgG] total, and [IgG1] and [IgG2]. The [IgG]-ELISA kit was assumed to react with the heavy chain of both IgG1 and IgG2. [IgG3] in colostrum is not present in relevant quantities so [IgG1]+[IgG2] was supposed to be as high as [IgG]. (1, 62) Watching averages, the hypothesis was right, but IgG1+IgG2≠IgG for many individual cases. Differences can be caused by test variation, but the duplo’s of the test were always <10% nearly. Cross reactivity with other milk constituents was not impossible, but will be comparable for all samples and equal in both groups.(55) Nevertheless, results are mainly in accordance with previous studies and all samples were treated in the protocol, so results are reliable for comparing groups.

Effect of continuous milking on colostrum quality

A significant difference in colostrum quality between the group continuous milked cows and cows after a dry period was detected for all parities. Colostrum quality in continuous milked cows has not been thoroughly studied, but lower colostrum quality after continuous milking was also reported by Rastani, although the main focus of that study was milk production and energy balance. (92) Also Remond (unpublished data) suggests negative effects of CM on colostrum [IgG]. (94, 95) Also half udder experiments suggested decreased colostrum [IgG] after skipping a dry period, although performed in just five low productive cows in the seventies. (14)

Changes in udder secretion from about two weeks before parturition corresponds with other studies. This may be explained by the fact that transfer of immunoglobulin towards the udder starts several weeks before parturition. (14) Thus, in CM cows, immunoglobulin accumulation is probably disturbed by removal of milk before parturition. This hypothesis was supported by the comparable loss of all antibody isotypes after continuous milking. Previous studies detecting decreased colostrum quality in CM-cows were performed with low numbers of cows or cows not managed for continuous milking. (23, 24) In this study CM cows were managed for continuous milking, and colostrum immunoglobulin concentrations were also inferior to DP cows.

Shortening dry period to ±30 days did not significantly affect colostrum [IgG] in most studies. (5, 41, 112) Although not thoroughly studied, decreased colostrum quality after CM was suggested by Rastani and is supported by our results. (92)

Studies about continuous milked cows should be carefully interpreted. Frequently, the continuous milked cows were not managed for skipping a dry period because of late abortion, twin pregnancies or missed breeding dates. Time and method of colostrum collection, parity distribution between groups and preservation and testing of samples can increases the variation of the immunoglobulin concentrations. In this study, continuous milked cows were managed for continuous milking and results were checked for parity effects within groups. No significant parity by treatment effect was detected in this experiment. ($p>0,102$) Even though higher parity cows were assumed to produce more IgG, this effect was insufficient to compensate removal of the immunoglobulins before parturition.

The natural variation of immunoglobulin concentrations in colostrum is quite large. (DP: $1,4\leq[IgG]\leq88,6$ -- CM: $1,7\leq[IgG]\leq69,2$) Variation is caused by several factors like dry period length, colostrum yield, milking time, probably genetic differences and many variations within cow and management factors. Large numbers of cows were required for adequate analysis, and the more participants, the more variables can be tested for influencing differences. In this study, herd differences

cannot be reliable tested because the uneven distribution of parities and treatments between herds. Herd differences were previously described to clarify up to about 13,7% of the variation, but in this study cannot explain the differences between the CM and DP groups. (43)

Survey results about decreased calf health correspond to lower quality colostrum. Higher disease incidence in calves of the CM cows was in accordance with the lower colostrum quality. The farm that reduced calf problems (Z) feeds the calves thrice daily. Decreased milk production and reduction of start-up problems after continuous milking corresponded to previous studies. (12, 22, 93, 106)

Colostrum supply calves

Survey of participating farmers by the questionnaires did not result in significant differences between calf survival rates because the low numbers and lacking data of a batch DP-control colostrum samples from previous experiments. Calves reported as diseased (n=5) were fed average quantity, but presumably lower quality colostrum (average [IgG] 17,8 mg/ml). The inconsistency of the colostrum protocol was remarkable for the farm with the highest disease-prevalence (G). Calf-mother separation, colostrum collection time, feeding time and quantity were not standardized. Colostrum quality estimations with a colostrometer were insufficient used in feeding protocols.

Standardized colostrum protocols covering every situation cannot be given because the huge variation of colostrum qualities and absorbance efficiencies. Correct estimates of the required colostrum quantities cannot be made without indications of colostrum quality. Colostrum quality is a keypoint in prevention of. Difference in IgG between CM and DP cows corresponds to at least 1,6 times more colostrum in the CM-group for the same IgG mass as the DP controls. The larger the volume provided to the calf, the lower the AEA through dilution of the antibodies. Instead of 2,5 liter, at least 4,2 liter will be needed for example. Decrease in colostrum quality results in increased risk of insufficient immunoglobulins which leads to shortage and administration problems.

A model for minimum colostrum feeding quantity was performed by us and displayed as decision tree for farmers. (Appendix 1) The decision tree implemented estimations calf weight for the serum quantity. Colostrum quality was estimated by colostrum quantity, based on produced amount of colostrum and parity of the dam and the difference between colostrum quality after continuous milking or a dry period.

For preventing FPT, minimal the recommended amount of colostrum must be fed in the first 24 hours of live and minimal half the quantity within two hours.

Effect of parity on colostrum quality

In this study, a trend in increased colostrum quality was detected for increasing age, but differences were only significant for IgA when comparing parity 1 and 2 with parity 5. The bigger chance of good quality colostrum in older cows was previously described. (43, 48, 78, 101, 108) Other studies reported no significant differences between parities. (16, 25) As suggested by others, this may be due to more concentrated colostrum in these cows, related to the longer exposure to pathogens. (8, 71) In this study, the heifer colostrum quality was comparable with 3th and 4th parities in contrast to other studies. (108) However, frequent good quality colostrum produced by heifers was previous reported and corresponding to our data. (8, 19) Eliminating heifer colostrum for supply to calves is unwanted. Generally, heifers start with low SCC’s and little volume which can be beneficial for colostrum quality despite of relative short exposure times to pathogens.

Quarter [IgG] differences

In this study, colostrum [IgG] differences were determined in colostrum of heifers. These heifers originated from one farm and samples were collected not sterile. Individual cow differences for quarters were not analyzed because the unrepeated measures/quarter and therefore the limited degrees of freedom. No differences in grouped quarter immunoglobulin concentration support the hypothesis of central regulation of the FcRn-receptor expression.(8) Small differences in quarter [IgG] may be caused by dilution through quarter different colostrum yields or differences in bacteriological clarity of the quarter. Results were comparable with a previous study. (37)

Colostrometer for [IgG] estimation

As described in the introduction, the colostrometer or hydrometer can be useful for estimation of colostrum quality but must be interpret carefully. In this study, the correlation (R^2=0,39) between colostrometer and ELISA was corresponding to a previous study (R^2=0,3-0,4). (70) On farm measurement of colostrum was not performed at standardized temperature and only on one farm, but indicated colostrum quality quite well.

Effect of paratuberculosis on colostrum quality

A total of 136 control samples were tested for presence of MAP antigen and antibodies in colostrum for another experiment. Comparison of colostrum IgG for different MAP test results showed significant difference between groups 0 (PCR and AB negative) and 3 (PCR and AB positive; p=0,041). Predominantly presence of antibodies seems to affect total colostrum [IgG] because MAP antibody positives have significant higher colostrum [IgG] (p=0,001). Parity differences were not observed (p=0,902), in antibody colostrum positivity. Higher colostrum [IgG] corresponds to studies that report a shift towards humoral immune response in later stages of MAP infections. (47, 105)

5. Conclusion

Continuous milking decreased average colostrum quality by 40%, compared to cows with a conventional dry period of at least 42 days. Providing adequate immunity to calves after continuous milking is possible in most cases, but the amount of colostrum fed has to be at least 1,6 times more compared to cows after a dry period. Large variations in immunoglobulin concentrations make it difficult to estimate individual colostrum qualities without information about colostrum quantity, cow parity, milking and feeding time and use of a colostrometer. A trend for higher colostrum quality for higher parities was visible, but heifers also produced good quality colostrum frequently. Late stage *Mycobacterium avium* subspecies *paratuberculosis* infection increases presence of immunoglobulin in colostrum, probably by immunologic shifting towards humoral responses.

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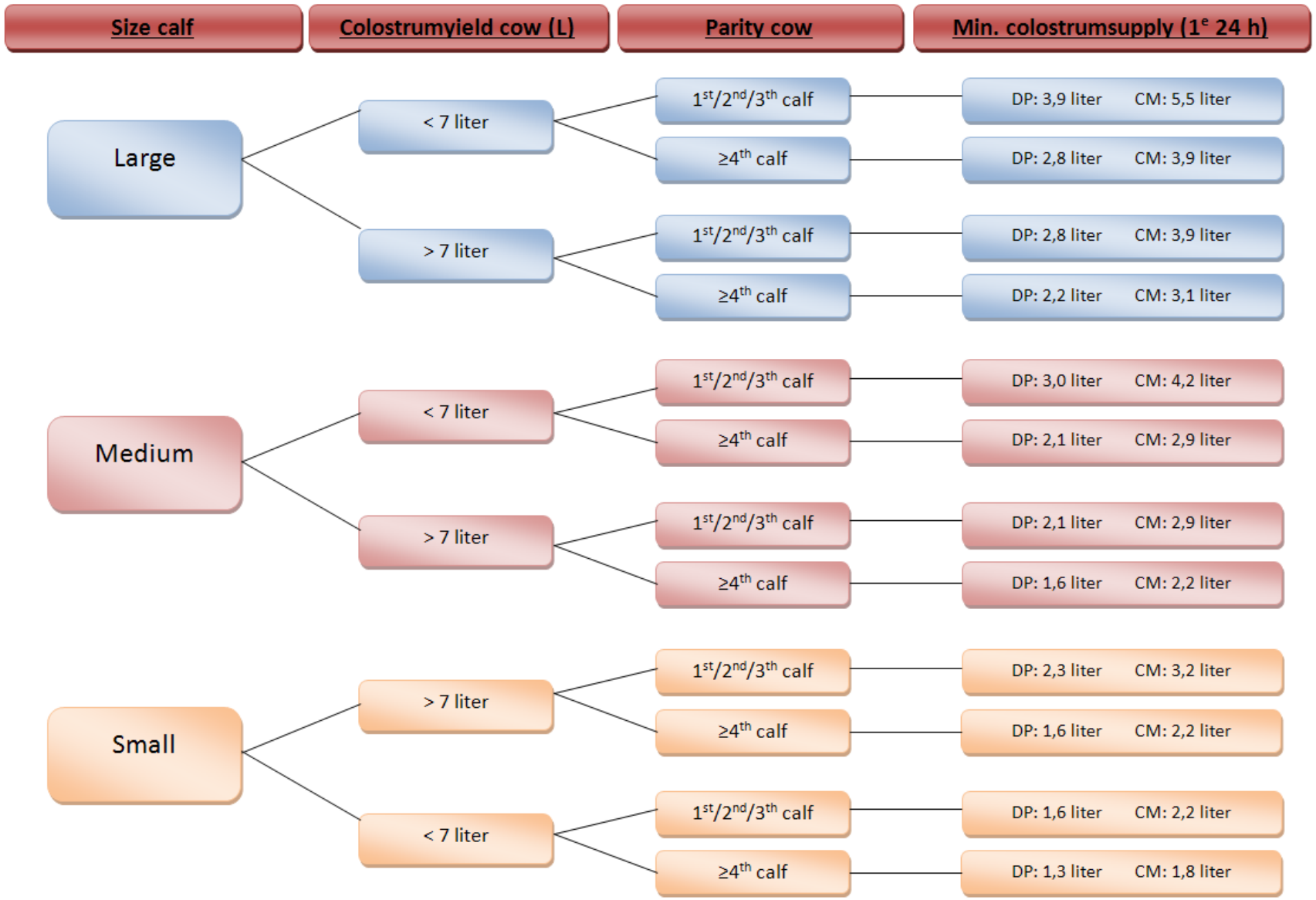

Appendix 1: Decision tree for minimal colostrum supply based on a model for cows after continuous milking (CM) compared to a dry period (DP). Decision tree displayed with assumptions for AEA (25%), blood volume (11,5% b.w.), hematocrit (0,35), required serum IgG (6 mg/ml). Calf sizes were large (60 kg), medium (45 kg) and small (35 kg). Higher parity increased average colostrum quality and higher colostrum yield decreased the colostrum [Ig] concentrations.

BUIS NUMMER	I&R KOE (123456789) (evt bedrijfs-nummer) Pariteit (omcirkel)	AFKALF DATUM (dd-mm-jaar)	I&R KALF (123456789) Geslacht (omcirkel)	BIESTGIFT (liters op eerste dag) (omcirkel)	OPNAME (hoe dronk het kalf de biest op) (omcirkel)	OVERIG (kalf gezondheid, eerste 6 weken/tot spenen) (omcirkel)
1	…. PARITEIT 1 (vaars) 2 3 4 5 of meer		… STIER VAARS	1 2 3 4 5 6 7 Liter	Vlot Normaal Traag	GROEI: (slecht) 1 – 2 – 3 – 4 – 5 (goed) ZIEKTE 0= niet ziek geweest 1=1x ziek geweest 2=vaker ziek geweest 3=dood gegaan op:…… AANDOENINGEN 1= hoesten 2= diarree 3=overig BEHANDELINGEN 1=antibiotica 2=overig

Appendix 2; Questionnaires corresponding to the colostrum sample number were replied by farmers.