

896/0301/09

2009

Experimental design:

1. Overall, 38 cows were included in this trial: 9 cows were tested in one year (group A) and 29 cows in a different year (group B). The cows were divided by their disease status (healthy or sick) and were given a disease score (0 = healthy, 1-3 = 3 is the most affected).
2. Milk was sampled from each cow between 3 to 6 times throughout the trial. The samples were taken before and after calving. The sample date was recorded and divided to 6 groups (DFC): 3 weeks or more before calving; 1 to 3 weeks before calving; up to 1 week before calving; up to 1 week after calving; 1 to 2 weeks after calving; and 2 weeks or more after calving.
3. Several parameters of the milk were determined from each milk sample: CD47 LYM - %; CD44 PMN - %; CD44 PMN - Δ MFC; CD47 PMN - %; CD47 PMN - Δ MFC; CD62 PMN - %; CD62 PMN - Δ MFC; CD18 PMN - %; CD18 PMN - Δ MFC;

Statistical models:

1. All statistical analyses were carried out with JMP software (SAS Institute, 2000).
2. The analyzed parameters was analyzed in one or two statistical models:
 - A. The disease status effect was determined by a three-way ANOVA model in a split-plot design, using the main effects Disease Status (healthy or sick) and DFC (Day From Calving: six groups). The third effect (a random effect) was the Group (A and B). The statistical model was:

$$\text{Model [1]: } Y_{ij} = \mu + \alpha_i + B_j + e^1_{ijk} + \gamma_l + \alpha\gamma_{il} + e^2_{ijklm}$$

μ = Mean of all data.

α_i = The difference between mean of Disease Status i from the trial mean (fix effect).

B_j = Variance between groups (random effect).

e^1_{ijk} = Variance between cows (Error1).

γ_l = The difference between mean of DFC l from the trial mean (fix effect).

$\alpha\gamma_{il}$ = Interaction between Disease Status and DFC.

e^2_{ijklm} = Residual variance between measurements (Error2).

In this model, the Disease Score effect and the Group effect was tested against the variance between cows and the rest of the sources were tested against the residual error.

B. The disease score effect was determined by a three-way ANOVA model in a split-plot design, using the main effects Disease Score (0, 1, 2 or 3) and DFC (Day From Calving: six groups). The third effect (a random effect) was the Group (A and B). The statistical model was:

$$\text{Model [2]: } Y_{ij} = \mu + \alpha_i + B_j + e^1_{ijk} + \gamma_l + \alpha\gamma_{il} + e^2_{ijklm}$$

μ = Mean of all data.

α_i = The difference between mean of Disease Score i from the trial mean (fix effect).

B_j = Variance between groups (random effect).

e^1_{ijk} = Variance between cows (Error1).

γ_l = The difference between mean of DFC l from the trial mean (fix effect).

$\alpha\gamma_{il}$ = Interaction between Disease Score and DFC.

e^2_{ijklm} = Residual variance between measurements (Error2).

In this model, the Disease Score effect and the Group effect was tested against the variance between cows and the rest of the sources were tested against the residual error.

3. Multiple comparisons between levels of disease status, disease score or DFC were done by Student t-test.

Results:

1. Analyzed parameter: CD47 LYM - %

Table 1: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) and in Model 2 (B, disease score), the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.2477
Disease Status	1	0.3239
Error 1*	34	<.0001
DFC	5	0.0604
Disease Status X DFC	5	0.0095
n	130	
R^2	0.691	
Variance between groups	-	
Variance between cows	46.5%	

B. Disease Score

Model 2		
Source	df	P value
Group	1	0.1408
Disease Score	3	0.8665
Error 1*	30	<.0001
DFC	5	0.1043
Disease Score X DFC	NA	
n	130	
R^2	0.622	
Variance between groups	-	
Variance between cows	43.1%	

* Error 1 in this model is the variance between cows.

Figure 2: Disease status X DFC interaction ($P = 0.0095$).

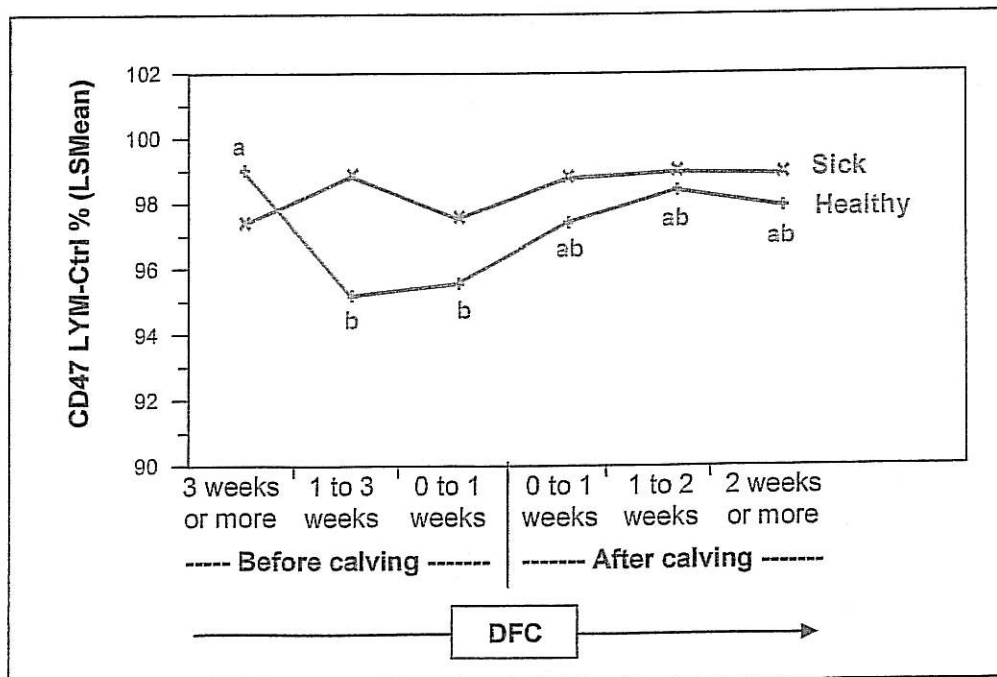


Table 2: The number of cows (n), CD47 LYM - Ctrl % LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

Also, CD47 LYM - Ctrl % means (Mean) of the disease status groups are detailed.

DFC		Healthy		Sick		<i>P</i> value	Sick					
		Score = 0 (18 cows)		All Scores (20 cows)			Score = 1 (8 cows)		Score = 2 (5 cows)		Score = 3 (7 cows)	
		n	Mean	n	Mean		n	Mean	n	Mean	n	Mean
Before Calving	3 weeks or more	18	99.0 ^a	15	97.3	0.0423	7	96.0	5	98.4	3	99.3
	1 to 3 weeks	7	95.2 ^b	10	98.8	0.0051	4	98.5	1	98.0	5	98.6
	0 to 1 weeks	12	95.5 ^b	13	97.5	0.0445	5	98.6	3	95.3	5	98.0
After Calving	0 to 1 weeks	17	97.4 ^{ab}	5	98.8	0.1205	6	99.0	4	99.8	5	100.0
	1 to 2 weeks	4	98.4 ^{ab}	11	98.9	0.7212	4	98.8	2	97.5	5	97.4
	2 weeks or more	1	97.9 ^{ab}	7	98.9	0.7070	3	100.0	0	--	4	97.8
<i>P</i> value		0.0009		0.4533								

2. Analyzed parameter: CD44 PMN - %

Table 5: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

Note: 3 records were excluded due to extreme values (lower than 10)

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.0566
Disease Status	1	0.4677
Error 1*	34	0.5483
DFC	5	0.0036
Disease Status X DFC	5	0.9635
n	126	
R^2	0.443	
Variance between groups	-	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 3: Disease status X DFC interaction ($P = 0.9635$).

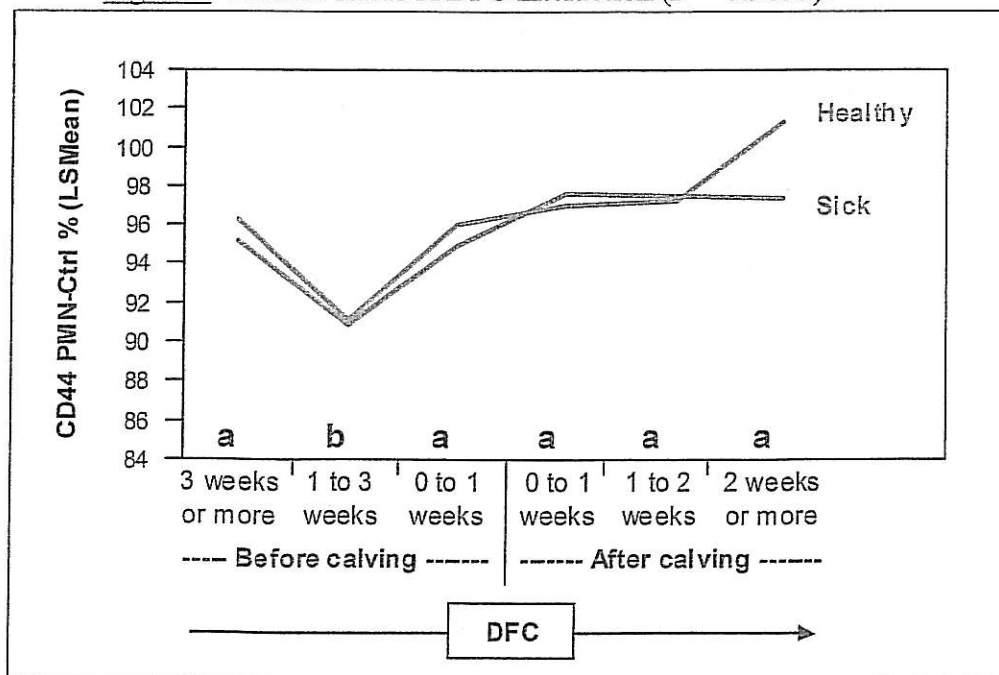


Table 6: The number of cows (n), CD44 PMN - Ctrl % LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before Calving	3 weeks or more	17	96.2	14	95.2	31	95.7 ^a
	1 to 3 weeks	7	91.1	9	90.9	16	91.0 ^b
	0 to 1 weeks	12	96.1	13	94.9	25	95.5 ^a
After Calving	0 to 1 weeks	17	97.0	14	97.6	31	97.3 ^a
	1 to 2 weeks	4	97.3	11	97.6	15	97.4 ^a
	2 weeks or more	1	101.4	7	97.4	8	99.4 ^a
<i>P</i> value						0.0036	

3. Analyzed parameter: CD44 PMN - ΔMFC

Table 7: The significance level ($P [F]$) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.0590
Disease Status	1	0.1388
Error 1*	34	0.9011
DFC	5	0.1694
Disease Status X DFC	5	0.2429
n	130	
R^2	0.330	
Variance between groups	-	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 4: Disease status X DFC interaction ($P = 0.2429$)

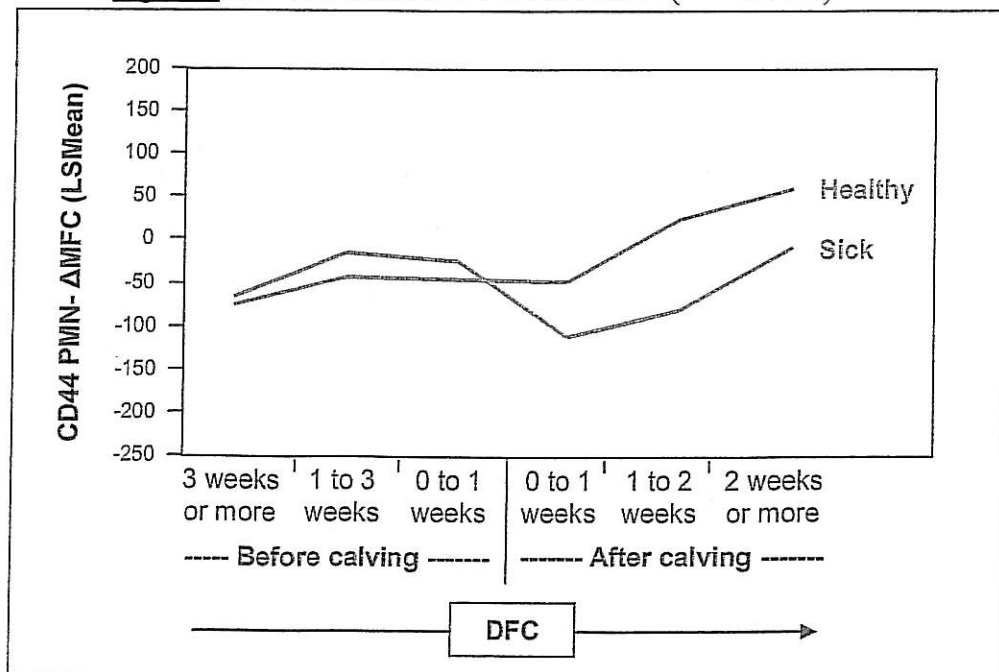


Table 8: The number of cows (n), CD44 PMN - Ctrl % LSmeans (Mean), and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before	3 weeks or more	18	-77	15	-67	33	-72
Calving	1 to 3 weeks	7	-42	10	-14	17	-28
	0 to 1 weeks	12	-44	13	-23	25	-34
After	0 to 1 weeks	17	-47	15	-113	32	-80
Calving	1 to 2 weeks	4	26	11	-80	15	-27
	2 weeks or more	1	62	7	-8	8	27
<i>P</i> value							

4. Analyzed parameter: CD47 PMN - Ctrl %

Table 9: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.2103
Disease Status	1	0.0152
Error 1*	34	0.7567
DFC	5	0.0001
Disease Status X DFC	5	0.0059
n	130	
R^2	0.442	
Variance between groups	-	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 5: Disease status X DFC interaction ($P = 0.0059$).

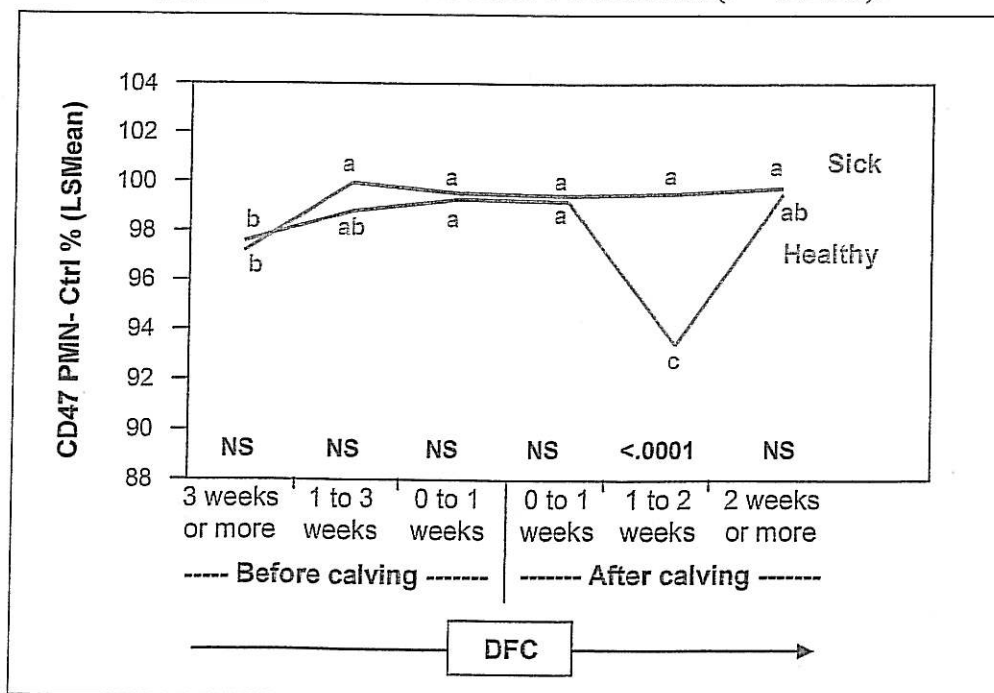


Table 10: The number of cows (n), CD47 PMN - Ctrl % LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		<i>P</i> value
		n	Mean	n	Mean	
Before	3 weeks or more	18	97.6 ^b	15	97.2 ^b	0.6707
Calving	1 to 3 weeks	7	98.8 ^{ab}	10	99.9 ^a	0.3578
	0 to 1 weeks	12	99.3 ^a	13	99.5 ^a	0.8305
After	0 to 1 weeks	17	99.2 ^a	15	99.5 ^a	0.7203
Calving	1 to 2 weeks	4	93.4 ^c	11	99.6 ^a	<.0001
	2 weeks or more	1	99.5 ^{ab}	7	99.8 ^a	0.9238
<i>P</i> value		0.0005		0.0348		

5. Analyzed parameter: CD47 PMIN - Δ MFC

Table 11: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.0187
Disease Status	1	0.1714
Error 1*	34	0.0229
DFC	5	0.0267
Disease Status X DFC	5	0.3576
n	130	
R^2	0.487	
Variance between groups	16.9%	
Variance between cows	15.2%	

* Error 1 in this model is the variance between cows.

Figure 6: Disease status X DFC interaction ($P = 0.3576$).

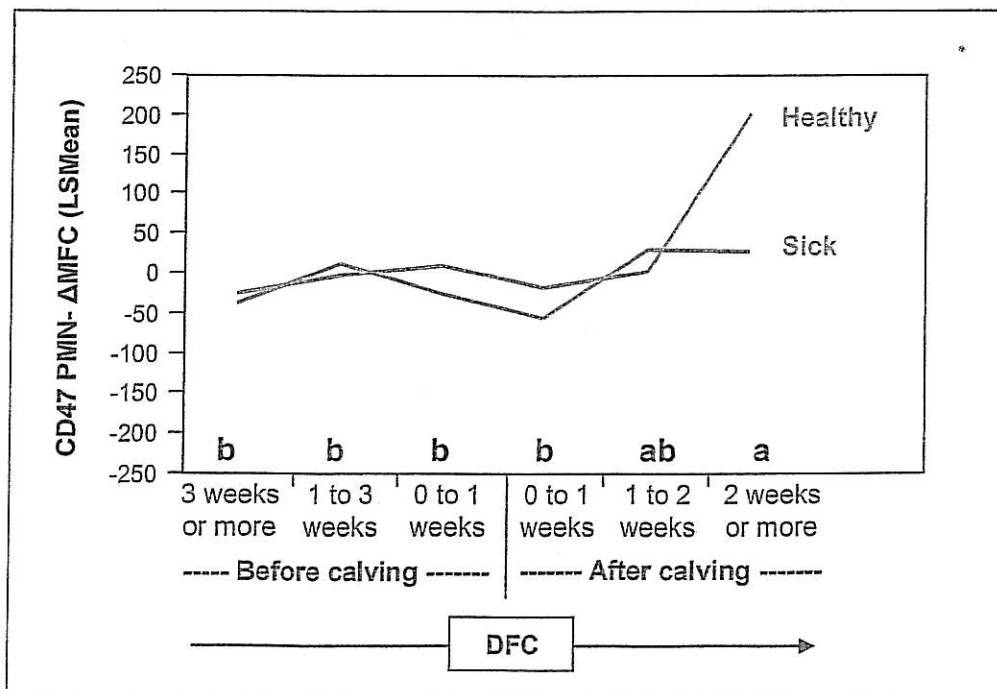


Table 12: The number of cows (n), CD47 PMN - Ctrl % LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before Calving	3 weeks or more	17	-24	14	-35	31	-30 ^b
	1 to 3 weeks	7	-1	9	14	16	7 ^b
	0 to 1 weeks	12	10	13	-24	25	-7 ^b
After Calving	0 to 1 weeks	17	17	14	-54	31	-35 ^b
	1 to 2 weeks	4	3	11	32	15	18 ^{ab}
	2 weeks or more	1	203	7	29	8	116 ^a
<i>P</i> value						0.0267	

6. Analyzed parameter: CD62 PMN – Ctrl %

Table 13: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.6277
Disease Status	1	0.9358
Error 1*	34	0.6856
DFC	5	0.0666
Disease Status X DFC	5	0.1229
n	130	
R^2	0.353	
Variance between groups	-	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 7: Disease status X DFC interaction ($P = 0.1229$).

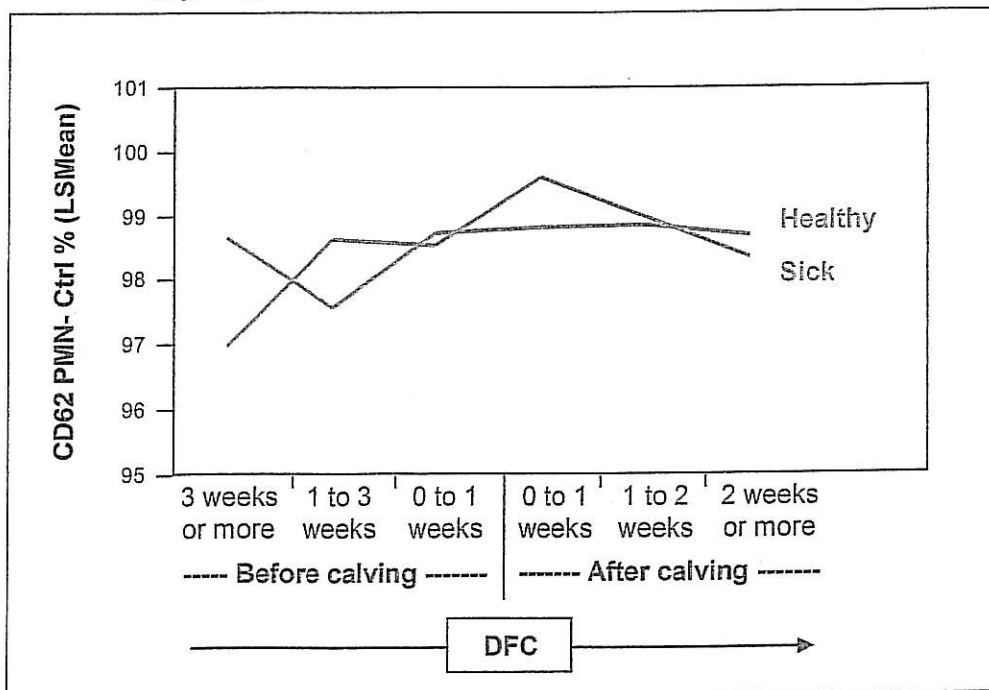


Table 14: The number of cows (n), CD62 PMN - Ctrl % LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before	3 weeks or more	18	98.6	15	97.0	33	97.8
Calving	1 to 3 weeks	7	97.5	10	98.6	17	98.1
	0 to 1 weeks	12	98.7	13	98.5	25	98.6
After Calving	0 to 1 weeks	17	98.8	15	99.6	32	99.2
	1 to 2 weeks	4	98.8	11	99.0	15	98.9
	2 weeks or more	1	98.7	7	98.3	8	98.5
<i>P</i> value							

7. Analyzed parameter: CD62 PMN - Δ MFC

Table 15: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.5133
Disease Status	1	0.1187
Error 1*	34	0.4996
DFC	5	0.5246
Disease Status X DFC	5	0.4181
n	130	
R^2	0.348	
Variance between groups	-	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 8: Disease status X DFC interaction ($P = 0.4181$).

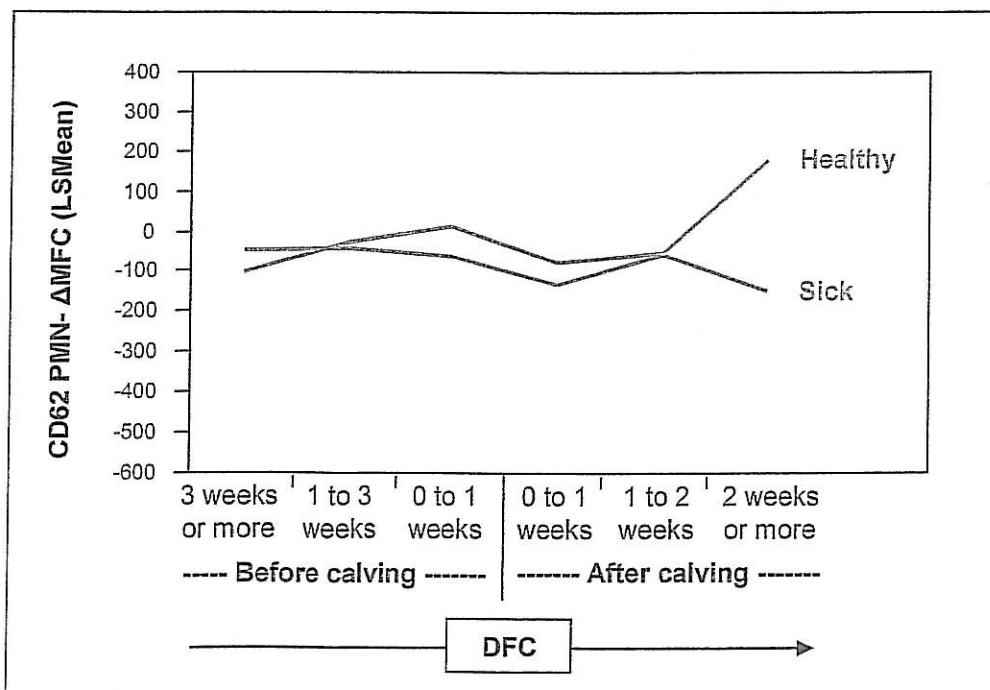


Table 16: The number of cows (n), CD62 PMN - Δ MFC LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before Calving	3 weeks or more	18	-102	15	-44	33	-73
	1 to 3 weeks	7	-26	10	-41	17	-33
	0 to 1 weeks	12	16	13	-60	25	-22
After Calving	0 to 1 weeks	17	-77	15	-130	32	-105
	1 to 2 weeks	4	-50	11	-55	15	-52
	2 weeks or more	1	180	7	-145	8	18
<i>P</i> value							

8. Analyzed parameter: CD18 PMN - Ctrl %

Table 17: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

Note: 2 records were excluded due to extreme values (lower than 76)

A. Disease Status

Model 1		
Source	df	P value
Group	1	<.0001
Disease Status	1	0.1521
Error 1*	34	0.6409
DFC	5	0.2922
Disease Status X DFC	5	0.9198
n	128	
R^2	0.419	
Variance between groups	36.3%	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 9: Disease status X DFC interaction ($P = 0.9198$).

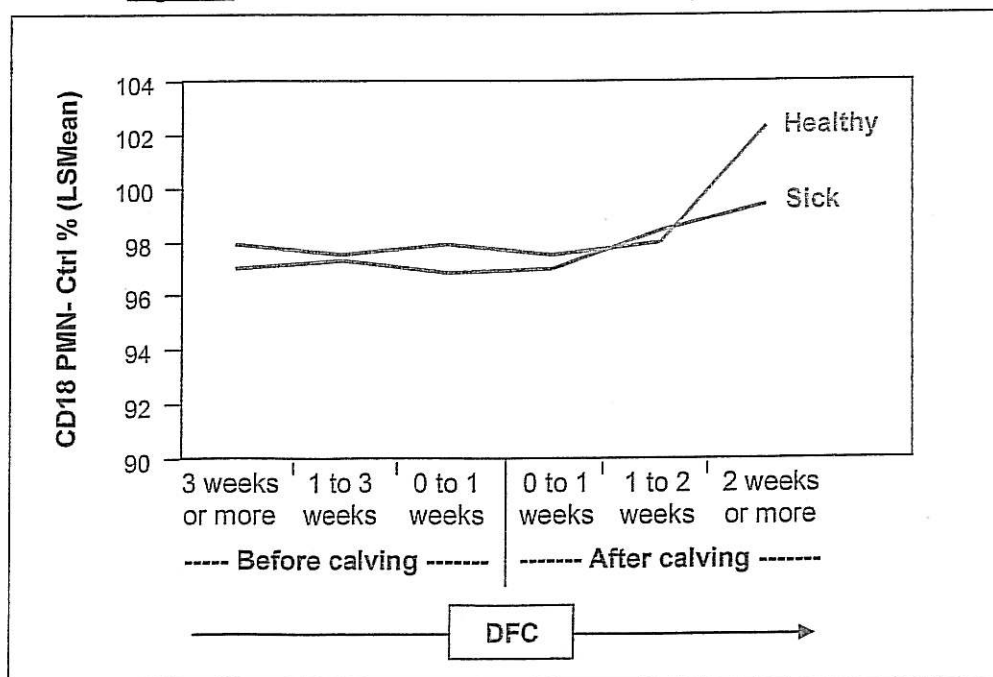


Table 18: The number of cows (n), CD18 PMN - Ctrl % LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before	3 weeks or more	18	97.9	15	97.0	33	97.5
Calving	1 to 3 weeks	7	97.6	10	97.3	17	97.4
	0 to 1 weeks	11	98.0	13	96.9	24	97.4
After Calving	0 to 1 weeks	16	97.5	15	97.0	31	97.3
	1 to 2 weeks	4	98.0	11	98.4	15	98.2
	2 weeks or more	1	102.3	7	99.4	8	100.8
<i>P</i> value							

9. Analyzed parameter: CD18 PMN - Δ MFC

Table 19: The significance level (P [F]) of the ANOVA Effects in Model 1 (A, disease status) the R square (R^2) and the percentage of variance between groups from the overall variance and between cows from the overall variance.

A. Disease Status

Model 1		
Source	df	P value
Group	1	0.0026
Disease Status	1	0.5188
Error 1*	34	0.3341
DFC	5	0.0299
Disease Status X DFC	5	0.3861
n	130	
R^2	0.501	
Variance between groups	22.3%	
Variance between cows	-	

* Error 1 in this model is the variance between cows.

Figure 10: Disease status X DFC interaction ($P = 0.3861$).

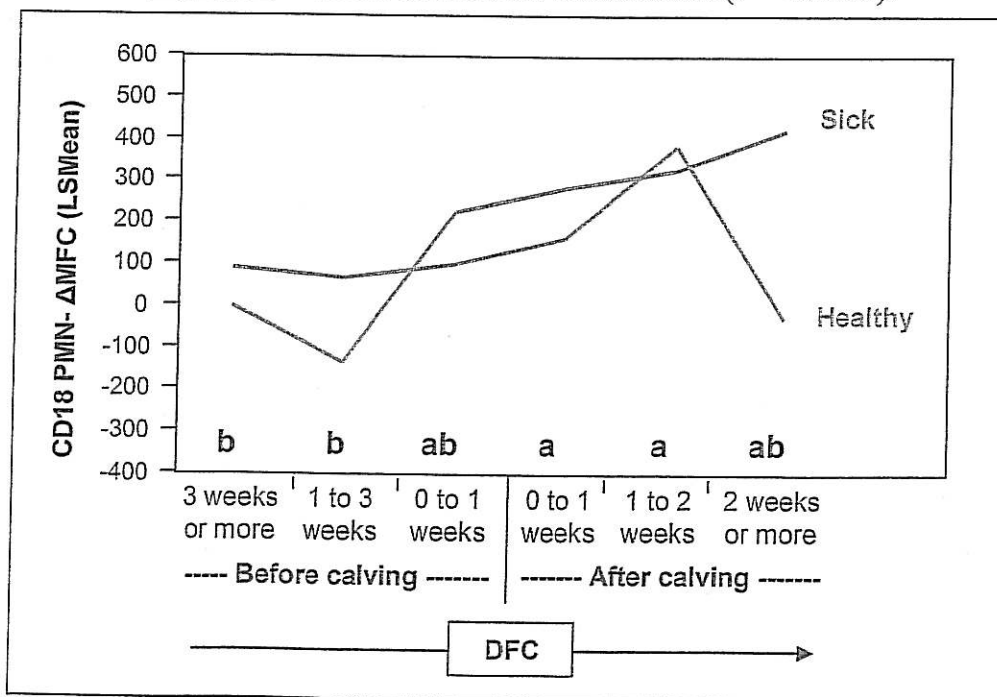


Table 20: The number of cows (n), CD18 PMN - Δ MFC LSmeans (Mean) and the significance (*P* values) between levels of DFC groups and Disease Status combinations.

DFC		Healthy (18 cows)		Sick (20 cows)		All (38 cows)	
		n	Mean	n	Mean	n	Mean
Before Calving	3 weeks or more	18	93	15	-1	33	46 ^b
	1 to 3 weeks	7	70	10	-132	17	-31 ^b
	0 to 1 weeks	12	101	13	230	25	165 ^{ab}
After Calving	0 to 1 weeks	17	164	15	285	32	225 ^a
	1 to 2 weeks	4	386	11	329	15	357 ^a
	2 weeks or more	1	-28	7	422	8	197 ^{ab}
<i>P</i> value						0.0299	