Is there a difference in the behavior of cows when they are immediately separated from their calves, versus separation after several hours or days?

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Abstract

Separating calves from the cow is a dairy management act that provokes the public debate. The intended health benefits for the calves are up for debate. While effects of separation on calf health and wellbeing are frequently investigated, the effect of separation on the wellbeing of the cows has not been well investigated. The aim of this study was to investigate whether there is a difference in behavior of cows after immediate separation, versus separation after several hours or days. In this study, cows of 8 farms have been equipped with sensors on their feet and neck. A period of 21 days was analysed. Data on eating, ruminating, activity, inactivity and lying was collected. As well as information from the farmers concerning calving and time of separation. Cows where divided in 5 groups dependent on moment of separation, G1 immediate separation, G2 separation within one hour, G3 separation within four hours, G4 separation within twelve hours, G5 separation after twelve hours. Cows who where immediately separated from their calves significantly differed from all the other groups in lying time. Cows in the group G1 spent around 610 ± 8.6 minutes a day lying, whereas the other groups spent around 640 minutes a day lying G2 629.5± 3.9, G3 654.2± 3.9, G4 646.3 ± 4.3. After 7 days of lower eating times the eating data increases and steadies at roughly the same level, so mean eating time differs from the level at which the groups stay after 7 days pp. Cows in G1 316.03 ± 5.83 had the highest eating time of around 350 minutes per day, G2 322.72 ± 2.41 and G3 317.81 ± 2.41 were slightly lower with around 330 minutes per day but with overall higher mean probably due to high daily mean on the first day owing to licking the calf. Eating time, after stabilizing, of G4 290.05 ± 2.65 was

around 330 minutes per day and of G5 189.59 ± 7.54 around 270 minutes per day. For ruminating time the course of the groups was almost the same. G2 376.14 ±2.62, G4 380.37 ±2.88 and G5 372.06 ± 8.18 were significantly higher than G1 366.42 ±5.57, G3 seemed slightly lower but did not significantly differ from G1. After stabilizing until around 7 days pp mean inactive time of G1 715.95 ± 8.95 was significantly lower than G2 700.93 ± 3.90, G4 730.27 ± 4.25 and G5 846.40 ± 12.08 while starting higher, G3 713.40 ± 3.91 did not significantly differ. Activity of G1 5361.37 ± 117.24 was higher from 7 days pp but mean overall activity was higher for G2 5469.45 ± 53.82 and G3 5450.23 ± 51.44 , G2, G4 4893.91 ± 57.18 and G5 3974.77 ± 162.06 significantly differed from G1.

Thus, in time eating, ruminating, activity and inactivity cows who had their calves removed immediately only did not differ significantly with the group who had their calves removed between one and four hours after calving. This study found a difference in behaviour, though further research is needed to investigate if the differences maintain during the whole lactation period.

Introduction

In dairy-farm practice cow calf separation at birth is common. By separating cow and calf after calving farmers intend to contribute to increasing health (circumstances) for the calf. There would be a decreased risk on faeco-oral transmission of disease and the farmer can provide for adequate colostrum intake (Flower, F. C., Weary 2003, Ridge, Baker et al. 2005). However, research is inconclusive about these allegations (Beaver, Meagher et al. 2019). This rearing procedure is one that provokes the public debate. Most people who are not involved in farming disapprove of cow-calf separation (Ventura et al. 2013, Cardoso et al. 2016).

Reasons to disapprove are often emotional, they feel separation of a mother and child is piteous. Another argument is that early cow-calf separation is unnatural, where living natural has the preference (Boogaard, Bock et al. 2011).

When a calf is born, a bond between the cow and calf has to develop. Forming this bond starts as early as 5 minutes after calving (Hudson, Mullord 1977). Soon after the calf is born, the cow starts licking off the amniotic fluids, which stimulates several physiological processes in the calf. Budding of the calf and vocalizations by the cow are also observed, these are innate maternal behaviors of the cow, which have probably developed for the stimulation of the newborn calf (von Keyserlingk, Marina A. G., Weary 2007a, Edwards, Broom 1982).

Separating of cow and calf, such as occurs in most farms, can induce behavioral responses by the cow (and calf). These responses can include: arousal, vocalizations, increasement of moving, increased time standing, keeping their head out of the pen and, in case of the calves, reduced play behavior (Flower, Frances C., Weary 2001, Lidfors 1996, Weary, Chua 2000, Johnsen, Mejdell et al. 2018).

It has been reported that the moment of separation has influence on the amount and intensity of the (separation induced) behavioural responses of the cow and calf. Lidfors (1996) first found that separation after 4 days versus immediately had an effect on the behavioral responses. Also, he did indicate that keeping the cow and calf together after calving stimulated them to a higher activity. Flower and Weary (2001) have found that separation after 14 days resulted in more vocalization, movement and placing the head out of the pen than when separation occurred after 1 day. This was also seen by Weary and Chua (2000) when they compared behavioral responses when separation occurred in 6 hours, 1 day and 4 days after calving. What has not been investigated yet, is if separation immediately, with no physical contact, versus separation after

several hours or days has an effect on the behavior of the cow. Thereby asking the question: Is it important for the cows' wellbeing to perform innate maternal behavior when the calf is born? This can be investigated by comparing the behavior of the cows after separation, focusing on behaviors which can give an indication of negatively affected well-being. For example eating and lying time of the cow, which have been found to be of high priority in cow behavior. When deprived of lying, social contact and eating for 15 hours, cows compensated most for lying time. Therefore, this was considered the highest priority. Eating time was compensated for by increasing the amount of food intake in a shorter period of time (Jensen, Munksgaard et al. 2004).

Deprivation of lying has an influence on the pituitary-adrenal axis and causes the blood cortisol/ACTH levels to rise. This did not occur when they challenged the cows with 75% food restriction (Fisher, Verkerk et al. 2002). Though this also appears relevant vice versa, stress and suboptimal environment influence the amount of time spent lying and eating. Stress and unrest in cows consequently decrease lying and eating time. Lying time has been reported to be a good indicator of cow welfare (Metz 1985, Cooper, Arney et al. 2007). Besides this deprivation of lying was found to have a slight negative effect on growth hormone (GH) levels (Munksgaard, Løvendahl 1993). GH plays a role in lactation, and high blood levels are associated with higher milk yield.

The objective of this study was to determine if there is a difference in behavior in cows who had there calves removed immediately, versus cows who had there calves removed after several hours or days. Differences in behavior could give some insight on the wellbeing of the cows. The wellbeing of the cows will be mainly assessed by looking at differences in time spent lying and eating. Time spent ruminating, non-chewing time and the amount of steps taken daily, have also been take into account. Knowing if immediate separation versus separation after several hours or days has an influence on the wellbeing of the cow, could help with developing management which is best adapted to the needs of the cow.

Materials and Methods

All cows (1533) on eight farms have been equipped with sensors (Nedap smarttag leg and neck, Groenlo, the Netherlands) on their feet and neck to register data 24 hours 7 days a week. The

sensors attached to their front leg registered: the activity measured by the amount of steps taken per day, overall time lying in minutes per day. Sensors attached to the collar around the neck registered eating related data: time spent eating in minutes per day, time spent ruminating in minutes per day, and inactive (with respect to eating) time in minutes per day. With these sensors deviating behavior is measurable, by plotting the information in graphs using excel. In this study, recorded data in the period from calving until twenty one days after calving was analyzed. Information concerning giving birth is recorded, day of birth and potential inconvenience or intervening by a vet while giving birth. After birth, the moment of separation was recorded. Farmers registered the time of cow-calf separation, in computerized forms. They recorded the separation interval time in 5 different groups (G1-G5, see below). All farms were visited weekly to obtain the data and also particularities were discussed. When cows had been ill, way of diagnosis and treatment was asked for.

Cows were divided in 5 groups; one (G1) in which separation has occurred immediately after calving, in the second group (G2) cow calf separation occurred within one hour, the third group (G3) contained the cows which separated from their calves after one and within four hours, in the fourth group (G4) separation occurred after four and within twelve hours, in the fifth group (G5) separation occurred after twelve hours. Of each group, the mean of measured data of the time spent lying, eating, ruminating and (in)activity, were determined with 95% confidence intervals. Analysis was performed using RStudio version 3.6.1, 2019, the R foundation of Statistical Computing. This was done with a random effects regression model. AIC-values were obtained to determine if the data was confounded and if the model used was just.

Inclusion criteria

Only multiparous cows were included. When a cow had twins, did not get up after calving or needed a calcium-bolus after calving it was not included. Cows that needed treatment or that died during the collection of data were not included either.

Graphs

The differences in activity, inactivity, lying, eating and ruminating time per group were plotted in graphs. The groups differed in the moment at which the cow and calf were separated and

contain all the cows from all the farms within these groups with the right inclusion criteria. For the descriptive data, the daily averages were obtained in Excel by (=AVERAGEIF), excluding all the columns without data. Between the different behaviors and between the groups, group size (n=) differs while sometimes not all data was available for all of the cows. When data of one cow for a certain behavior was missing for most of the days of the measured period, the data was excluded.

The graphs show the daily mean time of a certain behavior that was measured by the sensors on the cows. Statistics were based upon normal distribution of the data. Of all the behaviors AIC value was obtained to determined the model used. AIC value of the independent model was lowest hence this model was used.

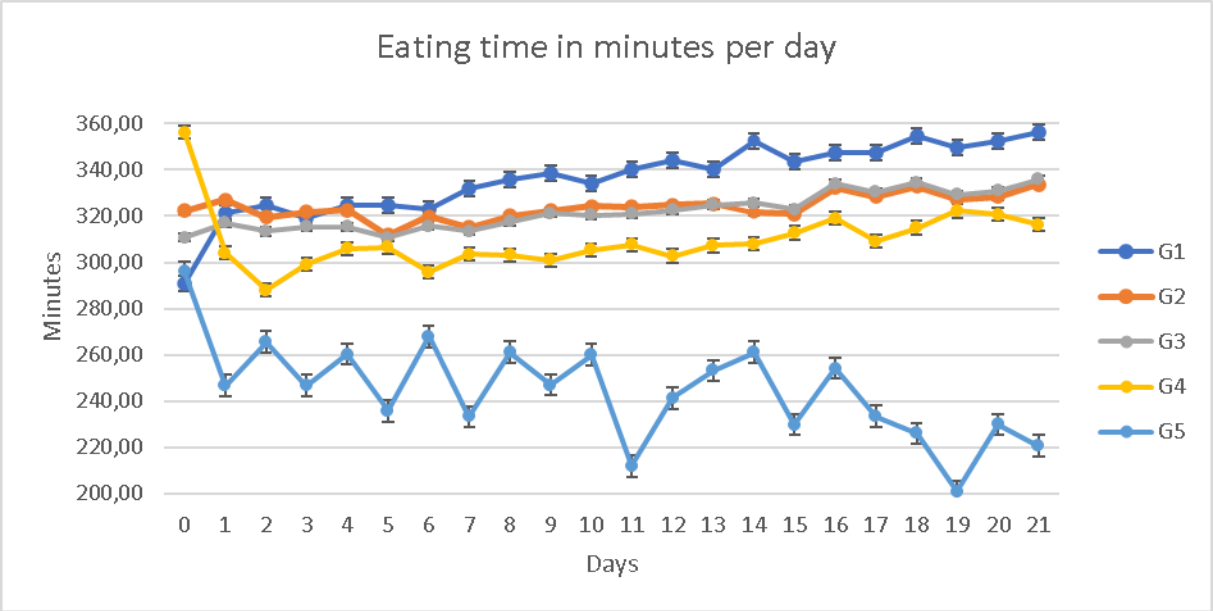
Results

The differences in activity, inactivity, lying, eating and ruminating time per group were plotted in graphs 1-5. The groups differ in size, while not all data was available for all cows. This was caused by the fact that for some days the sensors had not recorded the data properly, or something went wrong with uploading the data to the system. The appendix contains an overview (table 7) with the amount of cows per group and the distribution between the different farms. Graph 6-10 show the conditional means of group 1-4 obtained by ggplot, geom_smooth function in R-studio.

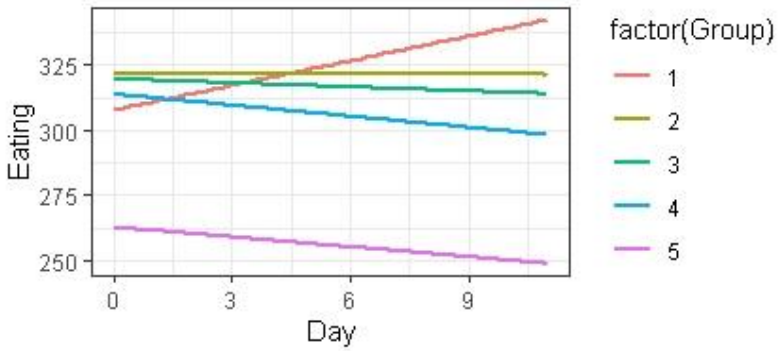
Eating time (Graph 1)

The eating times of G1 (n=98), G2 (n=180) and G3 (n=168) showed a rise from calving day until the first day after calving. Then eating time was stable in these groups until around day seven. After that, it increased gradually until day 21 (end of the study period). Eating time in G1 (316.03 ± 5.83) was highest from day 7 onwards. Eating time of G4 (n=88) and G5 (n=7) decreased after calving until first day after calving. G5 then showed an irregular declining pattern and a substantially lower eating time during the whole period. In G4 eating time rose somewhat in the following days than followed the same pattern as G1-3. Eating time of G2 and G3 was approximately 20 minutes lower, while eating time of G4 was approximately 40 minutes lower than G1. Confint (Table 1b) gave a difference between G1 and G2 (CI: 1.97- 11.40), a

difference between G1 and G4 (CI: -31.15- - 20.81) and also a difference between G1 and G5 (CI: -141.19 - -111.68). The difference between G1 and G3 was not statistically significant. Also in the graph 6a showing the conditional mean, the differences between G1 and the rest of the groups especially in the last days is evidently visible.



Graph 1. The mean Eating time in minutes per day from day of calving until 21 days after calving. *The lines represent the different groups, the cows that had their calf immediately removed (G1, n=98), the cows that had their calf removed within 1 hour (G2, n=180), the cows that had their calf removed within 4 hours (G3, n=168), the cows that had their calf removed within 12 hours (G4, n=88) and the cows that had their calf removed later (G5, n=7). Each value representing the mean eating time in minutes per day with the SD error bars.*



Graph 2. showing the conditional mean per day per group. *Lines 1-5 are respectively group 1- group 5.*

a)

Eating Fixed effects:	Estimate	Std. Error	t value
(Intercept)	316.03	5.83	54.25
factor(group)2	6.69	2.41	2.78
factor(group)3	1.78	2.41	0.74
factor(group)4	-25.98	2.65	-9.82
factor(group)5	-126.44	7.54	-16.78

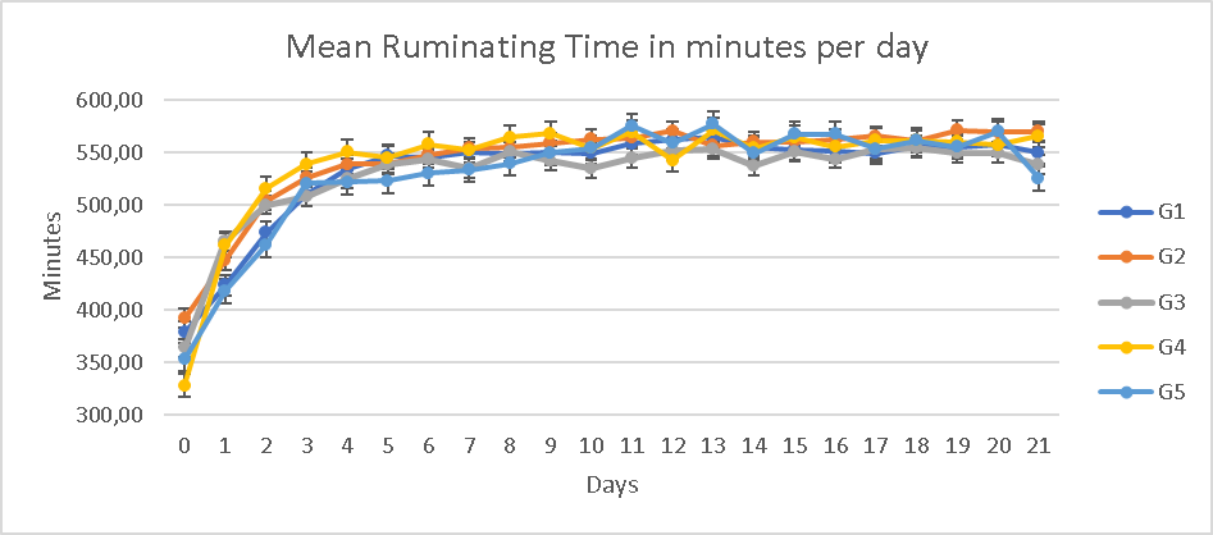
b)

confint(fit1) confidence intervals	2.5 %	97.5 %
.sig01	49.79	61.97
.sigma	80.82	82.96
(intercept)	304.60	327.43
factor(group) 2	1.96	11.41
factor(group) 3	-2.96	6.50
factor(group) 4	-31.16	-20.80
factor(group) 5	-141.19	-111.68

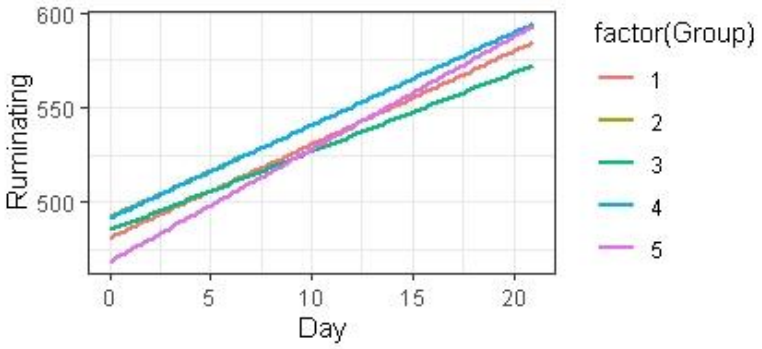
Table 1a. showing the fixed effect by fitting the data in R studio. 1b showing the confidence intervals of the groups *the groups G2-G5 are compared to G1 which is set as the intercept.*

Ruminating time (Graph 3)

Graph 2 shows the time spent ruminating per day. It can be seen that all groups G1-5 (n= 96, 177, 169, 87,7) show the same pattern. From the day of calving until about 8 days after calving the time spent ruminating rises. Then it stays approximately the same until the end of the studied period. Confint (Table 2b) was calculated for the whole period of 21 days and showed a difference between G1 and G2 (CI: 4.60- 14.84) as well as a difference between G1 and G4 (CI: 8.30- 19.60). The difference between G1 and G3 was not statistically significant.In Graph 3 the lines overlap, this makes interpreting the individual groups difficult. The appendix includes two graphs showing the mean ruminating time divided in two periods, from day one until day seven and from day eight until day twenty-one. So, the differences between the groups are easier to distinguish.



Graph 3. The mean ruminating time in minutes per day from day of calving until 21 days after calving. *The lines represent the different groups, the cows that had their calf immediately removed (G1, n=98), the cows that had their calf removed within 1 hour (G2, n=177), the cows that had their calf removed within 4 hours (G3, n=169), the cows that had their calf removed within 12 hours (G4, n=87) and the cows that had their calf removed later (G5, n=7). Each value representing the mean ruminating time in minutes per day with the SD error bars.*



Graph 4. showing the conditional mean per day per group. *Lines 1-5 are respectively group 1- group 5.*

a)

Ruminating Fixed effects:	Estimate	Std. Error	t value
(Intercept)	366.28	5.57	65.77
factor(group)2	9.72	2.62	3.71
factor(group)3	-3.48	2.62	-1.32
factor(group)4	13.95	2.88	4.84
factor(group)5	5.73	8.18	0.70

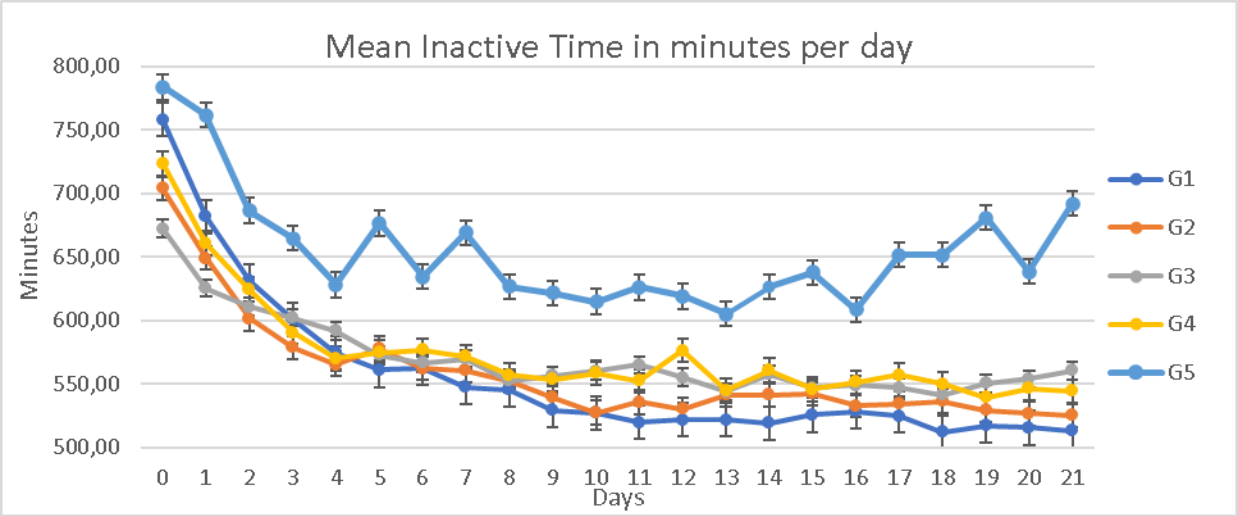
b)

confint(fit1) confidence intervals	2.5 %	97.5 %
.sig01	39.94	50.17
.sigma	87.68	90.00
(intercept)	355.37	377.18
factor(group) 2	4.60	14.84
factor(group) 3	-8.61	1.65
factor(group) 4	8.30	19.60
factor(group) 5	-10.30	21.74

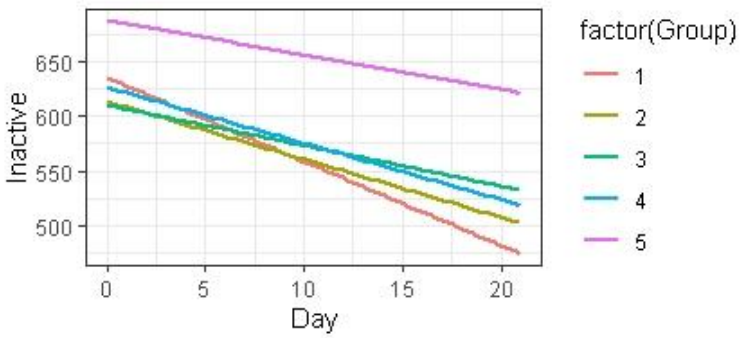
Table 2a. showing the fixed effect by fitting the data in R studio. 2b showing the confidence intervals of the groups *the groups G2-G5 are compared to G1 which is set as the intercept.*

Inactive time (Graph 5)

The inactive time (with respect to eating) of groups G1-4 (n= 96, 177,168, 88) decreased from day of calving until around 10 days after calving after which they stayed at a somewhat stable level. Inactive time of G1(715.95 ± 8.95) was lowest during the last ten days of the study period. The inactive time of G5 (n=7, 846.4 ± 12.08) decreased until day 4 after calving after which it had an irregular pattern for 4 days, then it was somewhat stable until day 13 after which it increased. Confint (Table 3b) showed a difference between G1 and G2 (CI: -22.66- -7.40), between G1 and G4 (CI: 6.03- 22.61), as well as a difference between G1 and G5 (CI: 106.79 - 154.10) The difference between G1 and G3 (CI: -10.19 – 5.11) was not statistically significant.



Graph 5. The mean inactive time in minutes per day from day of calving until 21 days after calving. *The lines represent the different groups, the cows that had their calf immediately removed (G1, n=96), the cows that had their calf removed within 1 hour (G2, n=177), the cows that had their calf removed within 4 hours (G3, n=168), the cows that had their calf removed within 12 hours (G4, n=88) and the cows that had their calf removed later (G5, n=7). Each value representing the mean activity time in minutes per day with the SD error bars.*



Graph 6. showing the conditional mean per day per group. *Lines 1-5 are respectively group 1- group 5.*

a)

Inactive Fixed effects:	Estimate	Std. Error	t value
(Intercept)	715.95	8.95	79.97
factor(group)2	-15.02	3.90	-3.85
factor(group)3	-2.55	3.91	-0.65
factor(group)4	14.32	4.25	3.37
factor(group)5	130.45	12.08	10.78

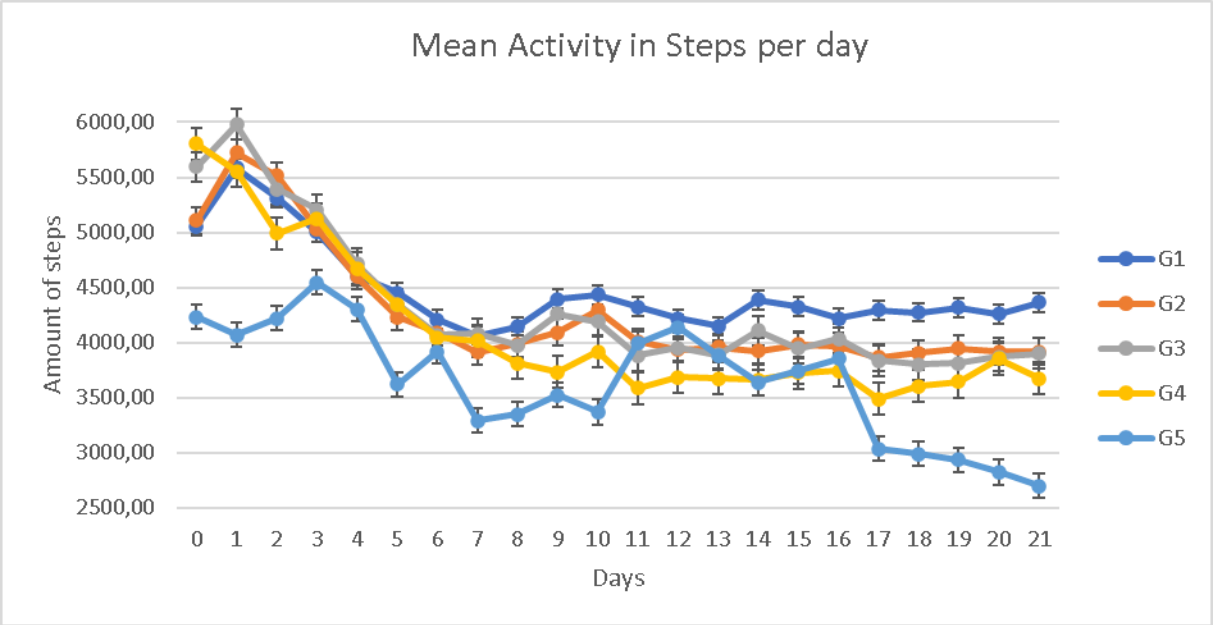
b)

confint(fit1) confidence intervals	2.5 %	97.5 %
.sig01	72.13	90.07
.sigma	129.25	132.68
(intercept)	698.42	733.49
factor(group) 2	-22.66	-7.38
factor(group) 3	-10.19	5.11
factor(group) 4	6.00	22.64
factor(group) 5	106.79	154.10

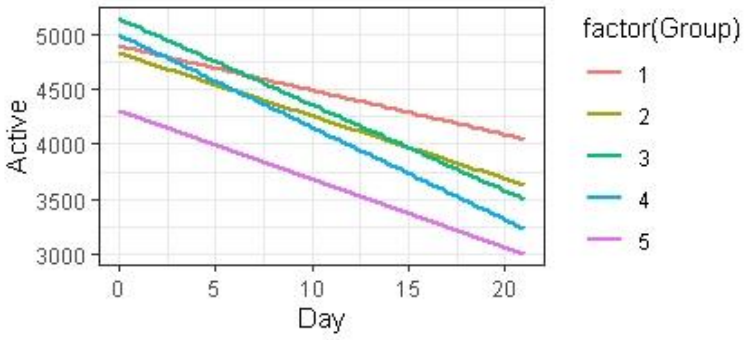
Table 3a. showing the fixed effect by fitting the data in R studio. 3b showing the confidence intervals of the groups *the groups G2-G5 are compared to G1 which is set as the intercept*

Activity (Graph 7)

All the groups showed an increased activity the day after calving. Then the daily activity decreased in approximately seven days. Thereafter, the activity stayed at about the same level for the following days, until the end of the study period, except for group 5. The cows in G1 showed more activity than the cows from other groups from 7 days pp. The difference between G1 and G2 (CI: 2.32-213.52), the difference between G1 and G4 (CI: -579.39- -355.46) and the difference between G1 and G5 (-1703.84- -1069.12) is statistically significant.



Graph 7. The mean activity in steps per day from day of calving until 21 days after calving. *The lines represent the different groups, the cows that had their calf immediately removed (G1, n=106), the cows that had their calf removed within 1 hour (G2, n=186), the cows that had their calf removed within 4 hours (G3, n=188), the cows that had their calf removed within 12 hours (G4, n=95) and the cows that had their calf removed later (G5, n=7). Each value representing the mean active time in minutes per day with the SD error bars.*



Graph 8. showing the conditional mean per day per group. *Lines 1-5 are respectively group 1- group 5.*

a)

Active Fixed effects:	Estimate	Std. Error	t value
(Intercept)	5361.37	117.24	45.731
factor(group)2	108.08	53.82	2.01
factor(group)3	88.86	51.44	1.73
factor(group)4	-467.46	57.18	-8.18
factor(group)5	-1386.60	162.06	-8.56

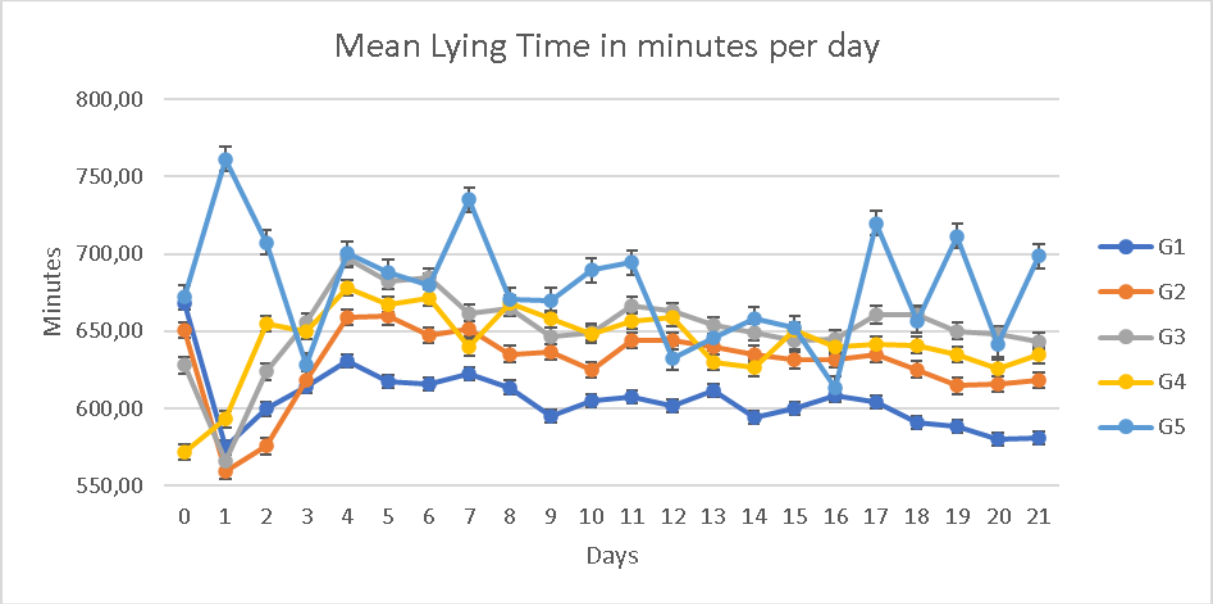
b)

confint(fit1) confidence intervals	2.5 %	97.5 %
.sig01	759.45	954.57
.sigma	1805.16	1853.45
(intercept)	5131.52	5590.94
factor(group) 2	2.32	213.52
factor(group) 3	-12.13	189.65
factor(group) 4	-579.39	-355.46
factor(group) 5	-1703.84	-1069.12

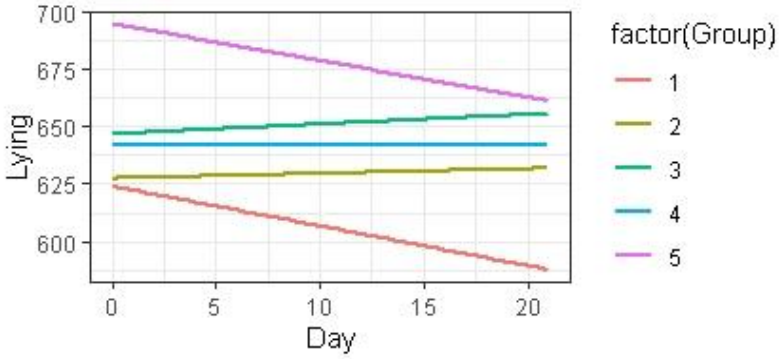
Table 4a. showing the fixed effect by fitting the data in R studio. 4b showing the confidence intervals of the groups *the groups G2-G5 are compared to G1 which is set as the intercept*

Lying time (Graph 9)

The lying time of groups G1-3 (n=105, 184, 186) decreased from the day of calving to the first day after calving, then it increased until day 4, thereafter it slowly decreased over the rest of the study period. Group G1 (609.90 ±8.55) had the lowest lying time overall and significantly differed from all groups. The lying time of G4 (n=94) increased from the day of calving until 4 days after calving, thereafter it slowly decreased taking an intermediate position between G2 and G3. G5 (n=8) showed a very irregular pattern, first increasing steeply and then an irregular pattern of decreasing with some peek days. Confint (Table 5b) showed a difference between all the groups relative to G1. Thus lying time in G1 was significantly lower compared with the other groups. It was also visible in the graph of the conditional mean (Graph 10) that lying time in G1 differed from the rest of the groups. It diverged from the other lines over time, meaning that the conditional mean of lying time of G1 decreased.



Graph 9. The mean Lying time in minutes per day from day of calving until 21 days after calving. *The lines represent the different groups, the cows that had their calf immediately removed (G1, n=105), the cows that had their calf removed within 1 hour (G2, n=184), the cows that had their calf removed within 4 hours (G3, n=186), the cows that had their calf removed within 12 hours (G4, n=94) and the cows that had their calf removed later (G5, n=7). Each value representing the mean lying time in minutes per day with the SD error bars.*



Graph 10. showing the conditional mean per day per group. *Lines 1-5 are respectively group 1- group 5.*

a)

Lying Fixed effects:	Estimate	Std. Error	t value
(Intercept)	609.90	8.55	71.30
factor(group)2	19.66	3.89	5.05
factor(group)3	44.29	3.90	11.37
factor(group)4	36.43	4.36	8.36
factor(group)5	117.43	12.31	9.54

b)

confint(fit1) confidence intervals	2.5 %	97.5 %
.sig01	63.72	79.79
.sigma	137.00	140.54
(intercept)	593.15	626.65
factor(group) 2	12.04	27.28
factor(group) 3	36.66	51.92
factor(group) 4	27.90	44.95
factor(group) 5	93.32	141.52

Table 5a. showing the fixed effect by fitting the data in R studio. 5b showing the confidence intervals of the groups *the groups G2-G5 are compared to G1 which is set as the intercept*

Group 1 and Group 3

The differences between G1 and G3 are not significant for activity, eating time, inactive time and ruminating time. Though on view it seems that G3 differs more or equally to the other groups in the last 10 days of the period. Therefore statistic tests comparing G1 and G3 for day 12-21 were performed, to find out if the groups did significantly differ in this period, like the other groups did. As is shown in Table 6 only for lying time (CI: 42.79 - 61.44) G3 did significantly differ from G1. Thus this similar to the test over the whole period.

Confint (fit1) G1, G3 day 12-21	2.5%	97.5%
Eating	-11.41	0.99
Ruminating	-5.18	7.36
Inactive	-4.13	13.15
Active	-132.05	143.74
Lying	42.79	61.44

Table 6 Showing the confidence intervals of G3 relative to G1 for day 12 until day 21 *obtained by confint fit function in RStudio.*

Discussion

The aim of the study was to investigate whether if there was a difference in behavior in cows when their calf was removed immediately, without contact, versus after contact for several hours or days.

The results showed some differences between the group of cows that had their calf removed immediately (G1) and cows that were separated after some time (G2-5). The data from the cows that had their calves for 12 hours or more (G5) showed much difference from the other groups. This was probably due to the fact that this group was from one farm, so farming management could have had a great influence, and the small group size (n=7), then outliers have much influence on the mean.

Eating

At the day of calving the graph showed for all the groups a higher eating time than G1, this could be due to the fact that the cows that had contact with their calf spent time licking and budding the calves. The sensors misinterpret this as eating. In the long term, cows in G1 seem to spent slightly more time on eating, this can also be seen in the conditional mean graph 6a. The statistical test for eating showed a significant difference between G1 and G2, G1 and G4 as well as G1 and G5, but not between G1 and G3. This could be due to the smaller group size of G3. Previous studies have found eating times to vary between 3 to 5 hours, with a positive correlation between higher feed intake and milk yield (Munksgaard, Jensen et al. 2005, Grant, Albright 2001, Løvendahl, Munksgaard 2016). In the present study, when eating time was somewhat stabilized. Eating times between the groups varied from slightly above 300 minutes per day in G4, to around 330 minutes per day for G2 and G3, to around 350 minutes per day for G1. G5 differed totally, eating times were lower dan 270 minutes per day from the first day after calving until the end of the study. The longer the cow calf contact the lower the eating times in minutes per day, except for G3 which does not significantly differ from G1 and is higher than G2. Perhaps increased unrest keeps the cows from going to the feed fence to calmly stand and eat there. If with the increased eating time the food intake is also increased, it could be of benefit for the cows health and wellbeing. In the first weeks after calving cows are at greater risk of diseases. These diseases are associated with NEB which is caused by inadequate energy intake

and increased energy demand due to and increasing milk yield (Drackley 1999). Limiting the risk of disease would benefit wellbeing of the cow and would benefit the farmer.

Ruminating

The pattern that ruminating showed in the graphs was almost the same for all the groups. Though there were significant differences between the groups. G1 (366.42 ±5.57) significantly differed from G2 (376.14 ±2.62) and G4 (380.37 ±2.88), these groups ruminated slightly more during the day.

G3 (366.42 ±5.57) had a slightly lower ruminating time and did not significantly differ from G1, for this there is no logical explanation. The lower ruminating time in G1 and G3 may be due to increased unrest as shown in higher activity in amount of steps per day. In the present study the ruminating time in minutes per day is around 550 minutes per day for all groups, this seems to agree with findings in previous studies. Other studies found ruminating times varying from 460-540 minutes per day (Bae, Welch et al. 1983, Stangaferro, Wijma et al. 2016, Goff, Hohman et al. 2020). Though it is peculiar that the groups which spend more time eating, spent less time ruminating. Perhaps the groups that have a lower eating time are eating more efficiently, taking up more food per bite.

Inactivity

Inactive time is eating-related behavior and gives the time in which the cows are neither eating nor ruminating. Inactive time showed the same statistical pattern as ruminating and eating where a difference between G1 versus G2, G4 and G5 is significant, but the difference between G1 and G3 (CI: -10.16- 5.08) is not. At the end of the measured period all groups seemed to spend more time inactive than G1. What is similar to the lying time of the groups.

Activity (walking)

For activity (number of steps taken) the difference between G1 (5361.37 ± 117.24) and G2 (5469.45 ± 53.82), the difference between G1 and G4 (4893.91 ± 57.18) and the difference between G1 and G5 (3974.77 ± 162.06) is statistically significant. The activity slightly increased the day after calving which could be explained by the fact that the cows have been put into the milking herd.

Lying

For lying time all groups statistically differed from G1, meaning that cows in G1 indeed spent less time lying. Which is a remarkable result.

In other studies mean lying times of 11 h ± 2.1 hours a day, with varying means between farms of 9.5- 12.9 h a day (Ito, Weary et al. 2009) and preferred lying time of 12-13 h a day were found (Jensen, Pedersen et al. 2005a). Lying time has been reported to increase as days in milk increased (Bewley, Boyce et al. 2010). This is not seen in the present study, though it could be that an increase in lying time could appear later in lactation. Cows in the group G1 spent around 610 ± 8.6 minutes a day lying, whereas the other groups spent around 640 minutes a day lying G2 629.5± 3.9, G3 654.2± 3.9, G4 646.3 ± 4.3, almost half an hour more. This is around 10 and 10.4 hours a day, which is not optimal if 12-13 hours a day is preferred (Ito, Weary et al. 2009, Jensen, Pedersen et al. 2005b). Yet many factors influence lying time of which one is days in milk. Research has shown that in early lactation, time spent lying is lower than in mid and late lactation (Bewley, Boyce et al. 2010). In other studies, an average lying time of 10 hours a day in early lactation has also been found by measuring in 10 day time samples and with constant measuring using accelerometers (Vasseur, Rushen et al. 2012, Maselyne, Pastell et al. 2017). So the mean of 10-10.4 hours in this study seems to agree with the findings in previous studies. In the present study only the first 21 days have been measured to find differences between the groups. It would be interesting if these differences persisted throughout the lactation period or that perhaps differences would only be seen in the first period after calving.

Implications for welfare

It seems there is a difference in behavior in cows who had their calf removed immediately. As mentioned before, lying and eating are of high preference for cows (Jensen, Munksgaard et al. 2004). In the present study it seems that eating time was higher in most G1 cows, which seems positive for their welfare. From the day of calving eating time also increased in the other groups, though it was significantly lower. Except for G3 which did not significantly differ from G1. Increased eating time in the first period after calving has also been found in other studies, who explained that by the fact that a cow needs much extra energy to meet the demands for milk production (Maselyne, Pastell et al. 2017, Vasseur, Rushen et al. 2012). Amount of food intake was not measured in the present study, so whether only the time spent on eating or also the

amount of food (DM) intake is increased cannot be concluded. This could be something to further investigate. In previous studies, it has been found that when cows are deprived in lying and eating, lying time is compensated for rather than eating time. Though food intake was found to be the same due to increased food intake in the reduced time (Jensen, Munksgaard et al. 2004). The time spent lying in G1, however, was lower compared with the other groups. It is not clear why this could be. Whether lying less is an indicator of diminished welfare is, as far as I know, not investigated. There is evidence that deprivation of lying causes stress in cows (Fisher, Verkerk et al. 2002), but here they could lie down but they did not.
So, there is a difference between the groups, but if this is directly linked with diminished wellbeing (due to stress) of the cows is hard to determine with current knowledge. Decreased lying time and increased activity could be a risk factor for the development of sole lesions (Colam-Ainsworth, Lunn et al. 1989, Ss, Wr et al. 1993), which would negatively affect wellbeing. The increased eating time, conversely, could be positively affecting wellbeing, when increased amount of food intake would have a positive effect on decreasing the risk of disease around transition.

Maybe the differences in the first hours after calving play a role in the behavioral differences between the groups. In the first hours after calving the expression of maternal behavior including licking, budding and sniffing is high (Jensen 2012). This maternal behavior and cow-calf contact causes an oxytocin release. Oxytocin has found to have, among many others, a stress reducing effect (Uvnäs-Moberg, Johansson et al. 2001). Maybe the oxytocin release in the first hours of cow calf contact has some stress- and pain reducing effect after calving, which is not experienced by cows who had their calves immediately separated. Perhaps the combination of the stress of the inability to perform maternal behavior and the reduced/lack of oxytocin release contribute to a difference in subsequent behavior. This is highly speculative off course.
In the present study there is no statistically significant difference in the amount of steps per day between G1 and G3. For the other groups there is a significant difference, and all the groups have a lower mean activity than G1 after 7 days pp.
Increased activity is a sign of unrest or distress (Moberg, Mench 2000). Therefore, this supports the idea that immediate removal has a negative influence on the wellbeing of the cows. However, it becomes only evident after a week pp. For this there is no sound conclusion.

What is remarkable in the present study is that for all of the measured behaviors, except for lying time, G1 and G3 do not significantly differ. Whereas the rest of the groups do. Group G1 and G3 spent more time eating and inactive had a higher amount of steps per day and spent less time ruminating than the other groups. Though when examining eating time G3 appears to be lower than G2, which does significantly differ from G1. This suggests the influence of group size of G3 on significance.
Only for lying time G1 and G3 significantly differed, cows in G3 spent less time lying than cows in G1. This would mean that there is no difference in behavior between cows who were separated from their calves immediately and cows who had contact for more than one and less than four hours. This cannot be explained so far.

Further investigating the link between no contact and lying less would be interesting while lying in cows contributes to cows health and production in various ways (Ito, von Keyserlingk, M. A. G. et al. 2010, Cyples, Fitzpatrick et al. 2012, Munksgaard, Løvendahl 1993). However, more studies investigated the effects of separation on calf health and wellbeing rather than cows health and wellbeing. Though if positive health and wellbeing can be proven, this could be of great advantage of the farmers.
That keeping cow and calf together benefits the animals, contradicts todays management in most dairy farms where separation immediately after calving is the norm. On the basis of recent studies, early cow-calf separation is in discussion, while the reasons for separation seem not as firmly proven (Beaver, Meagher et al. 2019, Meagher, Beaver et al. 2019). Thereby, there are several studies showing the benefits of prolonged cow-calf contact for calves (Bristow, Holmes 2007, Wagenaar, J. P. T. M., Langhout 2007, Rajala, Castrén 1995, Beaver, Meagher et al. 2019).
Some studies found some effects of suckling on reducing the risk of mastitis (Johnsen, Zipp et al. 2016, Walsh 1974), though here suckling for longer periods was observed.
As for the increased health risk which prolonged cow calf contact would protect from, consistent evidence cannot be found. Beaver and Meagher et al.(2019) found that with regard to the respiratory health no evidence can be found that prolonged contact increases the risk of the calf getting sick. However, further research concerning this risk is desired. For calf scours no

difference or a reduced risk was found with prolonged contact. An increased risk of Johne's disease by feaco-oral transmission was not found to be proven by several studies. Most important for decreasing the risk of that infection is good management and clean calving pens (Donat, Schmidt et al. 2016, Johnson-Ifearulundu, Kaneene 1998). As for colostrum intake and immunity also no persistent evidence supporting the benefits of early separation was found (Beaver, Meagher et al. 2019). Though they remarked that better monitoring of colostrum intake is needed. Adequate colostrum intake, independently of time of separation, should be focused on in dairy management. Also with prolonged cow calf contact, a farmer can make sure a calf gets enough colostrum, for instance by assisting drinking or feeding the calf colostrum while in contact with the cow.

In the present study we have found a difference in behavior in cows who had their calves removed immediately versus cows who had some contact time. This difference is best seen in time lying and eating and is visible for the entire period of measuring, 21 days. Except for the day of calving where mean lying time of G1 is higher and mean eating time is lower. Which could be explained by the fact that cows in other groups spent time licking their calves and perhaps ingesting the placental fluids (von Keyserlingk, Marina A. G., Weary 2007b) and did not lie down because thy were occupied with their calf.

That these effects are seen for the whole period is remarkable and could be a reason for investigating the effects of immediate separation on the cow further. While differences are definitely seen, but for the interpretation on how this affects the cows wellbeing further research is needed. Than it would be interesting to investigate if the differences continue to exist during the entire lactation period.

When (more) benefits for cow health and wellbeing are found and proven, adjustments in management will (in)directly improve the management of farmers. Simple use of a cuddle box (CowSignals®) can be a easily implemented in calving management routine by the farmer. The cuddle box provides an easy way to combine prolonged cow calf contact with the possibility of managing sufficient colostrum intake and a clean environment (without pathogens which could be found in manure or amniotic fluids) for the calf. This is done by putting the calf in a straw box at the side of the feed fence, the cow can reach, lick and bud the calf and subsequently, while already at the fence, start eating. Meanwhile the calf lies in clean straw and can be hand or bucket fed colostrum.

Increasing the time that cows and calves spent together would also benefit the farmers' position in the public debate and meets the proponents of a more natural way of farming. This could provide for a more positive view of dairy farming.

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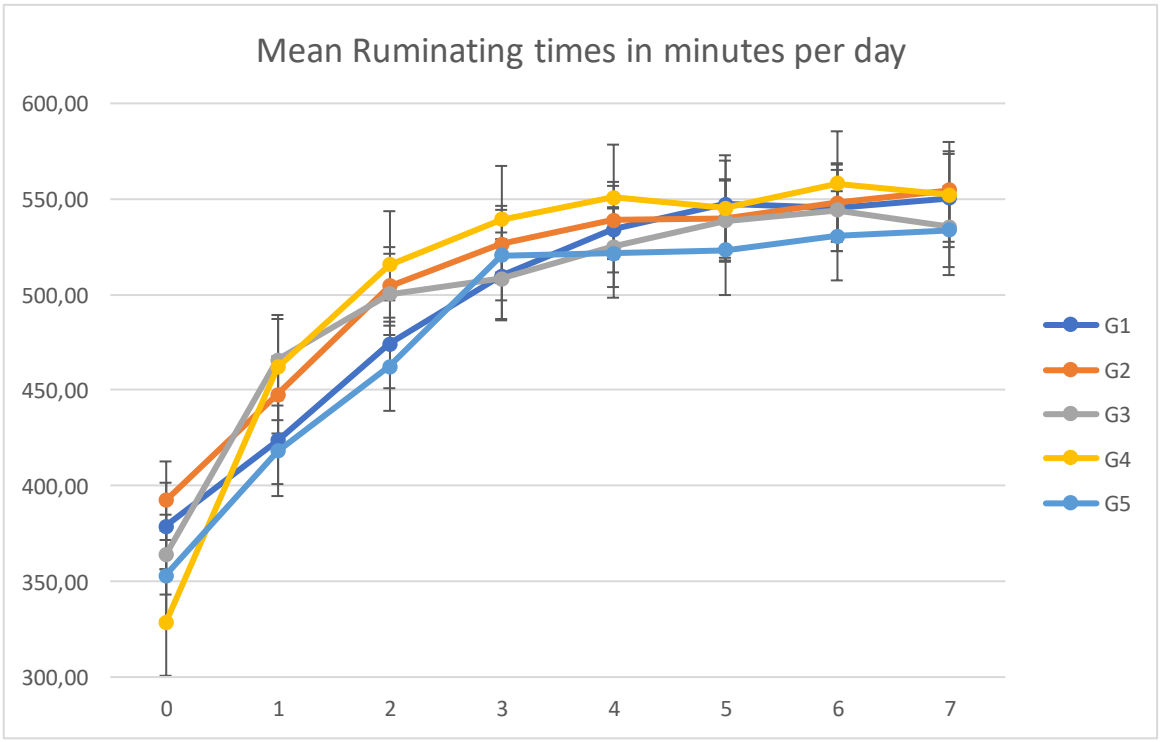
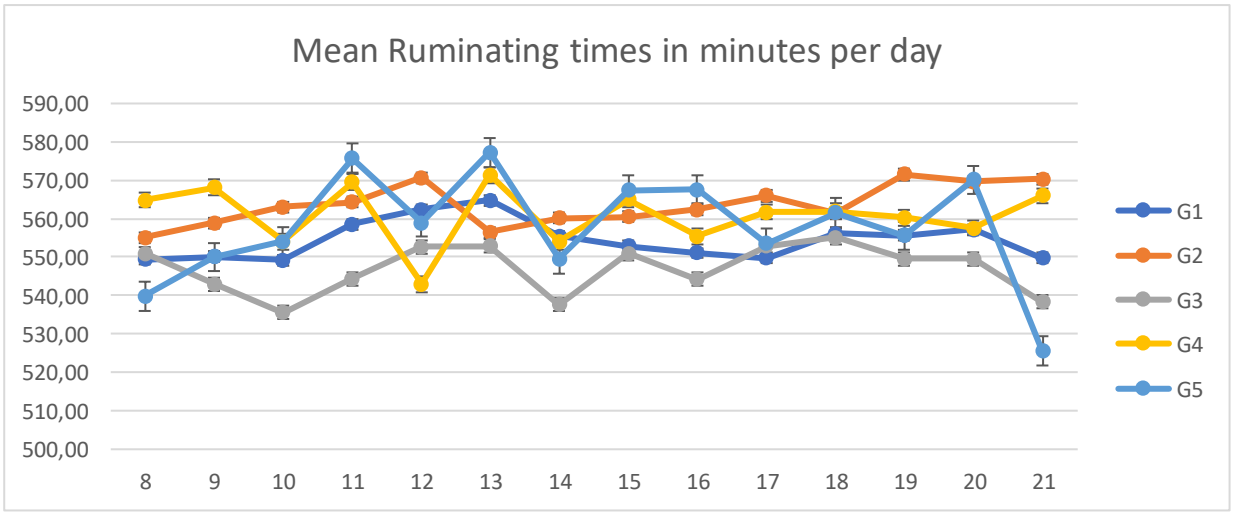
Appendix

a)



b)



Graph 2a-b. The mean ruminating time in minutes per day a) from day of calving until 7 days after calving and b) from day 8 until day 21. *The lines represent the different groups, the cows that had their calf immediately removed (G1, n=98), the cows that had their calf removed within 1 hour (G2, n=177), the cows that had their calf removed within 4 hours (G3, n=169), the cows that had their calf removed within 12 hours (G4, n=87) and the cows that had their calf removed later (G5, n=7). Each value representing the mean ruminating time in minutes per day with the SD error bars.*

	G1	G2	G3	G4	G5	Total
Farm 1	24	26	15	1		66
Farm 2	12	38	40	18		108
Farm 3	31	40	31	18		120
Farm 4	2	36	33	3		74
Farm 5	2	17	14	24	8	65
Farm 6	2	10	33	18		63
Farm 7	0	18	16	14		48
Farm 8	42	22	13	1		78
Total	115	207	195	97	8	**622**

Table 7. overview of the distrubution of the cows between the groups and farms. *Amount of cows per group can be different per graph, while sometimes data was lost due to fail in registration.*