# Effect of lying time on milk production of dairy cows in early lactation

Master Thesis – Research Internship Veterinary Medicine

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## Content

# Abstract

## Background

Cows have a high motivation to lie down, but the lying time of a cow depends on different factors. When testing if lying time is related to milk production these factors should be taken into consideration. These factors are environment related factors like cubicle characteristics or season and grazing, but also cow related factors such as lameness. In addition, factors that might influence the milk production like parity should be included in this relation. The aim of this study was to find a relation between lying time and milk production while correcting for these factors.

## Materials & Methods

A total of 83 cows of 7 farms that were 56±10 days in milk in the period September 2017 – February 2018 were used in this study. Cows that were on pasture in this period, and cows that were considered lame, were excluded.

The effect of lying time on milk production was tested using a linear regression line. To exclude influences of the farm and parity, another linear regression line was made with the relative lying time and relative milk production of each cow relative to the average of its own farm. At last, the effect of lying time on milk production was tested including the factors farm and parity in the statistics, using an ANCOVA test.

Two farms were tested individually using the ANCOVA test. The factor parity was included in this test. The same procedure was done as with the 7 combined farms.

## Results

Lying time did not have a significant linear effect on milk production. In the ANCOVA test, including farm and parity as factors, no significant effect of lying time on milk production was found in any of the groups (combined farms: $p=0.19$).

## Conclusion

In this study, the relation between lying time and milk production was not found significant, despite (or due to) the selection of cows and the correction on different factors.

# Introduction

Dairy cows in the Netherlands are often kept in free-stall barns. In this system the cows are milked in a milking parlour or with an automatic milking system and are free to walk around. The cows have individual cubicles and are able to choose in which cubicle they want to lie. Lying down is a behaviour that cows exercise a big part of the day. Gomez et al. (2010) found that cows spend an average of 11.9 h/d (SD ±2.4) lying. Jensen et al. (2015) found that heifers lie down for 12-13 h/d and the study of Ito et al. (2009) showed average lying times varying from 9.5 to 12.9 h/d per farm.

Lying is important for cows since depriving cows to lie down results in compensating behaviour afterwards (Norring and Valros, 2016; Tucker et al., 2018). Tucker et al. (2018) found that both cows being deprived to lie down for 4 h/d as non-deprived cows had an average lying time of 13 h/d. Reducing the time budget by depriving cows from lying down, eating and social contact for 1, 9 or 12 hours, resulted in a higher priority to lie down than time spend on eating or social behaviour (Munksgaard et al., 2005). Therefore we can conclude that cows have a high motivation to lie down. Besides meeting up to the needs or motivation of the cow to lie down a certain amount of time per day, it is important to favour this behaviour because of health aspects. For example: cows with lower lying times are found to have increased lameness, interdigital lesions and heel and sole lesions (Galindo and Broom, 2000).

### *Effect of lying time on milk production*

Except for the health aspect, promoting a longer lying time might also influence the milk production. Several studies compared lying time with milk production. Tucker et al. (2004) did not find a relation between cubicle width and milk production, even though lying time was affected. The duration of the test and sample size could have contributed to the lack of effect on milk production. On the contrary, Van Eerdenburg et al. (2013) found a positive relation between cow comfort and milk yield on Dutch farms. In their study, cow comfort was measured using different cow, environmental and health aspects. The factor lying time has not been measured but factors which are known to affect lying time were used. Bewley et al. (2010) found that milk production had a negative effect on lying time. They suggest that this could be caused by factors such as an increased requirement for food in high producing cows. However, in their study cows with different days in milk (DIM) where used. Cows with more DIM have lower milk yield and longer lying times (Deming et al., 2013; Munksgaard et al., 2005).

Grant et al. (2004) shows a graphic in which the lying time has a linear positive effect on milk production. Unfortunately, the full article of this study is not available on the internet so the material and methods of this study is unclear. Our expectation is that milk production increases when lying time is higher. Reason for this is that blood flow in the udder is higher in lying than standing cows (Metcalf et al., 1992). This increase in blood flow is associated with higher milk yield in the first three months of lactation (Berger et al., 2016). Moreover, energy wasted when standing possibly has a negative effect on milk production. Since energy intake and milk yield are correlated (Broderick et al., 2003) loss of energy could cause a decrease in milk yield. When testing the relation between lying time and milk production, different factors that influence lying time and milk production should be taken into consideration.

*Factors that influence lying time*

There are several factors that affect lying time of milking cows such as cubicle characteristics. Different farms have different cubicles regarding bedding and size. These variations have influence on the lying time of cows. Tucker et al. (2004) found that Holstein cows have 1.2 h/d longer lying time in cubicles measuring a width of 132 cm than 112 cm. Smaller cubicles cause longer time standing with two front hooves in the cubicle (Tucker et al., 2004) which also increases risk to lameness (Galindo and Broom, 2000). Tucker et al. (2003) compared preference and lying times of cows on 3 different types of bedding: matrasses, and sawdust and sand in deep-bedding cubicles. Cows preferred sawdust and sand bedding above mattresses and had longer lying time on sawdust than mattresses (Tucker et al., 2003). Also, the amount and quality of bedding material have effect. Cows spend more time lying down when more bedding is used (Tucker et al., 2009). A significant linear increase in lying time was found from 11.2 h/d to 12.4 h/d when increasing straw bedding from 1 to 3, 5, and 7 kg per cubicle (Tucker et al., 2009). However, when bedding is wet a decrease in lying time is seen (Fregonesi et al., 2007a; Reich et al., 2010).

Other factors affecting lying time are environmental aspects such as season and grazing. In the study of Reich et al. (2010) lying times of cows in summer and winter were compared. In the winter cows spent 2.2 h/d more lying in the cubicles than in the summer ($P < 0.0001$). However, grazing cows spent less time lying down than zero-grazing cows. From 7:00 till 13:00 h grazing cows spent 29.9% of the time lying down instead of 36.5% of the time in zero-grazing animals (Dohne-Meier et al., 2014). This lower lying time can be explained by a longer eating time. The grazing animals required more time to consume food than the zero-grazing cows (Dohne-Meier et al., 2014).

*Locomotion score*

Besides that lower lying times predisposes cows for lameness, interdigital lesions and heel and sole lesions (Galindo and Broom, 2000) as described above, an effect of locomotion score on lying time is also described by several studies. Cook et al. (2004) studied the effect of locomotion score on activity patterns of cows on cubicles with mattresses and sand bedding. They found that cows on sand bedding had little variation in activity patterns with increasing locomotion score. However, cows on mattresses showed a decrease in lying time between cows that were moderately lame and slightly and non-lame cows. The total lying time of all cows in the study combined decreased from 11.99 h/d to 10.59 h/d between non-lame and moderately lame cows. Ito et al. (2010) compared farms with mattresses and deep-bedding and found that cows with a locomotion score $\geq 3$ had longer lying times on mattresses than cows with a $<3$ locomotion score. The lying time increased from 10.9 h/d to 11.2 h/d. For deep-bedding farms a trend was seen in an increase in lying time with cows locomotion score $\geq 3$. For severe lame cows (locomotion score $\geq 4$) a significant increase in lying time was found (11.2 to 12.8 h/d) for cows on deep-bedding. However, no difference was found for cows on mattresses for severe lame cows. There seems to be an effect of locomotion score on lying time. However, the effect on lying time differs with different cubicle characteristics.

*Factors that influence milk production*

As is generally known, parity and number of days in milk (DIM) do also have effect on milk production as is shown in the following studies. Demming et al. (2010) found a negative relation of milk production and DIM. Yoon et al. (2004) also found a significant negative relation between milk production and DIM. Milk production was significantly lower in the first parity and tended to increase till the fifth lactation (Yoon et al., 2004).

All these factors that influence lying time and milk production make interpreting the relation between lying time and milk production difficult. Therefore, no univocal answer has been found regarding this issue. In this study we try to find a clear relationship between lying time and milk production while taking into consideration the influencing factors.

*Purpose of this study*

The aim of this study is to test the relationship between lying times and milk production. Only non-lame cows that had an average of 8 weeks in lactation were used in this study. Cows with a locomotion score >2 were considered lame. Only a limited period of the year, the barn period, was used. The factors farm and parity were included in the tests.

Secondly, to test the assumption that locomotion score affects lying time, lying time of cows with different locomotion scores were compared.

## Material and methods

*Housing and management*

Seven Dutch free-stall housing dairy farms have been selected for this study. The number of cows in milk per farm varied between 88 and 169 in December 2017. In perspective, one farm had <100 cows in milk, four farms had between 100-150 and two farms had >150.

Of these farms, three used an automatic milking system (Lely A3, Lely A3 next and Lely A4) and four farms used a milking parlour. Different kinds of bedding for the cubicles were used. Four farms used deep litter bedding filled with either straw or sawdust while the other three farms used mattresses. The size of the stalls varied between 110-120 cm width and 240-270cm length (Table 1).

| Farm | Bedding | Cubicle width (cm) | Cubicle length (cm) | Milking system | Grazing |
|---|---|---|---|---|---|
| 544 | Deep-bedding | 115 | 270 | AMS (Lely A3) | No |
| 2297 | Mattresses | unknown | 240 | Milking parlour | Yes, till 31 October |
| 2746 | Mattresses | 110 | 245 | Milking parlour | Yes, till half October |
| 3314 | Mattresses | 110 | unknown | AMS (Lely A4) | No |
| 2011 | Deep-bedding | 110 | 240 | Milking parlour | Yes, till 17 October |
| 2514 | Deep-bedding | 120 | 246 | AMS (Lely A3 next) | No |
| 5888 | Deep-bedding | 110 | 248 | Milking parlour | Yes, till 30 Augustus |

**Table 1**. The cubicle characteristics and management per farm.

Different management strategies were used. One farm fed the cows with a TMR while the other farms gave concentrates separately. Four farms had a grazing period while the other farms kept their cows inside all year round.

*Sensors and lying time*

The lying time was measured in minutes lying down per day (minutes/day). This data was measured with use of sensors made by Nedap (Groenlo, The Netherlands) called Smarttags. Each cow of every farm wore a sensor around the leg that is able to detect whether a cow is standing or lying down.

The average lying time (h/d) of each cow in the period between 50 and 56 days in lactation was used in this study. Cows with 3 or more missing daily lying time data in this period were excluded. Lying time of each cow was measured one week before the MPR was taken or in the week the daily milk production was obtained.

*Selecting cows*

Of the seven farms, cows that were 56 ± 10 DIM in the period September 2017 till February 2018 were used. The cows that were still on pasture in this period were excluded from the tests. To minimize fluctuations in lying time by season a limited period of the year was chosen.

Trained observers scored gait of every cow when they were 8 weeks in milk of each farm using a numerical rating system. This scoring was also based on a 5-point scale in which cows with

a score of 1 showed no signs of lameness and cows with a score of 5 were severely lame (Sprecher et al., 1997). Cows with a locomotion score of ≤2 were considered non-lame and were selected for the test of lying time and milk production.

Individual milk production data was obtained from the milk production registration (MPR) that is performed every 3-6 weeks. Since few cows were exactly 56 DIM when the MPR was taken a spread of 10 days was used. Despite this range, some cows were not included in this study because the MPR was not taken when they were 56 ± 10 DIM.

Of the farms using AMS, two (farm 544, 2514) were tested combined with the other farms but also individually because these farms were able to provide daily milk production data from the AMS. These two farms had no grazing period and both used deep litter bedding cubicles. Of these farms daily milk production data was available of every cow since these farms used AMS. Therefore all cows of these farms were exactly 56 DIM in this study. One farm (3314), also using AMS, was excluded because of shortage of available cows in the chosen period.

Cows were selected if both milk production data and lying time data was available. A total of 220 cows were selected of which 137 had locomotion score 3≥ . This group was used to test the effect of locomotion score on lying time. 83 non-lame cows (locomotion score ≤2) were selected to evaluate the effect of lying time on milk production. The number of selected non-lame cows from the individually tested farms were 38 and 28 of farm 544 and 2514 respectively. The cows were divided in groups based on parity (1,2, ≥3).

*Statistical analysis*

SPSS statistics 24 was used for the statistical analysis. Using descriptive statistics, an overview was made including the number of animals and the variation, average and standard deviation of the lying time, milk production and parity.

The effect of lying time on milk production of the combined farms was tested in three ways. First a linear regression line was made using only the lying time and milk production data. Second, the linear regression line was made with the lying time corrected by the average lying time per farm. The differences in milk production were corrected for the average production per parity per farm. This was done in order to obtain a linear regression line in which the factors farm and parity were included. Third, the relation of lying time on milk production was tested with the factors ‘farm’ and ‘parity’ added in the statistics. An additional test was done to test the effect of lameness on lying time. This test differed from the other tests in the compared group by using not only the non-lame cows but also the animals that scored locomotion score ≥3.

Of the two farms that were separately tested the relation was tested adding ‘parity’ in the statistics. A test was considered significant if $p<0.05$ and a trend was assumed if $p<0.1$.

Two linear regression lines of the 7 farms combined were made using lying time as independent variable and milk production as dependent variable. The absolute lying times and milk productions were used and the relative effect was tested. The relative line was used to correct for farm differences in average lying time and milk production. The milk production was corrected by the average milk production on that farm using the MPR. Each parity (1,2, ≥3) had their own average production in the MPR.

Relative milk production = absolute milk production – MPR average prod. per parity per farm

The lying time was corrected by the average lying time of the cows used in this study per farm.

Relative lying time = absolute lying time – average lying time on that farm (of the cows used in this study)

To test the relationship between milk production and lying time an ANCOVA test was used. The milk production was added as dependent variable. This test allows to add multiple variables. Farm and parity were added in the test as fixed factors. The test measured the significance of the linear relation between the lying time and milk production considering the effect of the other independent variables (Field, 2013)

Milk production was tested for normal distribution using an Q-Q plot. To use an ANCOVA test two important assumptions had to be considered. These are the independence of covariate and treatment effect and the homogeneity of regression slopes. The homogeneity of regression slopes determines whether the relation lying time-milk production differs significantly per group (different farms or parities). It was tested by adding the interactions (farm*lying time), and (parity*lying time) to the ANCOVA. The slope of regression found between the covariate (lying time) and the dependent variable (milk production) should not significantly differ within each group (farm and parity) added in the test (Field, 2013). The independence of covariate means that the factors included in the test (farm and parity) should not have significant influence on the covariate (lying time) (Field, 2013). To test this assumption, an ANOVA was used with lying time as dependent variable and farm and parity were added as factors.

The effect of lameness on lying time was determent using an independent T-test to test for difference in mean and variance between lame and non-lame cows. Also, a graph was made to illustrate the effect of lameness on lying time.

Furthermore, two individual farms were used to test the effect of milk production on lying time. For the individual farms a descriptive statistic was made. Then an ANCOVA test was used to evaluate the relation between lying time and milk production including the factor parity. The procedure of the individual farms was the same as with the combined farms.

# Results

## 7 farms combined

*Descriptive statistics*

Of 83 non-lame cows, lying time varied between 343 and 820 minutes/day with an average of 649±97 (mean±standard deviation) minutes/day. Milk production varied between 23.1 and 58.4 kg milk/day with an average of 38.2±7.4 kg milk/day and the average parity was 2.6±1.6 when combining all farms. Average lying time and milk production between farms varied between 583 to 677 minutes/day and 35.6 to 42.3 kg milk/day respectively. The number of cows per farm used in this study varied between 7 and 29.

*Linear regression of the absolute and relative lying time and milk production*

The linear regression line of the absolute lying time and milk production had a coefficient of -0,011 which was not significant (p=0.18) (Fig 1). When comparing the relative lying time and relative milk production no significant (p=0.13) linear effect was found either (Fig 2).

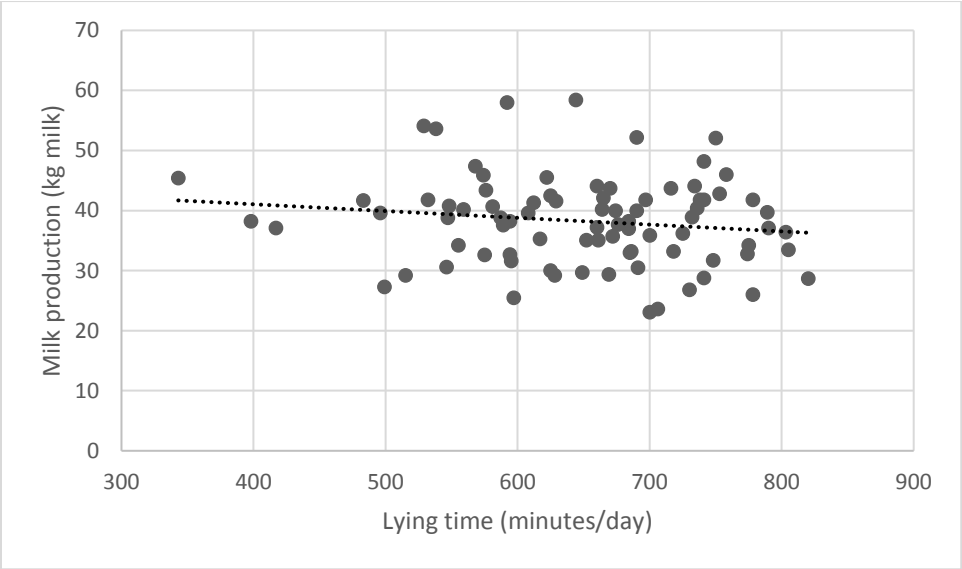


**Fig 1:** The linear regression of the lying time on the milk production per cow. The linear regression line is not significant. $R^2$ linear=0.022

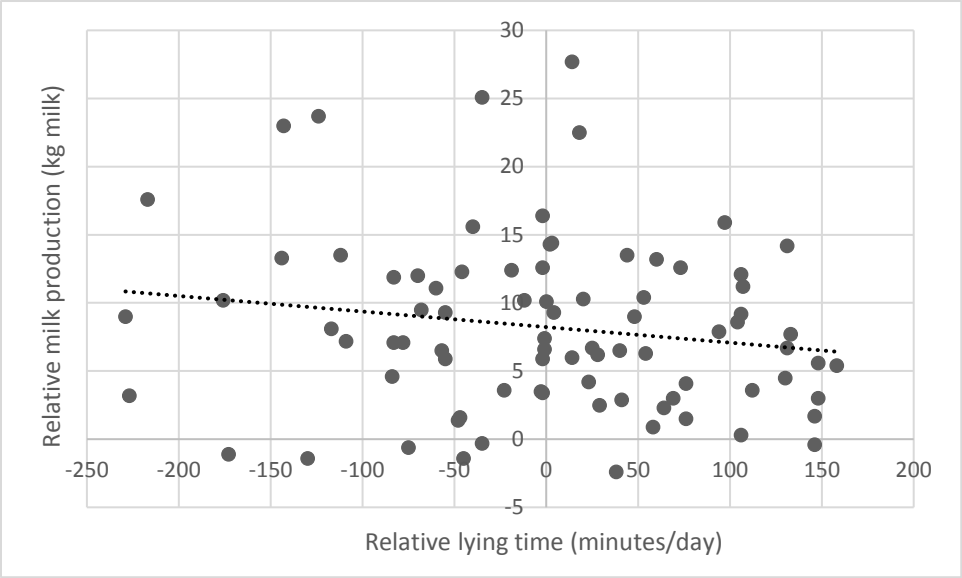


**Fig 2:** The linear effect of the relative lying time on the relative milk production of non-lame cows. The linear regression line is not significant. $R^2$ linear=0.028.

*ANCOVA test*

The Q-Q plot and the results of the 'homogeneity of regression slopes' and the 'independence of covariate' are added in Appendix 1. The results of the ANCOVA are shown in Table 2. The adjusted mean of milk production of parity 1 and 2 was respectively 9.0 and 6.8 kg milk lower than the production of parity 3. No further significant relations were found. The adjusted coefficient of lying time on milk production was -0.009 but this was not significant (p=0.190). A trend was seen in differences in adjusted mean between farms (p=0.094).

| **7 farms combined** | |
|---|---|
| Dependent variable: milk production | |
| **Independent variable** | **Sig.** |
| Lying time | 0.190 |
| Farm | 0.094 |
| Parity | 0.000 |

**Table 2**: The results of the ANCOVA showing the relation between the independent variables and the dependent variable (milk production).

*Effect of locomotion score on lying time*

The results of the effect of lameness on lying time are added in appendix 2.

## Individual farms

*Farm 544*

The 38 cows selected from farm 544 had an average parity of 2.21. Lying times varied between 404 and 825 minutes/day with an average of 696±95 minutes/day. Milk production varied between 25.1 and 55.1 kg milk/day with an average of 39.0±6.3 kg milk/day.

The assumptions are added in Appendix 1. A significant effect of parity on milk production was found (p=0.045). The adjusted mean of parity 1 and 2 were respectively 6.6 and 4.1 kg milk lower than for parity ≥3. Lying time (coefficient= -0.017) was not significantly related to milk production (p=0.12).

*Farm 2514*

The 28 selected cows on farm 2514 had an average parity of 2.14±1.30. The lying time varied between 533 and 802 with an average of 660±81 minutes/day. Milk production varied between 32.0 and 58.8 kg milk/day with an average of 43.9±6.0 kg milk/day.

The assumptions are added in Appendix 1. A significant effect of parity on milk production was found (p=0.005). The adjusted mean of parity 1 and 2 were -8.6 and -3.8 kg milk respectively lower than parity ≥3. Lying time had a coefficient of 0.003 but was not significantly related to milk production (p=0.8).

## Discussion

*Combining farms and testing farms separately*

The goal of this study was to relate lying time and milk production of cows that had similar environmental and cow factors. Therefore, cows with almost the same genetics, with the same DIM in the same period of the year (excluding the pasture period) were used. It would be ideal if the environmental factors had been the same for all cows. However, since the selection of cows was strict, not enough cows were selected per farm to test al farms individually. Therefore, the cows of different farms were combined and the factor farm was included in the statistical analysis. Only two farms using AMS were chosen to test individually since more milk production data was available of these farms.

*Effect of fixed factors on lying time*

The factors farm and parity were not related to lying time. Stall width and bedding are known to influence lying time (Tucker et al., 2003; Tucker et al., 2004). Deming et al. (2013) found that feeding frequency tended to influence lying time as well. Since the different farms had different cubicle characteristics and different management, a difference in lying time per farm was expected. In the present study the average lying time per farm varied between 9.7 to 11.3 h/day. Ito et al. (2009) found a mean lying time per farm varying from 9.5 to 12.9 h/d. The lower variation might be caused by the exclusion of lame cows. Ito et al. (2009) concluded that the within farm variation in lying time was bigger than the variation between farms. The within farm variation might have caused the lack of significant effect. A larger number of cows per farm might reveal a difference in lying time between farms. The lack of significant effect of parity on lying time agrees with the results of Bewley et al. (2010).

*Effect of fixed factors on milk production*

A trend was found in adjusted mean milk production between farms. This was expected since all farms had different management systems (feeding, grazing and milking). In all groups (combined farms and the two individual farms) there was a positive relation between parity and milk production as expected.

*Lying time and milk production*

Two graphs were made, one showing the absolute lying time and milk production of the cows of the 7 farms combined (fig. 1). The other showed the effect of relative lying time on relative milk production (fig. 2). The difference between these graphs is that the absolute variable did not consider farm and parity differences as possible effect. The relative variable showed how the cows were performing in lying and milk production, relative to the other cows of their own farm. Both showed a regression line that was not significant. The chosen method to correct lying time per farm was not ideal since the average was calculated using only the selected animals in this study. The average milk production per farm was based on the MPR in which all lactating animals of the farms were used. Therefore, the average milk production per farm was more reliable than the average lying time per farm in this study. Using the lying time of all cows per farm would have improved the reliability of the average lying time.

Including variables parity and farm as fixed factors in the ANCOVA did not result in a significant effect. Despite not being significant, the relation between lying time and milk production showed a negative relation in all tests except for farm 2514 which showed a small

positive effect which was not significant either. The expected positive effects of a higher lying time did not result in a higher milk production. Fregonesi and Leaver (2001) found that high yielding cows had higher eating time than lower yielding cows. Norring et al. (2012) did not find a difference in eating time but suggested that a lower lying time with higher milk production might be caused by udder discomfort making them less willing to lie down. Even though we did not find a positive effect of lying time on milk production, we consider lying time as in important factor for milk production since lower lying time increases the risk of lameness (Galindo and Broom, 2000) which has a negative effect on milk production (Archer et al. 2010; Warnick et al. 2001; Green et al. 2002).

*Potential effect of selection and the effect of BCS on milk production*

The potential bias in scoring locomotion score by observers was minimalized using standard scoring systems. In this study only non-lame animals were selected to test the effect of lying time on milk production. Selecting on locomotion score might have caused a selection for other cow related factors like parity and BCS. Previous studies found that lameness is associated with a lower BCS (Solano et al., 2015; Randall et al., 2015). Also cows with higher parity have an increased risk for lameness (Solano et al., 2015). So selecting cows on locomotion might have resulted in a group with younger animals and with a higher average BCS. In the statistics we included parity as factor. However, the factor BCS was not included in our study since there was no significant relation between BCS and milk production (data not shown). However, other studies did find a relation between BCS and milk production. Roche et al. (2007) found that BCS at calving and lowest BCS in lactation were non-linearly related to milk production. The greatest 60-d and 270-d milk yield was obtained from cows with a BCS at calving of 3-3.25. The review of Roche et al. (2009) points to an optimum BCS at calving between 3.0 and 3.5 for Holstein-Friesian dairy cows. In the first one-third of the lactation cows have a negative energy balance which is compensated with the body reserves of the cow (Bauman and Currie, 1980). Therefore it can be expected that cows with higher BCS at calving have more reserves and, therefore, more milk production. However, high BCS at gestation is negatively related to dry matter intake (DMI) after parturition (Roche et al., 2009). This results in a lower energy intake. BCS score in these studies was based on a 5-point scale in which cows with a score of 1 were very lean and cows with a score of 5 were very fat (Edmonson et al., 1989). Not only the BCS at a certain time is important but also the change in BCS during the dry period and early lactation influences the cows health status, reproduction and milk production (Chebel et al., 2018; Barletta et al., 2017; Sheehy et al., 2017).

*Effect of locomotion score on lying time*

In the present study, a total of 137 of 220 cows were considered lame. Archer et al. (2010) also found that the majority of cows had abnormal locomotion and scored only 1.7% out of 11,735 records as "good locomotion" in UK dairy farms. They found a mean prevalence of cows scored as lame of 62% varying between 48% and 72% per farm. In the present study it was found that lame cows had a bigger variance in lying time but no significant difference in mean lying time compared with non-lame cows. This means that the effect of lameness on lying time is both negative as well as positive. Cook et al. (2004) state that lame cows experience pain and, therefore, have an increased desire to lie down. However, Ito et al. (2010) found a different effect of lameness on lying time with deep bedding cubicles than when mattresses were used. So the comfort a lame cow experiences when lying down and standing up might influence the

lying time. Cook et al. (2004) proposed that lame cows experience pain and fear of slipping when rising and lying down and, therefore, have longer standing bouts. This effect might be more relevant on mattresses than on deep bedding cubicles. The type of hoof lesions might also influence lying time. Chapinal et al., 2009 found that cows with sole ulcers had longer lying times than cows with no lesions, sole haemorrhages or digital dermatitis. So the disease causing the lameness affects lying time. However, the cows having ulcers also scored higher in locomotion score than the other groups.

*Recommendations for further studies*

Due to strict selection many animals were excluded in this study. Using less selection criteria would increase the amount of data. However, if for example a full year of data was used or locomotion score was excluded as a selection criteria, more uncertainty could rise about those factors influencing the milk production or lying time. It was chosen to eliminate possible factors influencing milk production and lying time in order to specify the results of increased lying time on milk production.

In future studies, the factor BCS and the change in BCS before and after parturition, could also be taken into account since these factors are known to affect milk production.

Since this study failed to show a significant relation between lying time and milk production, in future studies, an interesting approach could be a test design where cubicle characteristics are optimized in order to increase lying time in half of the study group. This could possibly create a bigger difference in lying time between both groups and show if a significant relation between lying time and milk production can be found. If there is a positive relation, this would increase the need for better lying time management in free-stall farms.

## Conclusion

In this study, lying time did not have a significant effect on milk production. Including parity and farm as fixed factors did not result in a significant effect either. Apparently, the relationship between lying time and milk production was not found significant, despite (or due to) the selection of cows and the correction with different factors.

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# Appendix 1 – Testing the assumptions

*Combined farms*

The Q-Q plot of the milk production showed a linear line (Fig. 3). The homogeneity of regression slopes was tested. The interactions (farm*lying time) and (parity*lying time) were not statistically significant (Table 3). The ANOVA used to test the independence of covariate showed no significant effect. So lying time is not significantly related to farm or parity (Table 4).

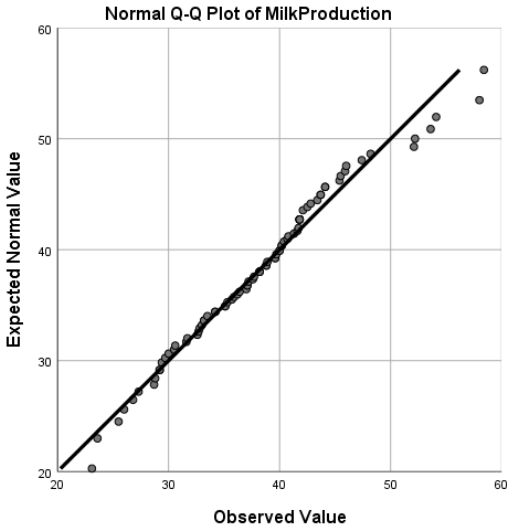


**Fig 3**: This graph shows the Q-Q Plot of the cows in the combined farm group.

| **Homogeneity of regression slopes** | |
|---|---|
| Dependent variable: milk production | |
| **Independent variable** | **Sig.** |
| Farm*lying time | 0,183 |
| Parity*lying time | 0,844 |

**Table 3**: This table shows the results of the homogeneity of regression slopes. Sig. is the statistical significance of the relation between the independent variable and the dependent variable.

| Dependent variable: lying time | |
|---|---|
| **Independent variable** | **Sig.** |
| Farm | 0,384 |
| Parity | 0,729 |

**Table 4:** This table shows whether the fixed factors (farm and parity) used in the ANCOVA, have a significant effect on the covariate (lying time).

*Farm 544*

The Q-Q plot of milk production showed a linear line (Fig. 4). The regression slopes did not differ among groups (p=0,6). Lying time was not significantly dependent on parity (p=0,4).

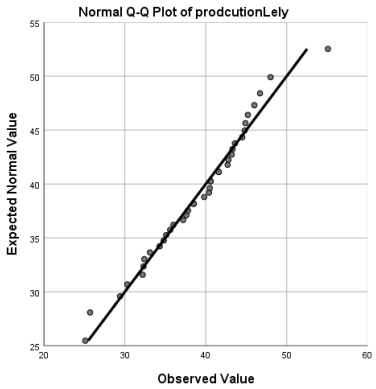


**Fig. 4**: Q-Q plot of milk production on farm 544.

*Farm 2514*

The Q-Q plot showed a line that approached a linear line (Fig. 5). The regression slopes did not significantly differ among groups (p=0,8). There was no significant relation between parity and lying time (p=0,7).

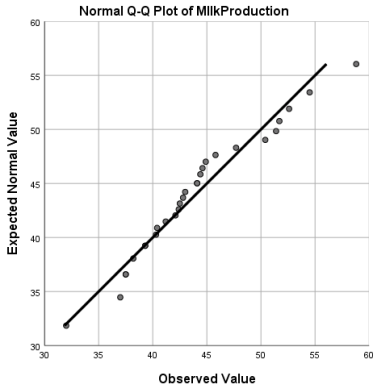


**Fig 5**.: Q-Q Plot of milk production on farm 2514.

## Appendix 2 – The effect of locomotion score on lying time

Of the 220 selected cows in this study, 137 cows were considered lame. Comparing non-lame and lame cows resulted in a difference in variance in lying time (independent T-test, p=0,001) but no significant difference in mean lying time (Fig. 6).

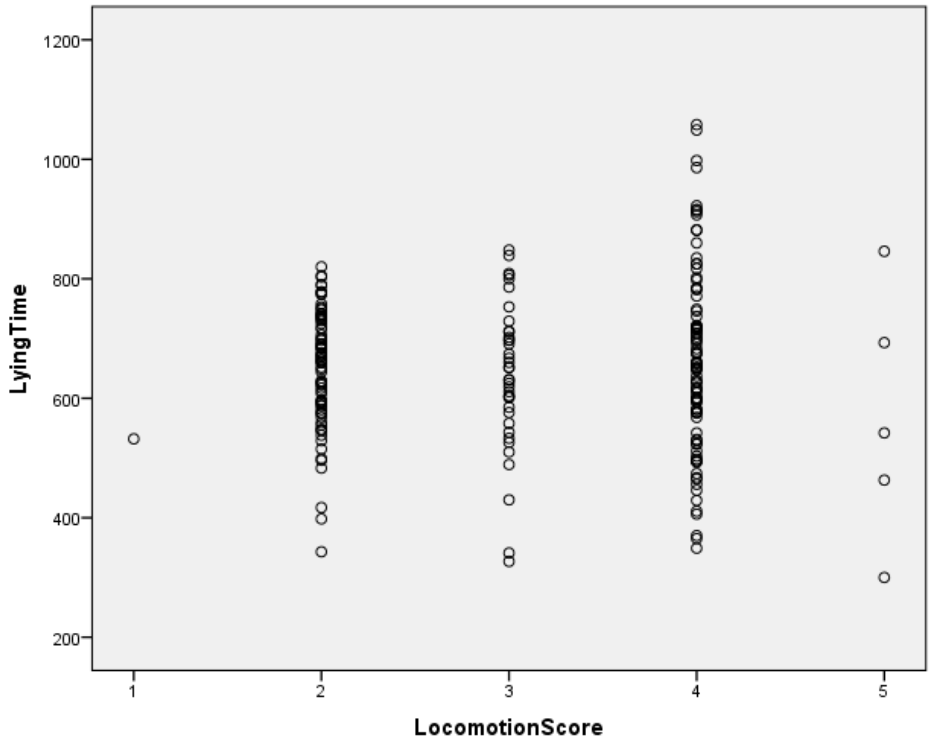


**Fig 6:** Effect of locomotion score on the lying time of the 7 farms combined. The variance in lying time of lame cows is higher than the non-lame cows.