

The effect of neighbouring cows within the milking parlour on a cow's daily milk yield

Ida Hansson^{a,*}, Hector Marina^a, Freddy Fikse^{b,1}, Per Peetz Nielsen^c,
Lars Rönnegård^{a,d,e}

^a Department of Animal Biosciences, Swedish University of Agricultural Sciences, Box 7023, SE-750 07, Uppsala, Sweden

^b Växa, Swedish University of Agricultural Sciences, Ulls väg 26, SE-756 51, Uppsala, Sweden

^c RISE Research Institute of Sweden, Division of Bioeconomy and health, Department of Agriculture and Food, RISE Ideon, SE-223 70, Lund, Sweden

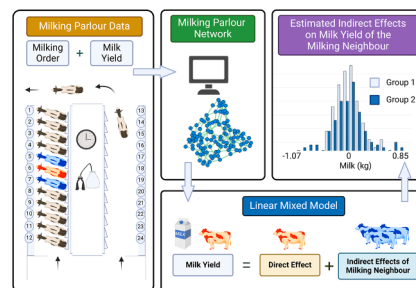
^d School of Information and Engineering, Dalarna University, SE-791 88, Falun, Sweden

^e The Beijer Laboratory for Animal Science, Swedish University of Agricultural Sciences, Box 7024, SE-750 07, Uppsala, Sweden

HIGHLIGHTS

- The neighbouring cow during milking may affect a cow's daily milk yield.
- The estimated indirect effects on milk yield ranged from -1.07 kg to 0.85 kg.
- Some cows seem supportive of their neighbours and others seem more disruptive.
- Weak negative correlation between the direct and indirect effects of milk yield.
- Regrouped cows changed to have a more negative indirect effect on their neighbours.

GRAPHICAL ABSTRACT



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ABSTRACT

Social interactions in a dairy herd are essential to maintain the herd's structure. Disturbances in social relationships can be stressful and may impact both animal welfare and production. Pathological and physiological changes, biological variations, but also the social environment induced by surrounding cows can affect variation in the daily milk production. This study aims to investigate the social interplay between cows during milking by examining the milking order in a milking parlour and determining if the individuals a cow stands next to will affect its daily milk yield. Milking order data from 234 individuals was collected from a two-sided herringbone parlour twice a day for 35 days. The indirect effect of the neighbour cows in the milking parlour was studied by fitting a linear mixed model to the daily milk yield residuals. The estimated indirect effects on milk yield ranged from -1.07 kg to 0.85 kg. We described a weak negative correlation of -0.26 (SE: 0.09) between direct and indirect effect estimates. The average of the indirect effects of neighbouring cows differed between different lactation stages and regrouped cows changed to a more negative estimated indirect effect in their new group. Our results show individual variation in the average indirect effect on the milk yield of the neighbour, with some individuals having a positive effect on their group mates, while others have a more negative effect. Further

* Corresponding author.

E-mail address: ida.hansson@slu.se (I. Hansson).

¹ Current address: AbacusBio, Roslin Innovation Centre, Easter Bush Campus, Edinburgh, EH25 9RG, Scotland.

investigation of these effects would be helpful in selecting the best individuals in a herd and optimising group composition and milking routines.

1. Introduction

Knowledge of the social interplay between cows in a dairy herd may improve herd management and enhance animal welfare and production. The social environment for cows living together in a group is one of the most central environmental impacts on an individual. There is a variation in sociality between individual cows (Hansson et al., 2023; Rocha et al., 2020), and it seems that cows create preferential relationships with individuals that have similar features (Boyland et al., 2016; Marina et al., 2024) and stronger relations depending on the time they have spent together (de Freslon et al., 2020; Gutmann et al., 2015; Rocha et al., 2020).

Social interactions between cows (i.e. affiliative and agonistic interactions) are essential in a dairy herd to maintain the structure of the group hierarchy and promote balance (Tucker, 2017). Agonistic behaviours are based in dominance and are expressed as aggressive acts (chasing, fighting, posturing, head butting) or responses to aggression (mainly avoidance). Insufficient space allowance or changes to the social environment, such as introducing new individuals and re-grouping animals, can be stressful, lead to social tension in the herd, and increase agonistic interactions (Bouissou et al., 2001). Affiliative behaviours, such as allogrooming and spatial proximity, are believed to have a calming effect, reduce aggression, and strengthen the social bond between individuals (Boissy et al., 2007).

Allogrooming has been associated with a higher milk yield for the receivers (Sato et al., 1991; Wood, 1977), while social tension in a herd can be stressful for cows and may impact both animal welfare and production since stressed cows produce less milk (Hedlund and Løvlie, 2015). Stress during milking also seems to directly affect the milk yield at that milking event with increased milk retained in the udder after milking, so-called residual milk (Rushen et al., 1999, 2001). This is probably due to delayed milk ejection, which inhibits milk yield (Bruckmaier and Blum, 1998). Deviations in milk production can indicate health disturbances in a cow or incomplete milking. The relative day-to-day variation in milk yield has been reported to range from 6–8 % in a review by Svennersten-Sjaunja et al. (1997), and this variation is caused by several unknown variance components such as pathological and physiological changes, biological variations, and sampling procedure (Forsbäck et al., 2010). However, it might also be that the daily variation in milk yield is associated with the social environment induced by the surrounding cows in the herd. In a study by Fadul-Pacheco et al. (2021), affiliative pairs of cows moving together were explored by using data from preselection sorting gates and social network analysis. The results suggested that the social relationships of the cows may impact the average daily milk yield, where milk production tended to be higher in groups during periods when affinity pairs were present. Also, when the affiliative cow pairs were separated, the daily variation in milk yield increased and this increase was argued to be a result of an increased stress level of the cows.

Our study aims to investigate the social interplay between cows during milking by examining the milking order in a milking parlour and determining if the individuals a cow stands next to will affect its daily milk yield. We hypothesize that if a cow stands next to a specific cow, which could have a potentially stressful effect, there will be a lower milk yield for that milking event. On the other hand, if a cow stands next to a specific cow, which could have a potentially calming effect, there will be a higher milk yield for that event.

2. Material and methods

2.1. Animals and data

Data was gathered from one Swedish commercial dairy farm; a farm description can also be found in Hansson et al. (2023). The farm had around 210 lactating cows of Holstein Friesian, Swedish Red, and crossbred breeds. The cows were housed in an uninsulated free-stall barn and grouped into two milking groups, G1 and G2. G1 had mainly cows in early to mid-lactation, while G2 primarily had cows in mid to late lactation. At approximately 170 DIM, the cows were routinely moved from G1 to G2 when confirmed pregnant or designated for slaughter. The gradual drying-off process in the herd started on Tuesdays when the cows were moved to a separate area and then milked only on the following Wednesday, Friday, and Monday morning. The cows were dried off two months before calving and were then moved to another building. Before calving, the cows were moved to calving boxes, and the timing of moving cows depended on the individual calving behaviour and current calving box occupancy. The cows were either housed individually or together with other calving cows, depending on the space available, and 24–48 h after calving, they were introduced to the milking groups.

The cows were milked twice daily in a two-sided herringbone milking parlour with 12 units on each side (2×12 GEA Euro class 800 with Dematron 75, GEA Farm Technologies, Bönen, Germany). A detailed description and layout of the milking parlour can be found in Hansson and Woudstra (2023). The milking sessions started around 0430 h and 1630 h, where cows in G1 were milked first, and the sessions lasted for around 1.5 h. In front of the milking parlour was a waiting area where the cows were gathered before milking, allowing them to move freely and position themselves towards the entrance to the milking parlour. The cows entered the parlour in a single row on one side at a time, with automatic identification at the entrance gate by radio frequency identification detection (RFID) technology. Each milking position within the milking parlour had a unique number to identify which cow was milked at each position. Twenty-four cows were milked in each batch (twelve on either side of the parlour). On the right-hand side of the parlour, the positions were numbered from 1 to 12, where the first cow that entered the parlour on the right-hand side occupied the 1st position and the last cow for that row within that batch occupied the 12th position. On the left-hand side, the positions were numbered from 13 to 24, where the first cow that entered the parlour on the left-hand side occupied position 13 and the last cow for that row within that batch occupied position 24. Details such as each cow's position in the parlour during milking and the timestamp of the milking cluster detachment at the end of the milking of a cow were transmitted from the milking equipment to the farm computer after each milking session. Due to changes regarding the storage of milking data on the farm computer, the milk yield per milking session was unavailable for the study period. A summarised milk yield of the morning and the previous day's evening session yield (in kg milk) was instead collected for each cow, referred to as the daily milk yield (DMY). The average milking interval between the morning and the previous day's evening session for the individuals in G1 was 12.8 h (SD = 0.47) and 12.8 h (SD = 0.49) in G2.

Data was collected from 70 milking sessions, corresponding to 35 days, between 31 August 2020 and 6 October 2020, for G1 and G2. Milk yield data was collected from cows with at least seven DIMs until the day of the drying-off process. Milking position within the parlour and time for milking were collected for all the cows during the study period, including newly calved cows and cows within the drying-off process. There were 234 cows with data on milk position and detachment

timestamp from the milking parlour, and the number of milking position records per cow ranged from two to 70, with a mean of 61.8 and a median of 70 records. The low number of records for some cows was due to their short presence during the study period, e.g., newly calved cows at the end of the study period or cows at their end of lactation at the beginning of the study. Merely 1 % of the collected milking events lacked information regarding the milking position in the parlour and the corresponding timestamp. Records from two milking sessions were missing for all cows due to a data transfer failure. The average proportion of missing position records for each cow was 1.6 %.

Individual DMVs that deviated more than three standard deviations from a 5-day moving mean were removed from the analysis. Out of 6896 milk yield records, 88 outliers in total were removed. Milk yield data were available for 219 cows; detailed descriptive statistics are shown in Table 1. The number of milk yield records per cow ranged from six to 35, with a mean of 31.4 and a median of 35 records. The low number of records for some cows was due to their short presence during the study period. The average proportion of missing milk yield records for each cow was 2.8 % and the median was 0 %. The parity of cows ranged from one to six in G1 and one to seven in G2 and were categorized into parity 1, 2, and 3+. All individuals were ordered into three lactation stages liable on the current DIM: Early (7–49 DIM), Mid (50–179 DIM), and Late (≥ 180 DIM) lactation. The DMV in G1 ranged from 9.0 to 67.6 kg of milk, with a mean of 37.5 kg. In G2, the DMV ranged from 7.4 to 51.2 kg of milk, with a mean of 29.2 kg (Table 1).

2.2. Statistical analysis

Data management and statistical analyses were conducted using the software R version 4.0.3 (R Core Team, 2020). The analyses were performed in two steps. To account for the dynamic characteristics of the lactation curve and the nonlinear relationship between milk yield and lactation stage, we first fitted a general additive model (GAM) (Eq. (1)) using the gam function from the mgcv package (Wood, 2011) to fit a lactation curve for each parity group:

$$\mathbf{y} = \beta_0 + \beta_1 \mathbf{x}_1 + \mathbf{f}(\mathbf{x}_2) + \mathbf{e}, \quad (1)$$

with N observations from n cows. Here \mathbf{y} is the response variable DMV (length N), β_0 is the intercept, \mathbf{x}_1 comprise fixed effects of parity, β_1 is the slope of \mathbf{x}_1 , \mathbf{x}_2 is DIM, \mathbf{f} is the smooth function of the DIM and \mathbf{e} is the residual term. One model for each group was fitted. The estimated effective degrees of freedom of the smooth term were 4.17 in G1 and 4.14 in G2, and the plotted smooths from the estimates of the GAM model can be seen in Fig. 1. The residuals from the outcome of the GAM model were then used as the response variable in the following analysis,

referred to as milk yield residuals, which represent the deviations of DMV dependent on parity and DIM.

In step two, daily adjacency matrices of who stood next to whom in each milking session were constructed based on the milking position and timestamp within the parlour. Only the closest neighbours, standing directly next to a cow (i.e., neighbouring cows that occupied the milking position numbered ± 1 of a cow's milking position), were defined as neighbours. A cow could have a maximum of 4 neighbours a day, 2 in the morning and 2 in the evening (i.e., a cow could at maximum have two direct neighbours during one milking session, one neighbour that occupied the milking position to the left of the cow and one to the right). The adjacency matrices were used to estimate an average indirect effect of the neighbour in the milking parlour on an individual's (residual) DMV by fitting a linear mixed model (Eq. (2)), with the hglm function in the hglm package in R (Rönnegård et al., 2010):

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_d\mathbf{u} + \mathbf{Z}_s\mathbf{a} + \mathbf{e}, \quad (2)$$

with N observations from n cows. Here \mathbf{y} is the vector of the response variable of milk yield residuals (length N), \mathbf{X} is the design matrix relating the observations of \mathbf{y} to the fixed effects given in the vector $\boldsymbol{\beta}$, which includes breed (class, $n = 3$), \mathbf{Z}_d is the incidence matrix relating the own direct effect to an individual's milk yield residuals (size $N \times n$), \mathbf{u} is the vector of the random cow effect (length n), referred to as direct effects, \mathbf{Z}_s is the adjacency matrix relating the indirect effect of each neighbour cow to an individual's milk yield residuals (size $N \times n$) where the element in i -th row and j -th column is equal to 1 if the j -th cow was a neighbour in record number i during one milking session, and equal to 2 if it was a neighbour during both the morning and evening milking sessions. The random effect \mathbf{a} is the vector of the average indirect random cow effects of each neighbour, referred to as indirect effects, and \mathbf{e} is the vector of residual effects. The direct effects are assumed to be normally distributed with variance σ_d^2 (the between-individual variance of cows), the indirect effects are assumed to be additive, independent and identically normally distributed with variance σ_s^2 (the between-individual variance of neighbour cows), and the residual effects are assumed to be independent and identically normally distributed with variance σ_e^2 .

One model for each group was fitted, with 3415 observations from 123 cows in G1 and 3393 observations from 125 cows in G2. Twenty-nine of these cows were in both groups during the study period. In total, there were 141 unique cows in G1 with records of milking position and included in the \mathbf{Z}_s and 142 cows in G2. Forty-nine of these cows were in both groups during the study period. The confidence interval of the estimated variance components was calculated according to Rönnegård et al. (2010). A likelihood-ratio test (LRT) was performed using the lrt

Table 1

Descriptive statistics of data collected in G1 and G2 for cows¹ with daily milk yield records.

Trait	G1					G2				
	Number of individuals	Mean (kg milk)	SD (kg milk)	Min (kg milk)	Max (kg milk)	Number of individuals	Mean (kg milk)	SD (kg milk)	Min (kg milk)	Max (kg milk)
Breed	123	37.5	8.6	9.0	67.6	125	29.2	5.8	7.4	51.2
CROSS	53	41.5	8.2	17.3	67.6	53	28.7	6.6	8.2	51.2
HOL	35	35.0	9.1	9.0	59.4	35	30.2	4.4	12.5	41.1
RDC	35	34.3	5.7	18.7	52.2	35	29.2	6.0	7.4	45.4
NA							28.4	2.6	19.6	32.5
Parity										
1	36	29.4	4.7	9.0	39.1	36	27.9	5.2	7.4	39.8
2	34	39.0	6.4	18.2	58.7	34	29.3	4.9	10.9	40.9
3+	53	43.0	7.3	19.0	67.6	53	29.8	6.9	8.2	51.2
Lactation stage										
Early	46	35.6	9.9	9.0	67.6	46	26.4	9.1	12.5	45.3
Mid	95	38.6	8.2	18.7	64.7	95	32.1	5.3	7.4	45.4
Late	14	36.9	3.6	29.2	45.2	14	28.8	5.8	7.7	51.2

¹ There were in total 219 unique cows with daily milk yield records. HOL: Holstein Friesian; RDC: Swedish Red Dairy Cattle; CROSS: Crossbred, NA: Not Available; Early: 7–49 Days in milk, Mid: 50–179 Days in milk, and Late: ≥ 180 Days in milk.

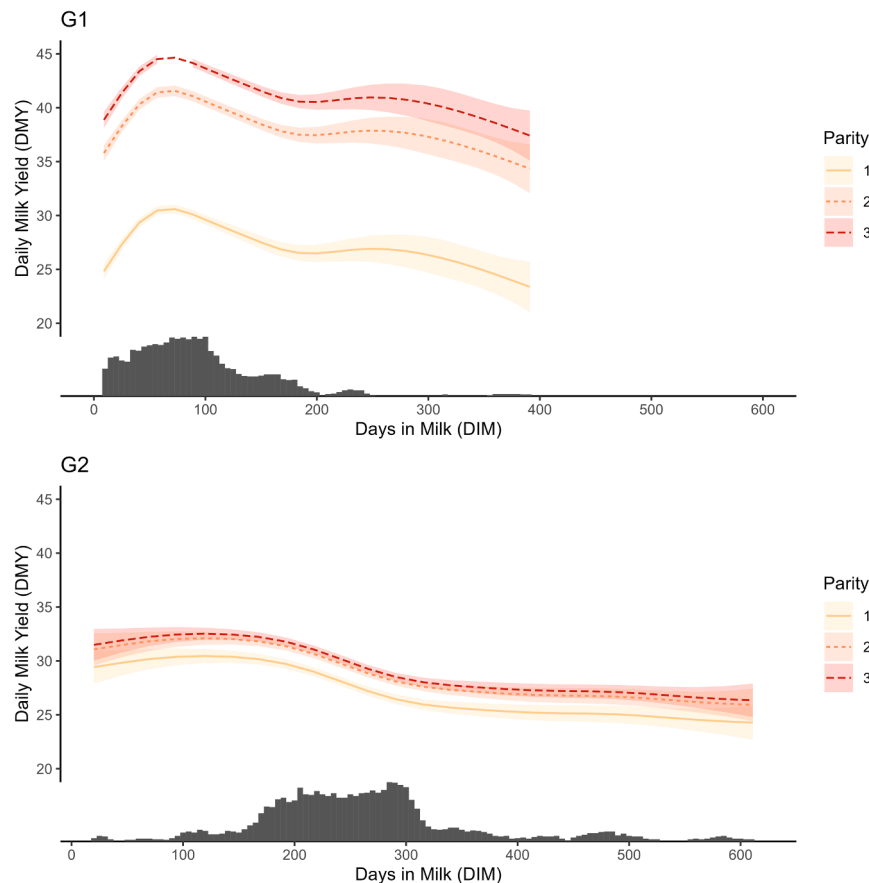


Fig. 1. Plotted smooths from the estimates of the GAM model for G1 and G2, representing the lactation curves for each parity group. The black marginal distribution above the x-axis shows the data availability for each time point.

function in the `hglm` package in R (Rönnegård et al., 2010) to test the significance of the indirect effects. The function compares the likelihood (L_1) of the fitted model with the likelihood (L_0) from a simpler model, where the indirect effect was removed, by computing the likelihood ratio statistic $\lambda_{LR} = -2(\log(L_0) - \log(L_1))$. The `lrt` function assumes that the test statistic follows a χ^2 -mixture distribution (Self and Liang, 1987) under the null hypothesis of a variance component for the indirect effects equal to zero. A significance level of 0.05 was used. The correlation between the estimated direct and indirect effects was calculated using the Pearson correlation coefficient. To test if the estimated effect size for the indirect effects was related to parity or lactation stage, an additional model was fitted for each group. A linear model was fitted with the `lm` function in the `stats` package (R Core Team, 2020), with the resulting indirect effects from the previous model as the response variable with the lactation stage and parity as fixed effects.

3. Results

The variance of the direct effects, σ_d^2 , was 28.8 kg milk (95 % CI: 22.4 – 37.2) in G1 and 27.5 kg milk (95 % CI: 21.3 – 35.5) in G2. The variance of the indirect effects, σ_s^2 , in G1 was 0.09 kg milk (95 % CI: 0.07 – 0.13) and 0.19 kg milk (95 % CI: 0.15 – 0.26) in G2, while the residual variance was 6.60 kg milk in G1 and 5.04 kg milk in G2. The indirect effects showed an association with the milk yield residuals in both groups, where the LRT statistic was 42.1 ($P < 0.001$) in G1 and 105.4 ($P < 0.001$) in G2. The estimated indirect effects on milk yield ranged from –0.56 kg to 0.58 kg for the individuals in G1 and from –1.07 kg to 0.85 kg for the individuals in G2 (Fig. 2). The sum of the indirect effects of all the neighbours for each observation ranged from –1.25 kg to 1.65 kg (mean: –0.05, SD: 0.40) in G1 and from –2.67 kg to 2.61 kg (mean:

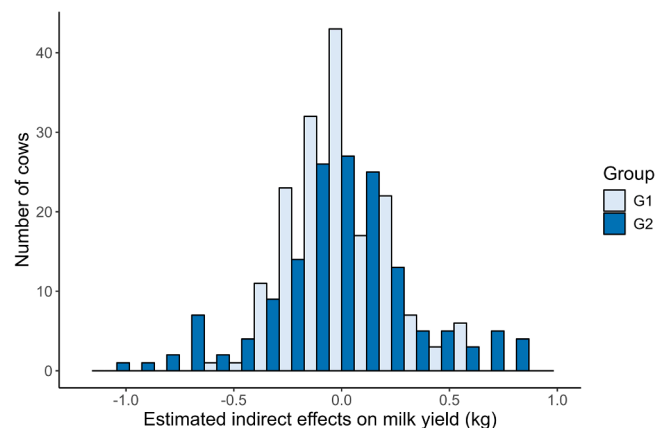


Fig. 2. Distribution of the estimated average indirect effects on milk yield for all the cows in G1 and G2.

0.01, SD: 0.58) in G2 (Fig. 3).

Lactation stage was associated with the estimated indirect effects on milk yield in both groups. In G1, cows in late lactation had on average 0.17 (SE: 0.06) kg ($P = 0.003$) larger indirect effect than cows in early lactation and cows in mid lactation had on average 0.11 (SE: 0.04) kg ($P = 0.01$) larger effect than early lactation cows. There were no differences between cows in mid and late lactation. In G2, cows in late lactation had on average 0.37 (SE: 0.14) kg ($P = 0.01$) larger indirect effect than cows in early lactation and on average 0.37 (SE: 0.07) kg ($P < 0.001$) larger effect than cows in mid lactation. There were no differences between cows in early and mid-lactation. There was no difference

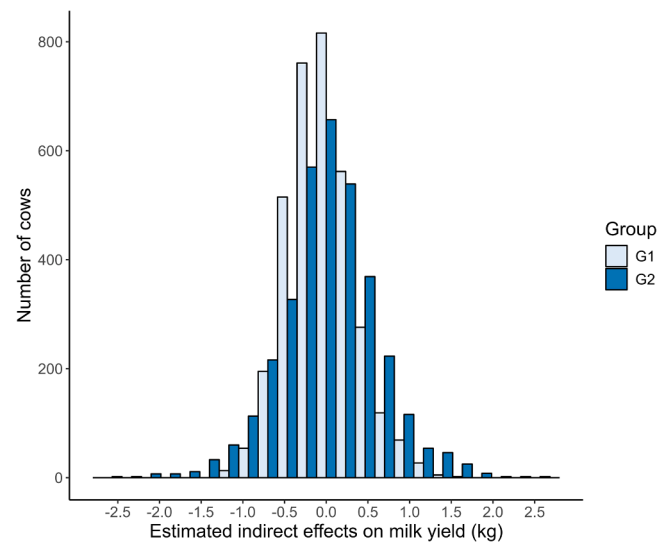


Fig. 3. Distribution of the sum of the estimated indirect effects of all neighbouring cows on one individual's daily milk yield, for each observation in G1 and G2.

in the effect size for the indirect effects related to parity in neither group. Forty-nine cows were moved from G1 to G2 during the study period; 17 of these cows, that had at least ten records in both groups, changed to a more negative estimated indirect effect on milk yield after the regrouping (Fig. 4). The estimated direct effect for these same 17 cows, changed for some of the cows, where most cows altered to a more positive effect and some to a more negative effect (Fig. 5). In G1, there was a weak negative correlation of -0.26 (SE: 0.09) ($P=0.004$) between the estimated direct and indirect effects. However, in G2, the estimates had no significant correlation, but the trend was in the same direction

(Fig. 6). In Fig. 7, the correlation between the estimated direct and indirect effects in G1 is grouped by parity and lactation stage, where the negative correlation seems to intensify with parity. The fixed breed factor was associated with the milk yield residuals in G1. In G1, cross-bred cows produced on average 3.7 (SE: 1.18) kg ($P=0.002$) more milk than the Swedish Red. There were no significant differences in production between the Holstein and the crossbreds and between the Holstein and Swedish Red. In G2, the breeds had no significant differences in the production level.

4. Discussion

We used the milking order within a milking parlour to assess the indirect effect of the neighbouring cows during milking on a cow's daily milk yield. We found an individual variation of the indirect effect during milking, with a larger variation in G2 compared to G1 and a weak negative correlation between the direct and indirect effect on milk yield in G1. As expected, the variance of the indirect effects was relatively lower than the variance of the direct effects. The impact of the individual with the largest indirect effect on her group mates was more than -1 kg of milk. We also found that the cows that regrouped from G1 to G2 went from having a more positive indirect effect on their group mates to a more negative effect.

There seems to be variation among cows in how much, on average, they affect other cows in the herd. Some individuals had, on average, a more negative effect on other cows. In our model, we estimate an indirect effect of standing next to a cow, but we do not model a biological causal effect per se. One explanation to these results could, however, be that a cow can be stressed during milking due to the cow standing next to her, leading to a delayed milk release, an increase in residual milk, and a decreased milk yield at that milking session. A study by Rushen et al. (2001) found that cows milked isolated in an unfamiliar place showed acute stress and had a lower release of oxytocin, higher residual milk, and less milk yield. Repeating event of stress at milking could potentially

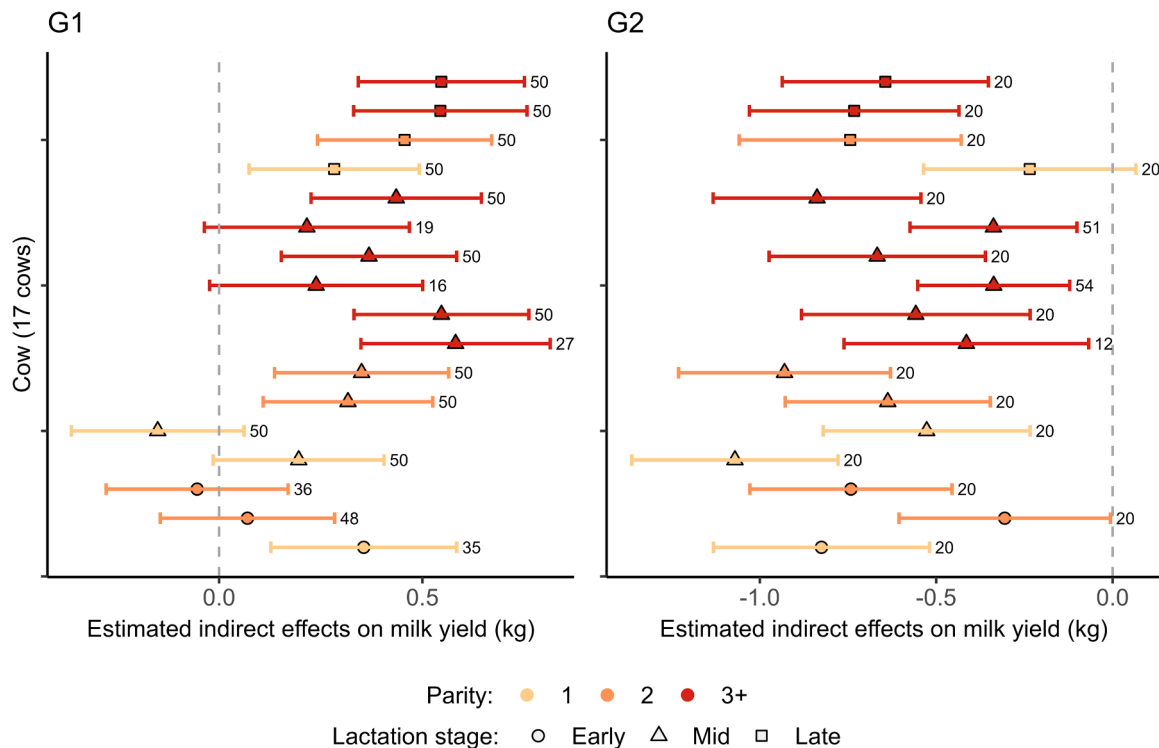


Fig. 4. Individual indirect effect estimates, with standard error bars for cows that regrouped from G1 to G2 during the study period. The figure shows the estimates for 17 out of 49 cows, which had at least ten records in each group. The estimates are presented with circles, triangles, and square symbols in light orange, orange, and red to represent the cow's parity and lactation stage. The numeric value after the error bars represents the number of records for that cow in the respective group.

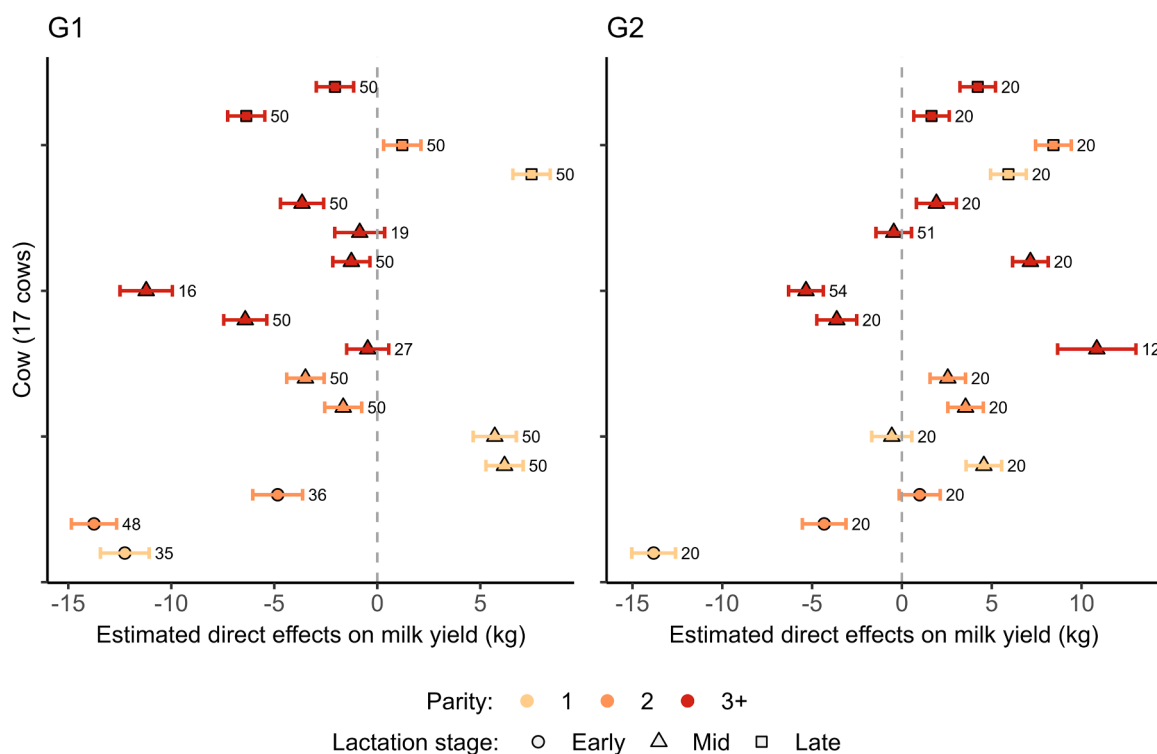


Fig. 5. Individual direct effect estimates, with standard error bars for cows that regrouped from G1 to G2 during the study period. The figure shows the estimates for 17 out of 49 cows, which had at least ten records in each group. The estimates are presented with circles, triangles, and square symbols in light orange, orange, and red to represent the cow's parity and lactation stage. The numeric value after the error bars represents the number of records for that cow in the respective group.

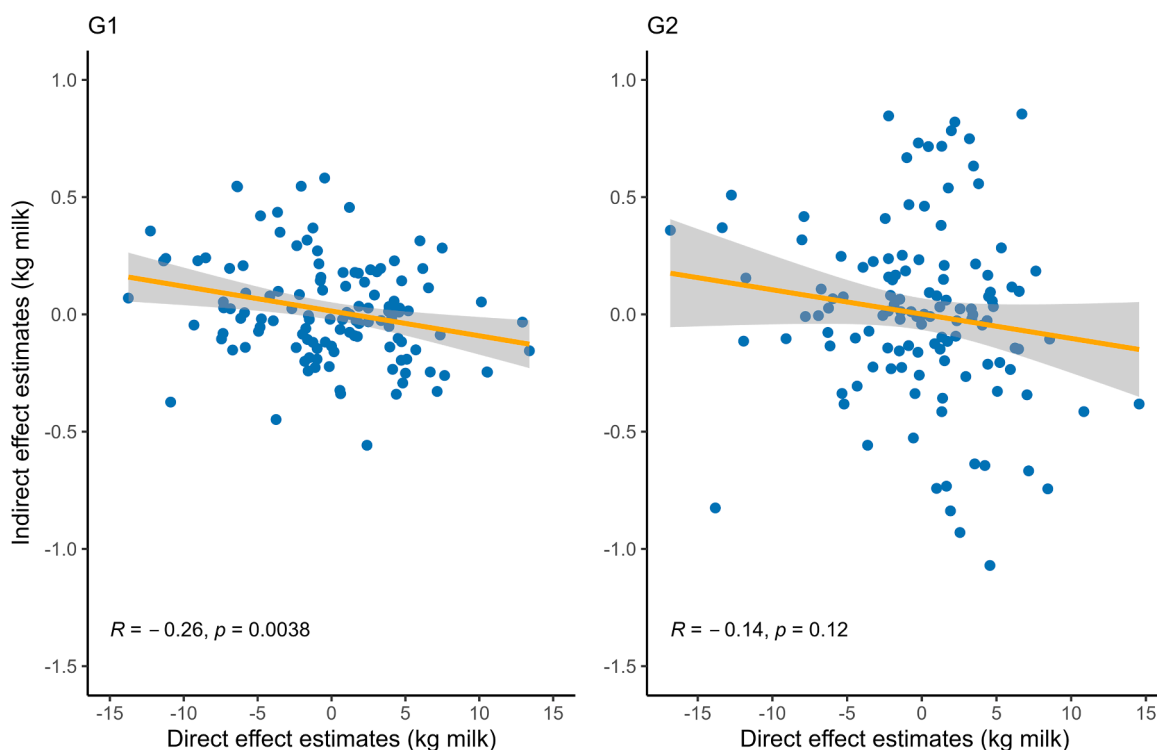


Fig. 6. Correlation plot of the estimated direct and indirect effects on milk yield for cows in G1 and G2, with the Pearson correlation coefficient, R, and corresponding p-value.

decrease the milk yield for a longer period. In our study, we also observed that some other cows had an average positive effect on another cow's DMY. A cow with a typical stressed and nervous state during

milking due to e.g., the milking procedure or the human performing the milking (Rushen et al., 1999) might be less stressed if the neighbouring cow has a calming effect on that cow. The milk yield will therefore

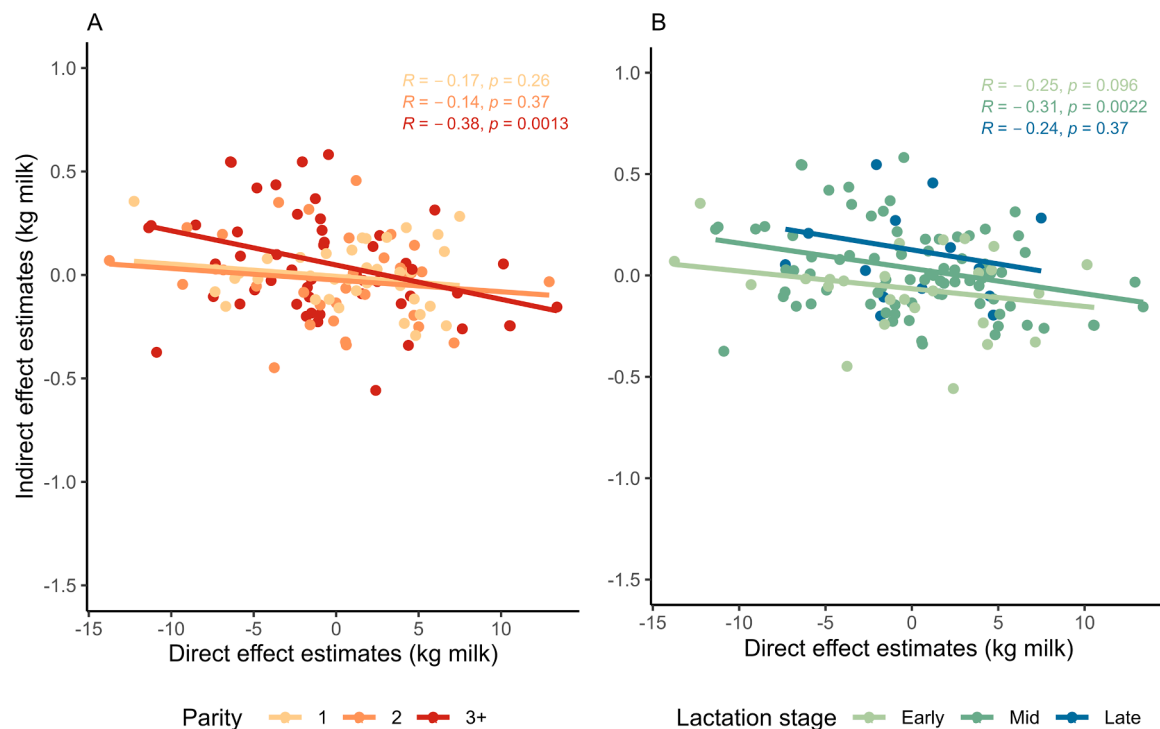


Fig. 7. Correlation plot of the estimated direct and indirect effects on milk yield for cows in G1 with a regression line for each parity group (A) and lactation stage (B). The estimates are presented in light orange, orange, and red to represent each parity group and in light green, green, and blue to represent each lactation stage. R = Pearson correlation coefficient.

increase compared to the normal state. Several factors appear to influence the provision of social support, such as the familiarity between the animals and the identity and emotional state of the partner (Rault, 2012), and the result of this study indicates that some individuals might, on average, be better social supporters than others.

Regrouping has been demonstrated to affect feeding and social behaviour, reduce milk yield (Hasegawa et al., 1997; von Keyserlingk et al., 2008), and diminish the social buffering properties of the group (Mounier et al., 2006). Cows that regrouped from G1 to G2 during the study period tended to have a more positive effect on their neighbours in G1 and a more negative effect on their new group mates in G2. This might be related to a disturbance of social stability within the group in G2 due to introducing new individuals and establishing social ranks. Usually, establishing social ranks is observed within three to seven days after regrouping (Grant and Albright, 2001), after which the aggressive interactions decline, and the hierarchy stabilizes (Bouissou et al., 2001). However, creating strong relationships between newcomers and residents after regrouping seems to take longer, and individuals may need longer than a couple of weeks to form long-lasting relationships (Rocha et al., 2020). This might explain why even the regrouped cows that have been in G2 for over 20 days still have a negative indirect effect on their group mates. These results also indicate that the indirect effect estimates depend on the group composition and whether we have a stable or dynamic group. In G1, we also had a dynamic group composition due to newly calved cows introduced to the group, and these cows also had a more negative effect on their neighbours' milk yield (Supplementary Figure. S1). It would be necessary to investigate these effects in a longitudinal study and assess if regrouped cows will return to a more positive effect on their group mates.

Some of the regrouped cows in our study changed their direct effect to a more negative effect in the new group. In contrast, most of these cows changed to a more positive direct effect, which contradicts the idea that regrouping leads to a reduced milk yield. However, G1 primarily holds high-yield cows, while G2 has a lower average production level. The direct effects are relative to the group mean and at the same DIM the

mean milk yield level is larger in G1 than in G2. Moving a cow in Parity 2, with 35 kg milk at day 200 will go from having a milk yield below average to above average (Fig. 1). Also, except for cows confirmed pregnant, cows with specific problems designated for slaughter are moved from G1 to G2. In relation to the other high-yielding cows in G1, these problematic cows might produce much less, which leads to a negative direct effect in G1. However, concerning the lower-yielding cows in G2, these cows might produce better, leading to a more positive direct effect.

In G1, there was a weak negative correlation between the direct and indirect effects on milk yield, which seemed to intensify with parity. This would mean that high-yielding cows will negatively impact the milk yield of their group mates, while low-yielding cows will have a more positive effect on the others. Wood (1977) found that allogrooming was associated with a higher milk yield for the receivers, and removing "social groomers" from the herd could be unfavourable to the milk yield at the herd level. Some individuals also have a negative direct and indirect effect on milk yield, which seems like unprofitable cows to have within the herd, while others have a positive direct and indirect effect on milk yield. It would be helpful to identify these indirect effects to select the most optimal individuals within a herd and to optimize group composition and milking routines. For instance, an indirect effect of 0.5 kg for a cow with four adjacent cows daily leads to 60 kg more milk in 30 days. The dispersion of the indirect effect estimates on the y-axis in Fig. 6 suggests that there is room for improvement, and further research should assess whether these effects have a genetic component, i.e., indirect genetic effects (IGE) (Griffing, 1967; Moore et al., 1997), to see if we can raise the average effect in each generation. We applied a linear mixed model similar to the variance-component model used to estimate IGE (Bijma et al., 2007b, 2007a; Muir, 2005), with a possible future aim to extend our model with genetic information.

Our model assumes that the indirect effects are random and additive. Suppose a cow stands next to an individual with a negative indirect effect on one side and next to an individual with a positive effect on the other side during milking. In that case, the effect may be negated. Fig. 3

shows the sum of the indirect effects for each observation, and the mean in both groups is around zero, which means the effect was negated for some of the observations. Yet, there was still a variation, and a larger variation in G2, where the total effect of an individual's neighbours could either contribute or reduce with up to >2 kg of milk.

The possibility of choosing the neighbour during milking might affect the results. In this herd, the milking order is fairly constant; cows do not enter the milking parlour in a random order (Hansson and Woudstra, 2023). This also corresponds to the results from other studies on milking order (Berry and McCarthy, 2012; Grasso et al., 2007; Vargas-Bello-Pérez et al., 2020). Cows in their first parity and cows in early lactation tend to be first in the milking order, while cows in late lactation and higher parity enter last (Hansson and Woudstra, 2023). Hansson and Woudstra (2023) speculated about their results that first-parity cows and cows in early lactation were presumable more subordinate or less familiar with the group members and, therefore, more motivated to leave the crowded waiting area in front of the milking parlour. Cows stay close to individuals with similar attributes, such as, e.g., parity, and create preferential bonds (Boyland et al., 2016; Churakov et al., 2021; Marina et al., 2024), indicating that they might choose the individuals they enter the milking parlour together with. However, the waiting area in front of the milking parlour is quite crowded at the beginning of the milking session, and the possibility of choosing a specific neighbour during milking is probably limited. The baseline production level of a cow could also probably influence how much the milk yield deviates due to social interactions (Jezierski and Podłużny, 1984). There are fewer opportunities for further improvement in milk yield for cows that already perform close to their physiological potential. In contrast, they are more susceptible to drops in production due to negative social experiences (Fielding et al., 2024).

In our study, the milk yield per milking session was unavailable, and the summarised DMY (the previous day's evening yield and the next day's morning yield) was used instead, together with the information on milking order per milking session. Neighbouring cows could impact a cow's milk yield for the current session, but also probably in the next session due to the potential increased or decreased residual milk. Using the milk yield per session would be preferable to get more accurate estimates of the individual indirect effects. To assess how the estimated variance components for the indirect effects would be affected if either the DMY or the milk yield per session was used, we performed a simple simulation (Supplementary Material S1). In the simulation, the residual variance was twice as big when using DMY as the milk yield from every session. Still, the variance components for the indirect effects did not change.

Several factors, such as interrupted milking, measurement errors, or pathological and physiological changes such as a cow being in oestrus, impact on health status, or feed intake, may cause day-to-day variation in milk yield. Our model did not adjust for cow's health, oestrus status or feed intake. The daily variation in milk yield can also depend on the sampling procedure, the milking equipment and the milking interval. We removed 88 individual daily milk records outliers that deviated more than three standard deviations from a 5-day running mean. Some of these outliers were half the amount of milk than the previous day and the next day, and it could be an error from the transfer from the milking equipment reporting the milk yield from one milking session instead of the DMY. The information on who milked the cows daily would also be important to consider in order to adjust for the farm staff's effect on milk release.

In our model, we accounted for indirect effects caused by the nearest neighbours standing next to a cow during milking. However, the nearest neighbours may in fact be affected by their nearest neighbours, which could have been modelled using a spatial autocorrelation, but in this first study on indirect effects we kept the model as simple as possible and chose not to include effects beyond the nearest neighbour. Additionally, the interactions in the parlour are only a subset of all interactions between cows, and the variation in daily milk yield might also be

influenced by what happens in the barn before milking. Social interactions during feeding (Foris et al., 2019; Val-Laillet et al., 2009) or the presence or absence of affiliative social partners in the group might have an effect on the daily milk yield (Fadul-Pacheco et al., 2021). Combining the milking parlour networks with social network analyses in the free-stall barn will provide further information on the social interplay between dairy cows and its impact on milk production might be interesting to include in future studies. Information on "kick-off" events or using cameras to capture how much the cows are moving during milking could also be used as an indirect measure of stress. Using cameras to capture specific interactions between individuals is also a possibility in the future.

Modelling lactation curves has been of interest in research for a long time, and numerous mathematical models have been proposed to account for the dynamic characterization of milk yield. Early studies proposed parametric functions to describe the standard lactation curve and can be fitted within the mixed model framework (Ali and Schaeffer, 1987; Wilmink, 1987; Wood, 1976), and later alternative functions such as Legendre orthogonal polynomials and smoothing splines have been proposed (Macciotta et al., 2005; White et al., 1999). There is an increasing interest in deviations from the lactation curves as indicators for resilience in dairy cows with different methods of capturing these perturbations (Ben Abdelkrim et al., 2021; Elgersma et al., 2018; Poppe et al., 2020). In our study, we used a smoothing function of the DIM to adjust for the lactation curve and used the milk yield residuals as the daily deviations in milk yield. Smoothing is usually applied to reduce noise in data but could also be used to identify short-time disturbances. Codrea et al. (2011) used differential smoothing procedures to capture perturbations in the lactation curve and found reduced milk yields during periods of nutritional challenge and additional deviations of unknown causes. However, in our study, we had daily milk yield records, which also make it possible to fit a smoothing curve, while other lactation curve functions are developed to estimate the curve based on test day records. Salamone et al. (2024) suggested that milk yield residuals can be used as indicators for the metabolic and health status of the cows at the start of lactation. In their study, they investigated the milk yield residuals in transitioning cows, where they subtracted the expected milk yield from the actual milk yield and found that more negative milk yield residuals were associated with lower dry matter intake and increased parity. Our study's results might suggest that part of these deviations from the lactation curves one can detect could be due to the indirect effects of neighbouring cows during milking.

5. Conclusion

The present study used the milking order within a milking parlour to estimate the indirect effects of the neighbouring cow during milking on a cow's daily milk yield. The findings of our study show individual variation in the average indirect effect on the milk yield of the neighbour, where some individuals have a positive average effect on their group mates' milk yield, while others have a more negative effect. In one of the groups, there was a weak negative correlation between the direct and indirect effects on a cow's milk yield, which tended to intensify with parity. Identifying these indirect effects would be helpful in selecting the best individuals in a herd and optimizing group compositions and milking routines. However, this effect seems to be disrupted when the cows are regrouped, and further studies are needed with additional farms to assess these effects for a more extended period.

CRedit authorship contribution statement

Ida Hansson: Writing – original draft, Software, Data curation, Visualization, Formal analysis, Conceptualization. **Hector Marina:** Writing – review & editing, Software. **Freddy Fikse:** Writing – review & editing, Supervision. **Per Peetz Nielsen:** Supervision, Writing – review & editing, Conceptualization. **Lars Rønnegård:** Supervision,

Conceptualization, Writing – review & editing, Methodology.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval

Not applicable.

Data availability statement

None of the data or the scripts were deposited in an official repository. The data and the scripts that support the study findings are available from the corresponding author upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors did not use any artificial intelligence assisted technologies in the writing process.

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Supplementary materials

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