The effect of transferring dairy cows from a group housing system to individual pens for research purposes on behaviour and milk yield

Abstract

Context. Cows are often subjected to different environmental and handling conditions for research purposes, and it is important to understand potential behavioural changes, as they may influence research outcomes. **Aim**. To investigate how a transfer from a group housing system to individual pens affected dairy cow behaviour. **Methods**. Lactating dairy cows (*n* = 24), housed in an open-sided barn, were transferred to individual pens for 10 weeks. For another experiment's purposes, cows were assigned to a control or an experimental diet. Measurements were taken before transfer (pre), after transfer to the individual pens (early), in the individual pens (late transfer) and on return to the barn (post transfer). Cows' behaviour was assessed every 10 min for 8 h per day, and whether cows were eating, ruminating or idling was recorded. Occurrences of social interactions were recorded continuously during the last 60-s of every 10-min observation. Lying time was recorded using dataloggers. Locomotory ability assessment and daily milk yields were also recorded. **Key results**. Ruminating and eating time was not affected by diet type, but decreased after transfer to the individual pens. Only eating time returned to pre transfer values at early transfer. Time that control cows spent idling increased in individual pens, whereas the experimental diet cows spent more time idling in the early transfer phase. Social interactions occurred more often during late and post transfer phases. Cow locomotory ability was not affected by the transfer or by type of diet, but total lying time increased from pre to post transfer, the number of lying bouts decreased from late to post transfer phases, and lying bout duration increased as the experimental period progressed. Milk yield gradually reduced over time, and it was affected by the type of diet. **Conclusion**. Behavioural changes in dairy cows during transfer between housing systems featured adaptive characteristics and did not seem to be detrimental to the major research. **Implications**. The transferring of cows from a group housing system to individual pens under the conditions used in this study did not jeopardise cow behaviour patterns to an extent that could affect other nutritional research outcomes.

Introduction

Scientific studies with dairy cows commonly demand specific experimental designs that require different environments and handling from what the cows are used to. An example is the use of individual housing (Branco *et al*. 2015) to enable collection of data on metabolic parameters, such as in respiration chambers, or individual DM intake when the use of weighing trough or automated feeders is not available. Modified environments or handling can be stressful to cows. However, cows are extremely adaptable animals (Jago and Kerrisk 2011), although they likely need a habituation period to adapt to abrupt changes (Grille *et al*. 2019). When cows are unable to adapt to new conditions or routines, they may have welfare issues and their productivity may be adversely affected (Grant and Albright 2001; Devries *et al*. 2004), thus compromising the experimental outcomes.

Cow behaviour results from an interaction between their internal state and the environmental stimuli (Beaver *et al*. 2019), and can provide an inference about their physiological and psychological state (Munksgaard and Simonsen 1996). Thus, temporary changes in cow behaviour could reflect their attempts to cope with a particular environment or handling (Schütz *et al*. 2013), suggesting whether they are stressed or not (Johns *et al*. 2015). This can be reflected in changes in normal behaviours, such as feeding (Devries *et al*. 2004) and social interactions (Tresoldi *et al*. 2015; Costa *et al*. 2016).

Cows are gregarious animals, and establish a complex and stable social rank within a group. Therefore, farm practices that cause disruption to social dynamics can be extremely stressful to cows (Proudfoot and Habing 2015). Practices, such as regrouping (Von Keyserlingk *et al*. 2008) or adding unfamiliar individuals to a group, can have behavioural and physiological effects, such as increased aggression, or in extreme cases, disease (Proudfoot and Habing 2015) and reduced milk production (Von Keyserlingk *et al*. 2008). Moreover, these effects are intensified for those cows that are reallocated to a new environment (Schirmann *et al*. 2011).

Housing type is a major factor that influences the daily lying time of cows (Charlton and Rutter 2017) and the risk of lameness in the herd, which are factors associated with cow welfare (Olmos *et al*. 2009). Lying behaviour is a priority for cows (Tucker *et al*. 2009), and changes in its pattern are a visible indication of whether cows are comfortable or not under a specific situation. Enriquez-Hidalgo *et al*. (2018) reported behaviour disruptions in cows transferred from a grass-based system to a tie-stall system. Lying times were reduced, and there was an increase in locomotion overall score (more impaired) due to the space and movement restrictions. However, Fregonesi and Leaver (2001) showed that housing system did not affect the locomotion score of cows, regardless of the duration of the experiment, and neither did the space allowance in a short period experiment (4 weeks), even when lying behaviour was affected (Fregonesi and Leaver 2002). Similar to Enriquez-Hidalgo et al. (2018), Broucek et al. (2017) reported changes in lying time of cows transferred from a tie-stall to a free-stall system, which implied a rapid adaptation, as they were moved to an environment with greater space allowance and freedom of movement. An immediate reduction in ruminating time was observed; nevertheless, ruminating time gradually increased as the cows became familiar with their new environment over a period of 10 days (Broucek et al. 2017). From this perspective, changes in the cows' environment may not always be adverse.

To our knowledge, no information is available regarding the effect that a transfer from a high-density group housing system to individual pens that allows free movement and greater individual area per animal, and vice versa, has on dairy cow behaviour, milk yield and locomotory ability. For the purpose of a study aimed at measuring methane production, 24 lactating dairy cows were submitted to that transfer condition (Enriquez-Hidalgo et al. 2020). We were concerned that changing the cows' environment could affect their pattern behaviour, influence milk yield and ultimately affect methane production. Therefore, the objective of the current study was to evaluate changes in behavioural patterns and milk yield in cows transferred from a group housing system to individual pens that, despite restricting cows to a smaller space, allowed them to move around the pen and socialise with their neighbours. We hypothesised that:

(1) moving cows from group housing to individual pens would change their behavioural patterns and thereby result in a reduction in milk yield. We believed changes in cows' behavioural patterns would mainly be reflected in abrupt reductions in lying (lying time, bouts and bout duration) and ingestive behaviour; however, despite the reduction of space availability, locomotory ability would not be impaired,

(2) cows would adapt to their new accommodation after a 5-week period of habituation, indicated by the increase in lying (lying time, bouts and bout duration) and ingestive behaviours and, (3) cows would show changes in social and other behaviours due to a change in accommodation, which is expected, but they would revert to their pretransfer behaviours as soon as they returned to the group housing

Materials and Methods

This study was part of the experiment reported by Enriquez-Hidalgo *et al*. (2020) that was approved by the Scientific Ethics Committee for Animals and Environmental Care of the Pontificia Universidad Cat´olica de Chile (protocol number 160511004). The experiment was conducted at the experimental farm of the University, Fundaci´on AgroUC, located in Pirque, (33°40'S; 70°36'W), from August to October 2018.

Animals and experimental design

Briefly, 24 lactating dairy cows were selected from a commercial herd of 220 cows that normally resided in a compost barn system allocated in two 84 m × 15 m sheds with open sides. Woodchips were added to the bedding area (84 m × 10 m) every other week and the beds were stirred using a rototiller three times per day to keep the top layer of bedding dry. The feed bunk was located along one side of the shed, separated from the bedding area by a 1.20-m concrete wall with four 6-m openings to allow cows free passage. A total mixed ration diet was delivered twice a day, and cows had *ad libitum* access to feed and to two 500-L water troughs located at the end of each shed. The cows were assigned to one of 12 blocks according to genotype (Holstein-Friesian and Montbeliard), lactation number (1.6 s.d. 0.76), days in milk (222 s.d. 84.7 days) and preexperimental milk yield (37.8 s.d. 4.2 kg/day). Cows within blocks were randomly allocated to one of two groups to evaluate the effect of two experimental total mixed ration diets on methane emissions (reported in Enriquez-Hidalgo *et al*. 2020). The diets were: (1) a control diet (CON) similar to the diet usually offered to the cows, where the forage fraction was made of corn silage and alfalfa hay; or (2) an experimental diet (MIX), where a fresh annual ryegrass and berseem clover mixed herbage was used to substitute 40% of the corn silage and alfalfa hay forage fraction of the CON diet. (Table 1). On Day 0, both groups were separated from the main herd for 10 weeks and randomly allocated to individual pens (6.0 m × 3.5 m) bedded with sawdust and separated with a wooden fence consisting of two rails spaced widely apart (50–60 cm), which allowed cows to interact with their neighbours. Cows had *ad libitum* access to their diet (regulated to 5% refusals) and to individual water troughs. After the 10-week experimental period, cows returned to the compost barn after morning milking on Day 71. Cows were milked three times per day with the rest of the herd at 0630, 1330 and 1930 hours while in the compost barn system; and at 0800, 1500 and 2100 hours while in the individual pens, right after milking of the herd that remained in the compost barn. Due to this criteria, duration of milking was 105 s.d. 13.0 min/day while cows were in the compost barn and 50 s.d. 8.5 min/day when in individual pens.

Table 1. Ingredients (% of DM) and chemical composition (g/kg DM) of the diets offered to the cows in the compost barn during pre and post transfer phases, and of the two diets offered to the cows in the individual pens: a control diet and experimental diet.

(%ofDM)	Compost barn	CON	MIX
Diet ingredients			
Groundcorn	14.7	16.7	20.2
Wheatbran	6	14.4	18.2
Canola	9	23	11.2
Cornsilage	36	34.2	25.2
Alfalfa hay	10.2	8.4	–
Annual rye grass and berseem clover fresh herbage	–	–	25.2
Brewersgrain	12.5	–	–
Lupin	4.5	–	–
Freshalfalfa	18	–	–
Diet chemical composition (g/kgDM)			
DM	533	518	408
CP	166	170	148
NDF	392	325	330
ADF	210	175	162

Animal behaviour

The experiment evaluated behavioural changes in dairy cows following a longitudinal arrangement similar to that used by Enriquez-Hidalgo *et al*. (2018) and Pereira *et al*. (2021). Observations of cow behaviour were performed during four phases: (1) in the compost barn for three consecutive days before transfer (pre; Day −5toDay −3); (2) immediately after transfer to individual pens for 3 days (early transfer; Day 2 to Day 4); (3) later in the individual pens for 3 days (late transfer; Day 37 to Day 39 for six cows from Group 1 and six cows from Group 2, and Day 52 to Day 54 for the remaining cows); and (4) in the compost barn for 3 days immediately on return (post transfer; Day 71 to Day 73), to the compost barn system. This yielded 576 observations per cow over the entire evaluation period. In late transfer, cow behaviour was assessed over two different periods for two different groups due to equipment availability to estimate methane emissions, as described by Enriquez-Hidalgo *et al*. (2020) and Pereira *et al*. (2021). In all phases, observations were performed between the milking times: 0930–1330 hours and 1530–1930 hours while in the compost barn; 1000– 1400 hours and 1600–2000 hours while in individual pens. In the compost barn, the 24 cows were individually identified with collars of different colours, and their behaviours were recorded through visual observation, according to a scan sampling procedure of 10-min intervals (Altmann 1974). The following behaviours were recorded: ingestive (eating, ruminating or idling), walking and 'other behaviours' (drinking water, interacting, defecating, urinating and grooming). Affiliative and agonistic occurrences were recorded during the last 60-s period of every 10 min of observation (Hessing *et al*. 1993). Descriptions of all the observed behaviour are detailed in Pereira *et al*.(2021).As cows were allocated between two sheds while in the compost barn, two observers watched the groups simultaneously. Both observers were trained before the study to ensure concordance in behaviours determination, and they were balanced across sheds and observation days, so that every observer recorded equally both groups. In the individual pens, behaviours were video recorded (H.264 network digital video recorder, Enforcer DR-1; SECO-LARM; Irvine, CA, USA) and assessed using the same methodology as for the direct visual observations. Cameras were positioned in the top corner (right or left) of each pen to allow a full view of two pens. One of the people who observed the cows in the compost barn watched all the videos to record the behaviours. Additionally, lying time, and number and duration of lying bouts per day were recorded at 5 min intervals on the following days: Day −7to Day −1 (pre transfer), Day 0 to Day 7 (early transfer), Day 37 to Day 44 for Group 1 and Day 50 to Day 57 for Group 2 (late transfer), and Day 71 to Day 74 (post transfer) using dataloggers (HOBO Pendant G Acceleration; Onset Computer Corporation, Bourne, MA, USA) fitted below the hock on the outside of the right hind leg or on inside of the left hind leg of each cow and secured using a Vetwrap™ bandage, as per O'Driscoll *et al*. (2008). Collected data were extracted using software supplied with the loggers (HOBOware Lite; Onset Computer Corporation), and data were processed as suggested by Ledgerwood *et al*.(2010) with adaptations made by Zobel *et al*. (2015).

Locomotion scoring was performed by the same evaluator in the four phases, after two consecutive morning milkings in each phase. Cows were individually released on a flat concrete surface, and then scored as they walked past (lateral view) and then away (posterior view) from the same observer. Five aspects of locomotion were scored (tracking, ab/adduction, speed, spine and head bob) between 1 (perfect) and 5 (most impaired), using the system described by O'Driscoll *et al*. (2010).

Milk yield of individual cows was automatically recorded daily during each of the four phases using milk metres (MM15; Delaval, Tumba, Sweden).

Statistical analysis

Data were summarised by cow and by day for each phase. Behavioural data, milk yield and locomotion score were analysed using generalised linear mixed models (Proc Glimmix) in SAS 9.4 (SAS institute, Cary, NC, USA), considering the phases, the diet type and the phase × diet type as fixed effects and the cow within block as the random effect. The group effect was initially considered as the fixed effect, but was removed from the model, as it had no significant effect ($P > 0.05$) on any of the analysed variables. The day nested within phase was used as the repeated measurement, and the cow was used as the experimental unit. Normality and homogeneity of variances were checked by residual analysis, and the distribution (normal, gamma or Poisson) was used according to visual evaluation. Differences in least squares were evaluated using the *t*-test, considering significant differences at $P < 0.05$. The results are presented as least square mean ± s.e.m. Daily lying time, lying bout duration and the number of lying bouts in each phase had normal distribution and were investigated using the same model. Individual datalogger data that were <20% and >70% of the daily time lying (3 out of 52 days, and one cow during the late transfer phase) were considered equipment errors and were not included in the analysis.

Results

The proportion of scans spent eating reduced by 12% after cows were transferred from the compost barn to the individual pens ($P < 0.001$; Fig. 1), increased by 5% ($P = 0.04$) from early to late transfer, and were then unchanged post transfer back to the compost barn. The proportion of scans of ruminating decreased by 5% after the transfer ($P = 0.01$), at early and late transfer phases, and returned to the initial level, when cows were back in the compost barn. Neither eating ($P = 0.21$) nor ruminating ($P = 0.73$) behaviours were affected by the type of diet or phase and diet interaction ($P = 0.50$ and $P = 0.44$ for eating and ruminating, respectively).
The proportion of scans spent idling was 15% greater when cows were housed in individual pens compared with in the compost barn (40.0 vs 29.4%; s.e.m.: 4.67%; $P < 0.05$), and it was even greater during the early than the late transfer phase ($P < 0.001$; Fig. 1). There was an interaction between phase and diet ($P = 0.02$) for the proportion of scans spent idling. The proportion of scans that cows on the CON diet spent idling increased when they were in the individual pens compared with when they were in the compost barn

(40.5 vs 20.3; s.e.m.: 4.93%; $P = 0.05$). Whereas cows on the MIX diet increased the proportion of scans spent idling only during the early transfer (47.6 vs 29.7; s.e.m.: 4.88%; $P < 0.001$) compared with any other transfer phase. was approximately 3% greater during late and post transfer phases compared with pre and early transfer phases ($P < 0.05$; Fig. 1), and tended to be greater for cows on the CON diet than for those on the MIX diet (8.5 vs 7.4%; s.e.m.: 0.41%; $P = 0.057$).

Agonistic and affiliative interactions were affected by phase ($P < 0.001$). A greater occurrence of agonistic interactions was observed when cows were in the compost barn, being greater for the post transfer phase than pre transfer phase (1.8 vs 1.3; s.e.m.: 0.10 occasions/h; $P < 0.05$). Whereas in individual pens, there was no difference between early and late transfer phases (0.6 ± 0.18 occasions/h, $P = 0.48$). The occurrence of affiliative interactions was lowest in early transfer (0.6 ± 0.26 occasions/h; $P = 0.04$) and greatest in post transfer (1.8 ± 0.21 occasions/h; $P < 0.01$), with intermediate values in pre and late transfer phases (1.2; s.e.m.: 0.20 occasions/h; $P = 0.45$).

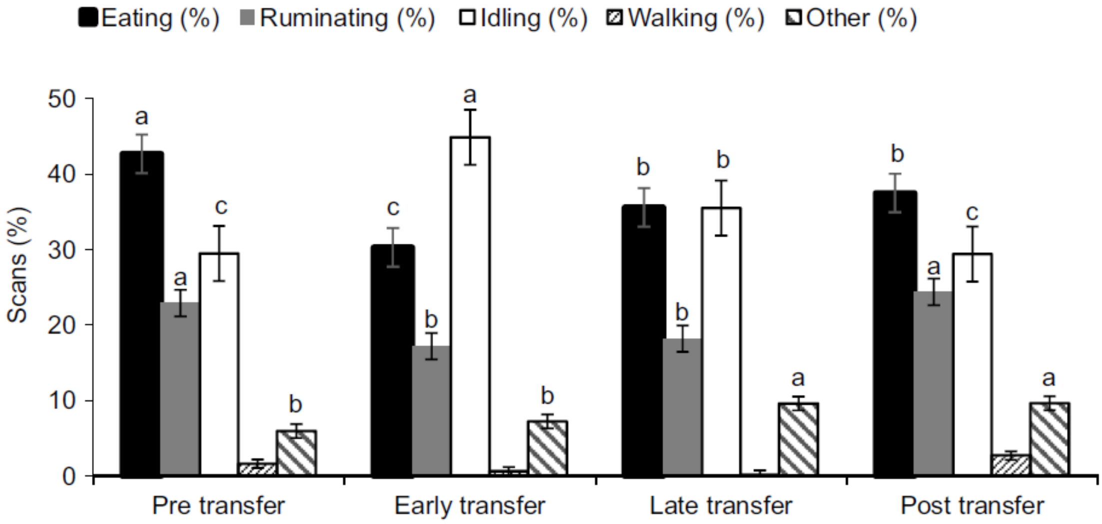

Fig. 1. Percentage of scans of each behaviour in each phase investigated. Before cows transfer to individual pens (Pre transfer), soon after transfer to individual pens (Early transfer), after the habituation to the individual pens (Late transfer) and on transfer back to compost barn (Post transfer). Columns with different letters within behaviour type differ between phases ($P < 0.05$).

Locomotion scores were not affected by the transfer, by diet type or the interaction between these factors. The mean score of all aspects of locomotion was 1.6 ± 0.08 ($P = 0.15$), and the scores of individual aspects of locomotion were 1.0 ± 0.18 ($P = 0.99$) for speed, 2.7 ± 0.31 ($P = 0.60$) for tracking, 2.2 ± 0.27 ($P = 0.64$) for ab/adduction, 1.2 ± 0.26 ($P = 0.64$) for spine curvature and 1.2 ± 0.19 ($P = 0.95$) for head bob.

Total lying duration gradually increased by 9% when cows were transferred, from pre to late transfer phase ($P = 0.04$), and returned to the same level as pre transfer phase when cows returned to the compost barn (Fig. 2). The number of lying bouts decreased by almost 30% during the post transfer phase ($P = 0.03$), and bout duration increased as the phases progressed after early transfer ($P < 0.05$).

The milk yield response showed an interaction between phase and diet type as a reduction in milk yield occurred as time progressed, but cows receiving the MIX diet had a greater reduction than cows receiving the CON diet (Table 2).

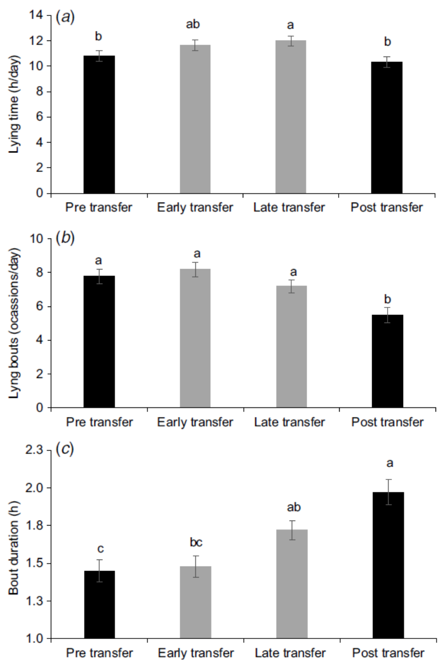

Fig. 2. Effect of different transfer stages: before cows transfer to individual pens (Pre transfer), soon after transfer to individual pens (Early transfer), after the habituation to the individual pens (Late transfer) and on transfer back to compost barn (Post transfer) on (*a*)total daily lying time (h/day), (*b*) daily number of lying bouts (no/day) and (*c*)lying bout duration (h/bout). Columns with different letters differ ($P < 0.05$).

Discussion

Moving cows from group housing to individual pens changed cows' behaviour patterns in the first few days, as indicated by a reduction in the proportion of time spent eating and ruminating, as hypothesised. However, contrary to our hypothesis, the proportion of time spent lying and lying bout duration were greater when cows were in the individual pens compared with the compost barn. Furthermore, the number of lying bouts and the locomotion scores were unaltered. This means we can only partially accept our first hypothesis.

Lying is described as a priority for cows and a strong indicator of animal comfort (Tucker *et al*. 2009). The fact that cows had longer total lying times and longer bout duration in individual pens could be partly explained by the greater individual area available than in the compost barn, 18.0 versus 11.6 m^2 per cow (Tucker *et al*. 2004), and probably indicates they were in a comfortable situation (Endres and Barberg 2007). In addition, there was no competition for preferred lying areas, and overall milking times were shorter due to the smaller herd size, which also explains the difference in lying times between the systems (Solano *et al*. 2016). The reduction in lying bouts during the post transfer phase can be explained by the increase in interactions, as cows were living socially and under a higher stocking density again (Charlton *et al*. 2014; Krawczel and Lee 2019).

Table 2. Milk production (kg/day) of the two groups investigated: group receiving the control diet and group receiving the experimental diet, according to the phases.

						P-value		
Milk Production	Pre transfer	Early transfer	Late transfer	Post transfer	s.e.m.	Phase	Diet	Phase × Diet
CON	38.3a	36.0ab	34.9ab	32.0bc	1.56	<0.001	<0.01	0.03
MIX	35.8ab	35.3ab	29.8bc	25.9c	1.55			

Before cows transfer to individual pens (Pre transfer), readily after transfer to individual pens (Early transfer), after the habituation to the individual pens (Late transfer) and back to compost barn (Post transfer). a–d, Means within a row with different lowercase letters differ ($P < 0.05$). s.e.m., Standard error of the mean.

Contrary to our study, a reduction in both lying time and bout duration was a consequence of the discomfort that cows experienced when they were transferred from a group accommodation (pasture system) to individual stalls (tie-stall system; Enriquez-Hidalgo *et al*. 2018). The difference between both studies may be due to our cows feeling more comfortable when housed in the individual pens compared with the individual stalls of Enriquez-Hidalgo *et al*. (2018), in which the bedding material differed substantially (rubber mats).

We hypothesised that cows would not demonstrate locomotion problems, even though they were individually restricted in pens. Cleanliness, good walking and standing condition prevent cases of clinical lameness in cows indicated by locomotion score (Fregonesi and Leaver 2001). Both systems used good-quality bedding material (Barberg *et al*. 2007), woodchips in the compost barn and sawdust in the individual pens. The bedding in the individual pens was changed daily and cleaned twice a day during milking times, to keep the cows clean and dry and the bedding soft, similar to conditions in the compost barn. The clean and dry surface facilitated hoof health of cows (reducing lameness; Bewley *et al*. 2017) and offered good traction (enhancing confidence of the cows when laying down or standing up; Whay and Shearer 2017). This could explain why locomotion scores were not affected by location.

The extent of the cows' ability to adapt to their new accommodation depends on the measure investigated. Lying behaviour indicated quick and complete acclimation. However, ruminating behaviour suggested that the cows did not adapt as readily. Changes in ruminating behaviour are generally described as the consequence of a change whether in the environment, management or routine (e.g. Broucek *et al*. 2017). Thus, the reduction in ruminating while cows were kept in individual pens could have been triggered by the change in housing system. Grille *et al*. (2019) also noticed a reduction in ruminating time as a sign of emotional disturbance when cows were changed from a different diet from a grazing plus total mixed ration mixed system to a confinement system. In the Grille *et al*. (2019) study, cows needed more time to fully adapt, as they only showed a gradual adaptation in ruminating behaviour 1 month after the transfer. In 5 weeks, the time given to the cows in the present study, our cows did not fully adapt, and the consistent reduction in rumination could indicate difficulty to adapt, stress and a challenge to cow comfort (Johns *et al*. 2015).

Stressful situations might trigger physiological reactions in cows, thus impairing milk production (Broucek *et al*. 2017). Nevertheless, we do not believe that the slight and gradually decrease in milk production in CON cows had a major effect from the transfer. In fact, the abrupt difference in the milk production of MIX cows was most likely affected by the nutritional composition (difference in crude protein) of the diet rather than by the transfer itself (more details related to the diets in Enriquez-Hidalgo *et al*. 2020) or also due to the cows stage of lactation that causes milk to gradually reduce over time, as the reduction was noticed in both groups. Likewise, feed characteristics and nutritional issues also have a great influence on ruminating time (Beauchemin 2018). Although the type of diet offered while in the individual pens did not affect eating or ruminating time, diets offered while in the compost barn and in individual pens had a considerable difference in neutral detergent fibre concentration (−16%). Furthermore, while cows need to compete for their food when they are in group housing systems, such as the compost barn, this is not a concern in individual pens, where the food is delivered specifically for each individual cow and they can eat in synchrony as soon as the food arrives (Asher and Collins 2012). In a competitive situation, there is crowding, fighting and low-ranking animals are jeopardised (Napolitano *et al*. 2009). Dominant cows take priority in accessing the feeders, and subordinate cows wait until the dominants are satisfied and vacate the feeders (Sowell *et al*. 1991). Therefore, eating time might be prolonged in the compost barn because of these social factors. We believe that, as cows in the individual pens could eat at their leisure, they chewed their food more during ingestion, thus requiring less rumination compared with the compost barn, where the emphasis may have been on consumption before other cows ate it. Therefore, we speculate that the decline in the proportion of scans spent ruminating could have resulted from the lower fibre content of the experimental diets compared with the diet offered at the compost barn and a reduced need to process feed eaten rather than being related to stress. Thus, it remains that our second hypothesis is plausible.

Cows showed changes in social behaviour due to a change in accommodation, but the frequency of social interactions post transfer was greater than pre transfer, so we cautiously accept our third hypothesis. Housing cows in individual pens removed competition for feed and isolated animals from bullying, while the fence design allowed continued social interaction through sight, sound and touch (Krawczel and Lee 2019). That could explain the decrease in agonistic interactions, as cows did not need to compete for resources. Similarly, affiliative interactions also decreased in the individual pens. Cows form bonds with their preferred social partners, while avoiding other individuals (Gygax *et al*. 2010). When cows were in the individual pens, they were placed next to random neighbours, and this may have promoted the reduction in affiliative interactions among them. According to Tresoldi *et al*. (2015), both agonistic and affiliative interactions are expected to increase when the space per animal is reduced. Although our cows were restricted to individual pens, they were provided with a greater area per animal compared with the compost barn, so we can agree with the authors. In the compost barn, cows needed to quickly readapt to living with a big herd again, competing for food, water and space, and thus reestablish their social status within the group (Tennessen *et al*. 1985), as noticed by increased agonistic interactions. Thus, affiliative interactions might have increased as a strategy to reduce social tension associated with the agonistic interactions (Laister *et al*. 2011). We did not evaluate cow behaviour to assess adaptation after transfer back to the compost barn. Further research is required to confirm if cows recovered or not from the initial difficulties of reintroduction.

The redistribution of cows' eating and ruminating behaviour caused expected changes in idling and 'other behaviours' scans, with an inversely proportional relationship between them (de Oliveira *et al*. 2017), as illustrated in Fig. 1. Cows on the CON diet spent more time idling when allocated to individual pens. Meanwhile, idling time of MIX cows increased only during the early transfer. During early and late transfer phases, the restriction of full social contact with other conspecifics can help to explain the increase in idling time, when affiliative interactions were lowest. Idle state is a deviation from other activities that the cows could be engaged in, such as eating, ruminating and social interactions, and could suggest a depression-like or boredom state (Meagher *et al*. 2017). Transfer between the housing systems might have caused an increase in this state, even though the cows had space to walk around in the individual pens, and walking behaviour was similar between phases, the space was still limited. However, the effect of offering a new diet to the cows (MIX) may have disguised the effect of the transfer by displaying a novelty, as cows that received this diet were able to return to their same basal level of idling during the late transfer phase.

Overall, and despite us not measuring any indicators of physiological stress or of the cows affective state, the behavioural changes we observed suggest that cows experienced no major discomfort that could trigger stress in the individual pens and with the management used in this experiment. Changes were in fact expected, as not only the environment was changed, but also diet and routine (milking time and length) of the cows. Cows were separated in this study specifically for methane emission measurements, which could require the use of respiration chambers. The use of respiration chambers is criticised, because cows are enclosed and all their natural behaviour patterns (grazing, walking, interaction with other animals etc.) are restricted (Pinares-Patino˜ *et al*. 2011). More importantly, this could potentially generate erroneous research data (Storm *et al*. 2012). Thus, we assume that the experimental conditions used in this study did not jeopardise cow behaviour patterns to an extent that could affect the nutritional research outcomes of the methane emission measurements research.

Conclusions

The pattern of behavioural changes noticed in cows resulting from a transfer between different environments for research purposes feature cows adaptation, as nutritional and social factors were of greater influence on behavioural changes and milk production than the transfer between systems by itself. Some behavioural changes appear to be positive, such as greater resting time and the lack of competition for resources. This supports the idea that a mid-term transfer for an experimental purpose is not necessarily adverse to cow comfort. Not all the behavioural changes reverted to their pretransfer values when cows returned to the compost barn, which indicates that although cows adapted to the individual pens, they had to readapt to living in a group again.

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