

Dr. Anina Vogt

*How to reduce weaning and separation stress in dairy cow-calf contact systems?
A comparison of a gradual process with the two-step nose flap method
and an evaluation of different stress indicators*

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Early separation of dairy cows and their calves shortly after birth is increasingly questioned by society, scientists and some dairy farmers. Cow-calf contact (CCC) systems, which allow prolonged contact between calves and their dam or a foster cow, are an alternative to this practice with many benefits for the animals' welfare. However, cows and calves form strong bonds and even at organic farms practicing CCC, separation is typically done earlier than it would happen under natural conditions. The weaning and separation process is therefore often highly stressful for the animals and remains one of the biggest challenges of CCC systems from a welfare perspective and also a main reason of farmers to discontinue with CCC. This thesis examined two separation methods with potential to reduce the stress for cows and calves during the process: *a gradual reduction in cow-calf contact time (GR) and two-step weaning and separation with nose flaps (NF)*. The NF method has the advantage that it separates the loss of nursing and complete loss of contact to the cow (or calf) in time and is also easy to use, while the GR method partly mimics the typically occurring reduction of milk intake during natural weaning and allows the animals to habituate to periods of separation. The aim of this thesis was thus to compare the behavioral and physiological stress responses of dairy cows and their 3-months-old calves during weaning and separation with either the GR or the NF method. As a secondary aim, different behavioural and physiological stress indicators were evaluated regarding their expediency for weaning and separation stress in cattle.

For the main experiment, 36 cow-calf pairs were weaned and separated either with the GR method (1 week half day contact, 1 week morning contact, 1 week fence-line contact before total separation, n=18 pairs) or with the NF method (2 weeks full-time contact while calves wore nose flaps, 1 week fence-line contact before total separation, n=18 pairs). The behavioural responses of pairs were monitored via direct observation in the cow area and via video observation in the calf area, which also included the selection gate where calves could switch between areas. Locomotor play levels and lying times of calves, as well as lying and rumination time of cows, were automatically recorded with sensors. Blood and fecal samples were collected from cows and calves for analysis of relative telomere length, immune responses and fecal cortisol metabolite concentrations. The cows' milk yield was recorded twice daily during milking, and calves were weighed weekly.

For the **cows**, there was no difference between the two separation methods in any of the examined behavioural and physiological indicators. However, both methods led to an increase in vocalizations and searching behaviour, as well as to a transient increase in physiological stress markers compared to baseline, indicating that both methods provoked stress for the cows. For the **calves**, the abrupt milk loss through the nose flap caused an energy deficit and likely compromised adaptation of the gastrointestinal tract (GIT) to the dietary change, as suggested by low weight gains, reduced play behaviour and an increase in inflammatory blood markers compared to baseline. Furthermore, a marked decrease in lying and play behaviour while wearing the nose flap, along with a high number of unsuccessful suckling attempts, pointed towards a negative emotional state of calves weaned with a nose flap. In contrast, we

saw higher weight gains, a lower decrease in lying bouts, as well as a lower decrease in locomotor play levels during the weaning process in GR calves when compared to NF calves, indicating a better adaption of the GIT as well as a presumably less compromised affective state with the GR method. In addition, all NF calves showed pressure marks and some even injuries at the nasal septum when the nose flaps were removed after the 14 days of usage. Video observations of the selection gate between the cow and calf area revealed, that it took 4 days after insertion of the nose flaps until the calves significantly reduced the time they spent in the cow area. Afterwards, there was no further significant reduction of the time spent in the cow area, as it remained at a constant lower level. This suggests that 3-month-old dairy calves need to wear a nose flap for at least 4 days in order to effectively reduce their suckling motivation, while there seems to be no benefit of using a nose flap much longer than this time. It could not be determined whether nasal septum injuries were already present in the nostrils of our NF calves after 4 days of usage, but similar injuries were reported in 8-month-old beef calves after 5 days with the nose flaps.

Given the overall benefits of the GR method for the calves and the missing difference between methods in cows, a gradual weaning and separation process is recommended for reduction of weaning and separation stress in CCC systems. Nonetheless, gradually weaned and separated pairs also showed strong behavioural responses indicative of stress, especially in response to introduction of fence-line contact in the third week that completely terminated nursing. This was probably caused by the fact that the removal of afternoon contact in the second week of the GR method led to no considerable reduction of calves' milk intake and also caused no relevant disruption of established nursing routines, as indicated by the similar evening milk yields of GR cows, as well as the similar amount of nursing behavior of GR pairs in the weeks with half-day and morning contact. Consequently, the GR method as implemented in the present thesis, worked in a rather stepwise than gradual manner and could also not achieve the intended low-stress separation of pairs. Recommendations for refinement of the GR method are given in the thesis and include, among others, the need to truly reduce the milk intake of calves along with the contact time and to focus more on loosening the established daily routines rather than on the reduction of total daily cow-calf contact time per se.

Regarding the tested stress indicators, results of this thesis showed that a low frequency of calves' vocalizations during weaning cannot be taken as evidence of low stress levels, if this is not backed by further indicators. Relative telomere length as sampled in the present thesis was not a valid indicator of the cumulative separation stress over the whole weaning and separation process, while fecal cortisol metabolite concentrations were found to be a generally valid stress marker for (unweaned) dairy calves, but unsuitable for comparison of pre- and post-weaning states.

In conclusion, this thesis provides valuable new insights for the further development of a low-stress weaning and separation procedure for CCC systems, guiding practical applications as well as future research on this topic.

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