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Assessing Adrenocortical Activity in Dairy Cows During Simulated Power Cuts in Milking Robots

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ABSTRACT: The effects of unsteady electricity supply (power cuts) to an automatic milking system (AMS) on cattle behaviour caused by load management, are crucial for the success of the future farming technologies summarized by "Integrated Dairy Farming –Stable 4.0". The influence of simulated power cuts on adrenocortical activity was assessed by a non-invasive stress monitoring method determining faecal cortisol metabolites (FCM).

This study was carried out on four commercial dairy farms in Bavaria, Germany, from March to August 2014. All farms were using 1 Lely AMS for about 60 dairy cows. 12 adult Fleckvieh, aged from 2 to 8 years (average 3.9) were randomly selected per farm. They had between 1 and 7 lactations (average 2.4). The cows under observation were given 6 days to get used to a daily routine. On days 7 to 9 baseline faecal samples were taken. On days 10 to 13 a power cut was simulated daily. Samples were collected manually from the rectum approximately 10 to 12 hours after the simulated power cut and another one approximately one hour later. The amounts of FCM were determined via an 11-oxoaetiocholanolone enzyme immunoassay, measuring 11,17-dioxoandrostanes. All data were initially collected with the program Excel 2010 and evaluated with R version 3.0.1.

Per farm, no differences between baseline and test days were detected (Kruskal-Wallis, all p-values > 0.1), thus FCM values indicated that cows were not stressed. There was no significant correlation between measured FCM values and the difference between expected and actual milk yield, with the exception of one farm, where actual milk yield was lower than predicted.It was not possible to show that a short malfunction of AMS causes stress in dairy cows. Nevertheless, new questions arose which can be elaborated in additional studies.

Keywords: Automatic milking system, faecal cortisol metabolites, milk yield, Stable 4.0, stress.

I. INTRODUCTION

In the future, highly mechanized and automated dairy barns will increasingly require energy in form of electricity and heat. To achieve a high proportion of self-produced electricity and to keep the load on the public electricity network as low as possible, the project "Integrated Dairy Farming - Stable 4.0" was created (figure 1).

Figure 1: Overview "Integrated Dairy Farming"

The concept "Integrated Dairy Farming - Stable 4.0" describes the idea of a comprehensive sustainable stable (Höld et al., 2015). It contains automatic production technology as well as regenerative energy resources and intelligent power grid connectivity (figure 2).

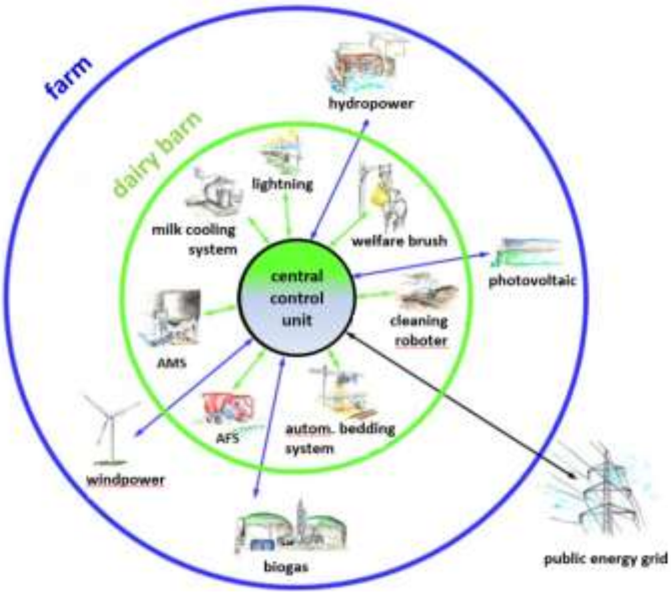

Figure 2: Energy management system (Höld, 2015)

Even nowadays, farms run by own family staff are trying to reduce physical work by greater flexibility through the acquisition of a milking implement which allows an animal individual milking. The result is a progressive increase in mechanization and automation, especially in Bavarian dairy farms. In 2015, 1,577 Bavarian farms were already using AMS (LKV Bayern, 2015). Applying automatic milking systems not only leads to organizational adjustments but also requires adapting energy supplies. The energy required must be available for 24 hrs/day.

Although surveys have shown that Europe provides quite a stable power network, there have been diverse irregularities in supply in different countries of the EU (Roon and Buber, 2013). The last significant power cut, on November 25th 2005 in North Rhine-Westphalia, Germany, left 250,000 people without power for up to four days; on November 4th, 2006 ten million people in vast parts of Europe were without power for two hours.

Parallel investigations of the relevant animal technology interactions assess the influence of automated technology on animal behaviour. Various effects of a temporary power cut to the automatic milking systems (AMS) on animal behaviour were analysed. In this study, using a non-invasive stress monitoring method (Palme, 2012), the activity of the adrenal cortex has been evaluated by measuring faecal cortisol metabolites (FCM).The objective of the project "animal/machine interaction" within the concept "Integrated Dairy Farming - Stable 4.0" is to quantify potential stress reactions of dairy cows to energy-related failures of milking robots (Höld et al., 2016). To detect stress reactions, also heart rate and heart rate variability rumination, movement and behaviour were recorded and evaluated. In this study, FCM concentrations of 12 arbitrarily selected focus cows out of the cattle of four again arbitrarily chosen farms were analysed before and after a simulated power cut. In addition the milk yield prognosis of the milking robot was compared with the actually extracted milk quantity.

II. Materials And Methods

2.1 Farms and Animals

This study was carried out on four different commercial Bavarian dairy farms using a Lely Astronaut milking system from March 2014 to August 2014. The number of milking cows varied between 52 - 71 animals on each farm (figure 3).

Farms	B1	B2	B3	B4
Numberofdairycows	64	71	68	52

Figure 3: Number of dairy cows on analysed farms

Note, that all farms are considerably different in the management of the cattle. For technical reasons, on each farm 12 lactating Fleckvieh cowswere selected randomly for the experiment by taking each forth cow from the increasingly order list of ear numbers to test their adrenocortical responsiveness. At each farm, both lactating as well as pregnant animals were observed. The number of lactation periods was in the range of one to seven lactations, on average 2.4 lactations. The age of the cows was between two and eight years. The average age was 3.9 years.

2.2 Basal measurements, simulated power failure

The total duration of the experiment was 13 days on each farm. For six days (day 1 to 6) before data recording the animals had to wear a chest strap for measuring their heart activity so that they could get used to it and to the whole experimental procedures (habituation).The period of data collection (figure 4) on each farm was seven days (day 7 to 13), split into three days of basal measurement (day 7 to 9) and four days of test measurement (day 10 to 13).

Figure 4: Experimental design

The basal measurement detected the condition of the animals without the influence of an energy-related failure of the AMS. For the actual test measurement the usual milking behaviour of the focus cows was taken into consideration to inhibit the milking permission of the respective focus cow once within 24 hours for two hours (block time).

On the second day of testing the milking block time was extended to four hours to increase the likelihood of an AMS-rejection. On the next two test days the milking permission was set to two to four hours in order to keep the udder burden as low as possible and the milk yield steady.

2.3 Adrenocortical activity (concentration of faecal cortisol metabolites), milk yield

In this study, as in other studies (Bertulat et al., 2013), concentrations of a group (11,17-dioxoandrostanes) of faecal cortisol metabolites (FCM) were used for evaluation as an indirect stress parameter, (Palme and Möstl, 1997; Palme et al., 1999 ; Möstl and Palme, 2002).

FCM in cattle are excreted about ten to twelve hours after the actual stress event. Therefore, the faecal samples were taken individually between ten and twelve hours after the simulated power cut The FCM concentration reflects the cortisol secretion from the respective time before (Palme et al., 1999). Pursuant to Palme (2005) the faeces was bottled from various points of the glove into tubes. As the concentration of FCM samples can alter during storage at room temperature (Möstl et al., 2002), the tubes were immediately cleaned with cold water after removal and stored in a freezer box. The samples were stored at minus 20°C until steroid extraction which was performed with 80% methanol as described before (Palme et al., 2013). FCM were analysed with an 11-oxoaetiocholanolone enzyme immunoassay previously validated for use in cattle (Palme and Möstl, 1997; Palme et al., 1999).The data "milk" and "milk yield expected" were given by the company software program of Lely Holding S.à.r.l of the Astronaut milking system (Lely, n.d.).

2.4 Statistical analysis

All data were initially collected with Excel 2010, displayed graphically and evaluated using "R" (R version 3.0.1, The R Foundation for Statistical Computing). Since all farms are very different in the management, each farm was analysed individually. To assess the influence of the simulated power cuts, the difference of the second sample and the first sample was taken, so in case of an increase of the FCM the value should be significantly greater than zero. The days of both, the basal and power cut period, were treated as factors for an univariate analysis of variance. The Shapiro-Wilks-Test indicated for all farms normal distribution ($P > 0.1$), apart farm 4 ($P = 0.008$). The Bartlett-Test indicates the homogeneity of the variances for the samples in all farms ($P > 0.09$), apart farm 1 ($P = 0.03$). To treat all farms in the same way, the Kruskal-Wallis-Rank-Sum-Test was applied with the days of the FCM measurements as factor. Interactions between the factor farm and the factor day were excluded a priori, so the difference in the farms could be examined separately. Assuming the normality of the samples – which holds for all farms except farm 4 – the 95 % confidence intervals for the differences were calculated to apply the duality of confidence interval and hypothesis testing for the comparison of the differences. For the dependency of the difference in expected milk yield to the milk yield predicted by the milking robot, again each farm was analysed on its own, assuming an univariate linear dependency, since in this case all possible factors of possible influence are summarized in the change of FCM.

III. RESULTS AND DISCUSSION

The results indicate no differences in the differences of the FCM measurements for all farms (P > 0.4). Looking at the differences in the farms and excluding a priori interactions between farm and day and assuming the normality of the samples – which holds for all farms except farm 4 – the 95 % confidence intervals for the differences are [0.26, 2.1] for farm 1, [0.38, 1.67] for farm 2, [-0.32, 2.65] for farm 3 and finally [-9.2, 9.35] for farm 4. To conclude, in farm 1 and farm 2 FCM were generally elevated, whereas in farm 3 and 4 no significant difference was expected. The reasons therefore are not obvious. A possible cause could be diversities in the herd management.

Thus a two to four hour power cut did not lead to detectable stress via FCM on AMS milked dairy cows. No significant correlation between the measured FCM values and the differences between predicted and actual milk yield was found. Only one farm showed that the actual milk yield after the power cut was lower than predicted.According to Palme (2012), faecal samples should be taken as fresh as possible and immediately cooled in order to slow down a possible metabolism by bacterial enzymes (Palme, 2005).In these experiments, the samples were fresh as they were directly removed rectally, then immediately cooled down, yet transport of the samples took significantly longer than 30 minutes. This might have had an adverse effect on the results obtained.

To avoid such interference during a following experiment the samples could be treated with alcohol immediately after removal (field test) to impede the bacterial metabolism. This could be a possible method to stabilize the metabolites, before performing the actual measurement (Palme et al., 2013).The experiments were conducted in spring and summer 2014. In August there was a significant rise of temperature. This could have had an influence on the FCM values. This was also found in the studies of Zähner et al. (2001) who discovered the impact of extreme climatic changes on cortisol concentration.To study the influence of stress on the milk yield, the difference of the expected and actually obtained milk yield, as well as the difference of the FCM concentration was used (figure 5).

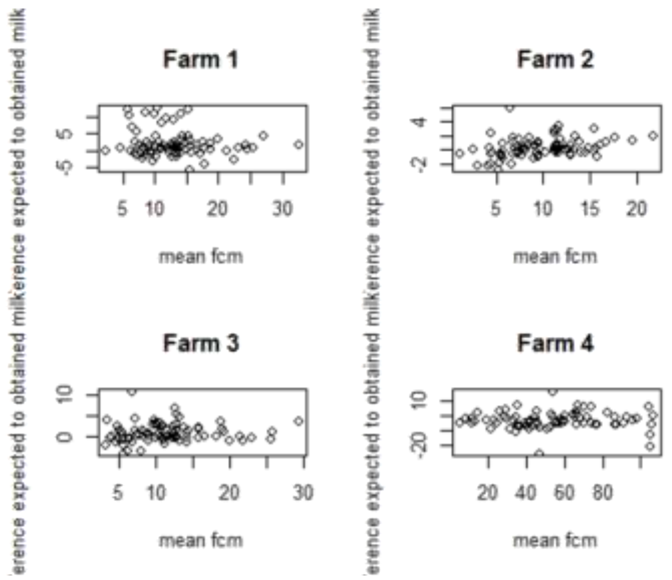

Figure 5: Relation between the FCM in [ng/g] and the difference between the expected and observed milk yield in [l/d]

So, if the difference between the expected and observed milk yield is positive, the expected milk yield is overestimated whereas in the case of a negative difference the expected milk yield is underestimated. It can be observed, that there is no linear dependency, since R2adjusted is throughout all farms smaller than 0.02 and all slopes are not significant (P > 0.1). The intercepts are all significant (P < 0.02) different from zero: for farm 1 the value is 1.97, for farm 2 0.36, for farm 3 0.9 and finally for farm 4 -3.1, which indicates an overestimation of the expected milk yield in the farms 1 to 3 and an underestimation in farm 4.

IV. CONCLUSIONS

The subproject "animal/technology interactions" should show a combined evaluation of this study and all other test results within the concept "Integrated Dairy Farming - Stable 4.0". Potential stress reactions of dairy cows caused by an energy-related failure of milking robots should be presented. The test results should give an explanation for whether variations or failures in the energy supply have effects on the cow behaviour or whether postponements in the usual daily routine, e.g., in the milking routine, cause stress reactions to the milk cows. Therefore, ethological criteria can be validated in a quantitative and qualitative analysis. Moreover, the behaviourpatterns—appropriate for animal species in an increasingly automated process of engineering in a dairy farm, can be evaluated while taking autonomous control options within the scope of a comprehensive on-farm-energy-management system into account.

Stress reactions can be detected via various hormones, by different physiological and/or behavioural parameters and therefore give information on animal welfare. The analysis of FCM is an effective way to evaluate stress, because collecting faeces samples is not influencing the cortisol values by the stress of an invasive sampling itself (for example, from the blood) (Palme and Möstl, 2001). This study attempted to consider FCM concentrations as part of those criteria. Certainly, a linking of all results of "Integrated Dairy Farming - Stable 4.0" is still required.

To summarize, it was not possible to show via FCM concentrations that a short malfunction of an AMS (up to four hours) caused detectable stress reactions in dairy cows. However, it must to be considered that the power cut was simulated for only 12 cows per farm. The consequence of a real power cut would be a lot worse, as this would affect all animals of the herd and not only a dozen focus cows. Nevertheless, new questions arose which can be elaborated in additional studies.

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Author contributions

AG designed the experiment with the assistance of HB. AG carried out the data collection. AG and HP analysed the data. RP contributed reagents and analysis tools. AG and HP wrote the paper. RP and HB criticallyreviewed and commented on the manuscript.

Ethical Approval

This manuscript does not contain experimental manipulation of the study animals by any of the authors; all samples and observations were collected noninvasively.

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