EFFECT OF ENVIRONMENTAL FACTORS ON STRESS INDICATING PARAMETERS IN CATTLE AT THE SLAUGHTERHOUSE

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Abstract: Farm animals experience various stressful situations, of which slaughter is a particularly sensitive event. Improvement of conditions during slaughter are essential to ensure good animal welfare.

We identified potential problem areas through guided interviews with slaughterhouse employees and a situation analysis at a Swiss cattle slaughterhouse. After implementation of improvement measures, data from 503 cattle slaughtered in August 2017 were evaluated with regard to the stress-indicating parameters cortisol content in exsanguination blood and proportion of vocalizing animals compared to data of a former study in 2012.

The mean cortisol content (3.50 ug/dl) was uncritical and significantly influenced by the driving person and the day. Whereas the high proportion of vocalizing animals of 19.1% was critical. The behaviour of the driving person was also decisive here.

We conclude that employee training is the key to further reducing stress-inducing situations during slaughter.

Introduction: Farm animals experience various stress-triggering situations of which those linked to slaughter are particularly sensitive. Cortisol levels in the blood are often used to measure physiological stress (Gruber et al., 2010), and were reported to increase with an increasing number of human prods (Hemsworth et al., 2011). Vocalization is a suitable indicator for measuring the affective state of cattle (Grandin, 1998), and is correlated with physiological stress levels (Hemsworth et al., 2011).

Our aim was to identify problem areas and to check if implementation of improvement measures at a Swiss cattle slaughterhouse had a positive effect on the stress indicators cortisol content in blood and vocalization frequency. Results were compared with those of an earlier study conducted at the same slaughterhouse (Probst 2013). We also checked if factors identified by slaughterhouse staff in interviews had an influence on the two stress indicators.

Material and methods: Interviews and Animals
In order to identify critical areas and derive improvement measures, guideline based interviews were carried out in June 2016 with all employees of the slaughterhouse who drive the animals to the stunning box (n=5, 10-15 minutes/ interview).
Based on the evaluation of the interviews and on our own observations, the following measures to improve slaughter conditions were proposed in spring 2017:
- Mounting a mirror in front of the stunning box
- Elimination of irregular light rays on the floor in the gangway
- Reduction of the number of animals (from two to one) in the area before the stunning box in order to reduce gate contacts because of little space
- Brighter illumination of the rearmost division of the waiting area

Following the implementation of these measures, surveys were carried out from 21 to 25 August 2017, to investigate the influence of the changes on stress-indicating parameters in 10 months old Natura Beef labelled cattle (n=518). The animals were driven to the stunning box with a rubber stick or an electric driver by the slaughterhouse employees. Each animal was individually locked into the stunning box. A sample of 10 ml blood leaving the neck section during exsanguination was taken from each animal and its cortisol content (ug/dl) in serum was determined at the University of Zurich using the electrochemiluminescence immunoassay method (Documentation Roche, E170).

**Statistical analysis**
The influence of the explanatory variables slaughter day (1, 2, 3, 4 or 5), driving person (person 1, person 2 or person 3), use of rubber stick (yes or no), use of electric driver (yes or no), contact with the gate in the drive gear (yes or no), contact with the gate in the area of the stunning box (yes or no), occupation of the area in front of the stunning box with more than one animal (yes or no) and occurrence of turn around trials (yes or no) on vocalization frequency and on cortisol content (ug/dl) in the exsanguination blood was examined on complete observations of 503 animals in R (R Core Team, 2018, Version 3.5.1). Explanatory variables were selected by stepwise backward regressions using the Akaike information criterion in the MASS package. Driving person and the day of slaughter remained as explanatory variables in the model for cortisol content. For vocalization frequency a logistic regression model for binomially distributed data was calculated, in which the explanatory variables driving person, drive with an electric driver and the interaction between drive with a stick and contact with the gate in the area of the stunning box were found to be significant. Post-hoc tests were calculated as Tukey contrasts with the lsmeans package. The statistical significance level was assumed to be $P < 0.05$.

**Results:** The median of the cortisol content in slaughter blood was 3.24 (mean value 3.50 ug/dl). Only the driving person and the slaughter day had a clear influence on the cortisol content (Figure 1).

On average 19.1% of the animals vocalized. Although the influence of the driving person on the proportion of vocalizing animals was significant over the whole model, the results of the post hoc tests between the driving persons 2 and 3 ($P = 0.054$) and 2 and 1 ($P = 0.097$) showed only a tendency in favour of person 2, while person 1 and 3 did not differ significantly ($P = 0.653$).

Animals vocalized significantly more frequently when an electric driver was used (with electric driver: LSM 22.8% ± 5.4% SE vs. LSM 11.3% ± 2.7% SE without electric driver).

Most animals (75.5%) were driven with a rubber stick, while an electric driver was only used in 19.5% of the animals. In the drift area less animals had gate contact compared to the stunning box area (6.4% vs. 19.5%, respectively). Animals with gate contact in the stunning box area were more often driven with a stick (15.1% with stick drive compared to 4.4% without stick drive had gate contact). However, cortisol content in the slaughter blood of the animals with or without gate contact did not differ significantly.
Less than a quarter of the animals (23.1%) were not alone in the area in front of the stunning box. Single animals had significantly more gate contact compared to animals with a companion (14.5% versus 5.0%). Neither cortisol content of the blood, nor vocalization frequency were significantly affected by this parameter. A proportion of 13% of the animals tried to turn around in the gangway. However, no statistically verified influence of this behaviour on the stress indicators could be determined.

**Discussion:** The mean cortisol content we found was clearly below the mean value of > 7.0 ug/dl described as stress indicator (Grandin, 1997). It was comparable with the results of Probst (2013) for 10-month-old Natura Beef cattle (median: 3.22 ug/dl, mean: 3.59 ug/dl). In agreement with our findings, Probst (2013) also found no statistically verified influence of the drive with electric driver on the cortisol value, whereas Hemsworth et al. (2011) could establish a correlation between the increase in the cortisol content and increasing numbers of electric prods. Results on driving equipment indicate a shift in driving device, as in a former study 75.5% of the animals had been driven with an electric driver (Probst 2013).

Proportion of vocalizing animals in our study was higher than in the 13% reported by Probst (2013). This situation is not satisfactory, since more than 5% vocalizing animals are interpreted as a sign of problems at the slaughterhouse (Grandin, 2001). By contrast to our finding, Probst (2013) found no statistical impact of number of driving events on vocalization frequency.

The fact that we could not establish difference in cortisol level in animals with or without gate contact might be due to the fact that the time between gate contact and a traceable impact on the cortisol level was probably too short. Although the reduction in the number of animals in the area before the stunning box was successfully implemented, gate contact was not reduced. The usefulness of this measure is therefore questionable. In particular because the slaughterhouse employees reported fewer stress reactions in accompanied animals compared to those being alone. Overall, we conclude that the measures taken probably had a slight stress-reducing effect. However, the situation with regard to the proportion of vocalizing animals remains critical. Reduced use of electric drivers could have a positive effect here. Since the influence of the driving person, who also decides on the use or non-use of the electric driver, is great, the training of employees is to be regarded as key factor.

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Probst, J.K. 2013 Stress reduction in slaughter cattle by improving the human-animal relationship. Diss ETH No 21357.
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