Short Communication

A note on the physiological and behavioural consequences of cannibalistic toe pecking in laying hens (*Gallus gallus domesticus*)

Bemerkungen zu den Konsequenzen von kannibalistischem Zehenpicken auf die Physiologie und das Verhalten von Legehennen (*Gallus gallus domesticus*)

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Manuskript eingegangen am 1. Juli 2010, angenommen am 31. Juli 2010

Introduction

Feather pecking and other types of allopecking have been examined in many studies (JENSEN et al., 2008; ALBENTOSA et al., 2003; Buitenhuis et al., 2003; Leonard et al., 1995; HUGHES and DUNCAN, 1972). Cannibalistic pecking which is directed to the rear parts of conspecific bodies can lead to serious injuries, stress and even to death (e.g. CRAIG and LEE, 1990). However, less is known about toe pecking, which also can be regarded as a type of cannibalistic allopecking behaviour in fowl. Toe pecking is in general assumed to be most likely caused by damages to the feathers or from blood at the feet (Buitenhuis, 2003). It may also be that toes from elevated perching hens stimulate conspecifics to peck on the toes (WALSER, 1997). Once toe pecking has resulted in toe injuries, these injuries act as a trigger for more toe pecking (Buitenhuis, 2003). Toe pecking in laying hen flocks occurs less often than feather pecking or cloacal cannibalism (Buitenhuis et al., 2003; Leonard et al., 1995). This low incidence of toe pecking makes it more difficult to study it empirically.

Toe pecking is a potential threat to animal welfare. Often very serious injuries occur immediately with bleeding and tissue loss (Buttenhuis, 2003). Long term consequences on health and behaviour of victims of toe pecking have, to our knowledge, not been investigated so far.

Therefore, we studied physiological and behavioural responses of adult laying hens which had been exposed to toe pecking for several weeks. We expected that toe pecking victims would show more physiological stress in comparison to control hens, manifested in increased weight of the adrenal glands (Carsia and Weber, 2000; Höhn, 1983) and increased blood cell heterophil to lymphocyte (H/L) ratio (Gross and Siegel, 1983; Beuving et al., 1989). We further expected toe pecking victims to be more fearful by having longer tonic immobility (Jones and Waddington, 1992; Albentosa et al., 2003) and longer latency to leave a platform in a modified step down test (Krause et al., 2008) than non pecked control hens.

Materials and Methods

Fifty weeks old Lohmann Selected Leghorn laying hens were subjects in the present investigation. They were housed in five adjacent litter floor pens with ad libitum access to food and water. Hens were kept in groups of 7-9 birds in each pen. All compartments were 1.6m wide and 3.8m long and littered with wood-shavings (maximal stocking density: 1.48 hens/m²). Perching facilities (1.8 m perch per pen in a height of 0.15-0.6 m) were provided. The light-dark rhythm was 16L:8D hours. Hens were kept in the compartments as groups from an age of 20 weeks on. The hens were bought from a commercial breeder where they were reared in cages from day old until transfer to the experimental pens at 20 weeks of age. In one of the compartments toe pecking began 15 weeks prior the experiments, i.e. at an age of 35 weeks. Toe pecking persisted in this pen until the experiments were conducted. All individuals in this pen had injuries at toes and feet (Figure 1). Conspecific toe pecking (one hen pecking the toes of another) was occasionally observed during the daily visual inspection whereas allo toe pecking (a hen pecking own toes) never was recorded. Beside the injuries at the toes no other injuries or damages from e.g. feather pecking were visible. Seven toe pecking victims (TPV, N_{TPV} = 7) from this pen were used for the experiments. Control hens (C, $N_C = 8$) were taken randomly from the other pens, two from each. Control hens had no injuries at toe and they have never been seen to toe peck or receive toe pecking, they had also no further injuries or damages. The control pens were visually separated from the pen where TP has appeared. All hens were tested in two behavioural experiments. A Step down test (SD) where hens were placed individually on an unfamiliar elevated (3 cm) platform with a grid in front, modified from a step down avoidance experiment (Krause et al., 2008). Latency to leave the platform and step down on the grid was recorded. Secondly, a tonic immobility test (TI) was conducted one week later. In the TI test the latency to stand was recorded (ALBENTOSA et al., 2003). Directly after the TI test hens were weighed and blood was collected (2µl/per hen). Blood was smeared on a glass slide and was stained using a Pappenheim staining (May-Grünwald/Giemsa, Gross and Siegel, 1983). On each slide one hundred leucocytes, including heterophils, lymphocytes, monocytes, basophils and eosinophils (BEUVING et al., 1989) were counted and the H/L ratio calculated (Gross and Siegel, 1983; Beuving et al., 1989).

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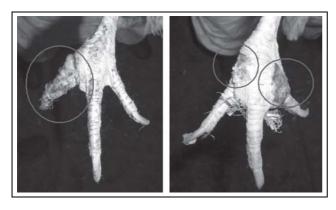


Figure 1. Injuries resulting from conspecific toe pecking at a) a single toe and and b) at the entire foot. Verletzungen welche durch Zehenpicken von Artgenossen an a) einer einzelnen Zehe und b) dem gesamten Fuß verursacht wurden.

Subsequently, hens were euthanized and both adrenals were dissected and weighed. A pair of adrenals from one TPV hen was lost during preparation. The relative weight of each adrenal gland was calculated (g adrenal gland/kg bodyweight). Three slices from each adrenal gland were stained using an adapted protocol of Masson Goldner. Slices were photographed (AxioCam camera, Carl Zeiss, Jena, Germany) and the sizes of adrenal gland cell area (A) and interrenal cell area (I) were measured (AxioVision LE Rel4.3, Carl Zeiss, Jena, Germany). We calculated the I/A ratio, to examine whether the celluar composition of the adrenal glands was affected. From four hens only slices from a single lateral side were obtained for the cellular analysis.

Statistical analysis

Adrenal gland weight was analysed using a linear mixed effect model (LME) with treatment (TPV, C) and side of adrenal gland (right, left) as fixed factors. Hen ID was included as random factor in the model. H/L-ratio from blood smears were analysed using independent t-tests. Behavioural data were analysed using non parametric Mann-Whitney U-test. The statistical analysis was run using R 2.9.0 and SPSS 17.0.0.

Results

Physiological parameters

The relative adrenal gland weight was significantly increased in the TPV hens over that of C hens, on average at 34.5%. In all hens the left adrenal gland was larger than the right (LME, Treatment: $F_{1,12}$ = 5.01, P = 0.045, lateral side: $F_{1,13}$ = 5.74, P = 0.034; Figure 2a).

Treatment and lateral side main effects did not significantly affect the I/A-ratio (LME treatment $F_{1,13}$ = 0.04; p = 0.84; lateral side $F_{1,60}$ = 0.33, p = 0.57, but the interaction of treatment and lateral side had a significant effect (LME, treatment * lateral side $F_{1,60}$ = 5.32; p = 0.025; Figure 2b).

Results from blood smears showed no significant differences in the H/L ratio (independent t-tests, T_{13} = -0.20, all P = 0.84) between TPV and C hens. The body weight of TPV hens (mean 1.55 kg \pm 0.17 SD) was not significantly different from C hens (mean 1.65 kg \pm 0.10 SD) (independent t-test, T_{13} = -1.34, P = 0.21).

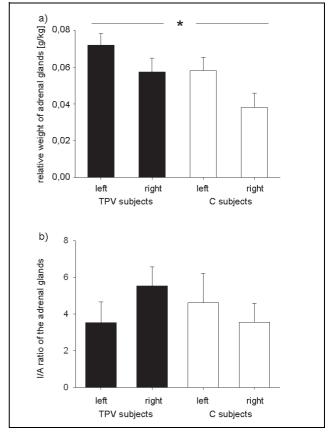


Figure 2. a) Relative weight of adrenal gland (g/kg body weight + SE) as affected by treatment and laterality. b) Ratio of interrenal to adrenal cell area (I/A-ratio) of the adrenal glands of toe pecking victims (TPV) and control (C) hens, from left respectively right adrenal gland.

a) Relatives Nebennierengewicht (g/kg body weight + SE) welches von den Versuchsbedingungen und der Lateralisation beeinflusst wurde. b) Verhältnis der interrenalen und adrenalen Zellbereiche (I/A-Verhätlnis) der Nebennieren von Zehenpick-Opfern (TPV) und Kontroll-Hennen (C) für die linke und rechte Nebenniere.

Behavioural recordings

In the Step Down (SD) test, the latency to step down from a platform was significantly shorter for the TPV hens compared to C hens (U-test, Z = -2.64; P = 0.008; Figure 3). In the TI-test no significant difference was found between TPV and C hens (U-test Z = -1.74; P = 0.083).

Correlation between physiological and behavioural parameters

The SD time from all hens was significant negatively correlated to the mean relative weight of the adrenal glands (N = 14, $R_S = -0.54$, P = 0.045).

Discussion

Toe pecking is a type of cannibalistic allopecking behaviour in domestic fowl that has received relatively little attention. In the present investigation physiological and behavioural parameters were found to differ between toe pecking victims compared to control individuals, namely enlarged adrenal glands, as well as a shorter time to leave an elevated platform in a step down test. The increased

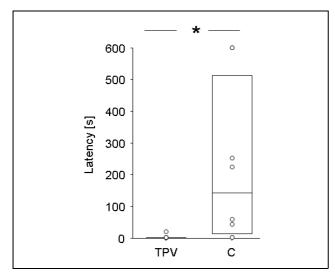


Figure 3. Latency (s) to leave an elevated platform in a step down test in victims of toe pecking (TPV) and control hens (C). Data points are indicated by circles, some data points overlap. Box plots indicate 25% quartile, median, and 75% quartile. Significant difference (P < 0.05) indicated by an asterix. Latenz (s) zum Verlassen einer erhöhten Platform in einem `step down' Test von Zehenpick-Opfern (TPV) und Kontroll-Hennen (C). Die einzelnen Datenpunkte werden von den Kreisen dargestellt, einige Kreise liegen übereinander. Der Box Plot gibt das 25% Quartil, den Median und das 75% Quartil an. Signifikante Unterschiede (P < 0.05) sind durch ein Sternchen markiert.

relative adrenal gland weight in toe pecking victims is an indicator of stress (Carsia and Weber, 2000). It is known that frequent exposure to stressful situations can increase adrenal gland weight (HÖHN, 1983). The weight of the adrenal glands is positively correlated to the adrenal-cortical production (Höhn, 1983) and corticosterone is one of the main steroids secreted by the avian adrenal gland. Besides increased corticosterone production, an increase in adrenal gland cell area can lead to increased adrenaline secretion which may induce or increase stress. We also have hints from the I/A-ratio that the cellular structure of the adrenal glands is altered from exposure to toe pecking. From the literature it is known that hyperplasia is the most likely underlying mechanism for such changes in the cellular structure which lead to adrenal gland enlargement (HÖHN, 1983). To clarify the finding on cellular level further studies will be needed.

The physiological stress reactions from toe pecking are also reflected in behavioural responses of toe pecking victims. The results from the SD test may indicate that toe pecking victims react with more arousal to the handling and to the experimental situation. It may also be that the platform in the SD test did not represent a safe area. If toe pecking victims have received toe pecks while perching in the home pen, they may not regard elevated areas as safe. Thus, a shorter latency for leaving the platform can be interpreted as an expression of a higher level of fear (fear of being toe pecked from conspecifics).

Conclusion

In conclusion, toe pecking has a negative impact on important parameters of stress physiology and might also induce fearful behaviour in the victims. Toe pecking seems to have a lower incidence than feather pecking and cannibalism and thus probably induces a lesser problem to the egg industry. Nevertheless it is still a very considerable threat to the welfare of affected animals. Taken together, toe pecking is an important welfare issue and ought to get more attention in future research on laying hen welfare.

Acknowledgement

We would like to thank Annemieke Looienga, Erika Nußbeck and Silke Werner for help and Lars Schrader for supporting the study. Werner Bessei provided helpful comments on the interpretation of the results.

Summary

Feather pecking and cloacal cannibalism are damaging allopecking behaviours well known and studied in the domestic chicken. They often lead to serious injuries or even death of the victims. Toe pecking is a less common phenomenon and much less is known about the potential effects on physiology and behaviour in victims of this type of allopecking. In the present study we found that exposure to toe pecking led to significantly enlarged adrenal glands which indicate increased physiological stress. The behaviour of toe pecked hens was also affected in that these hens stepped off a raised platform more quickly than Control hens, possibly indicating increased fearfulness of raised areas. Our results indicate that when toe pecking occurs in groups of hens, it represents an important welfare issue and more emphasis should be put on finding ways to reduce its occurrence.

Key words

Adrenal gland, cannibalism, chicken, H/L-ratio, stress response, toe pecking

Zusammenfassung

Bemerkungen zu den Konsequenzen von kannibalistischem Zehenpicken auf die Physiologie und das Verhalten von Legehennen (*Gallus gallus domesticus*)

Federpicken und Kloakenkannibalismus sind weithin bekannte und gut untersuchte Verhaltensweisen in Legehennen, die zu Verletzungen der Tiere bis hin zu ihrem Tode führen können. Zehenpicken ist hingegen ein weniger häufiger auftretendes Verhalten, über dessen Folgen auf Physiologie und Verhalten der Opfer wenig bekannt ist. In der hier vorliegenden Studie konnten wir zeigen dass Tiere die Zehenpicken von Artgenossen ausgesetzt waren eine signifikante Vergrößerung der Nebennieren aufweisen, was ein Indikator für physiologischen Stress ist. Des Weiteren war auch das Verhalten der an den Zehen gepickten Tiere in einem Step Down Versuch signifikant beeinträchtigt. Unsere Ergebnisse zeigen das Zehenpicken ein ernstzunehmendes Problem für die Gesundheit und das Wohlergehen von Legehennen ist. Es besteht die Notwendigkeit weitere Studien zu dieser Problematik durchzuführen.

Stichworte

Nebenniere, Kannibalismus, Legehennen, H/L Verhältnis, Stress Reaktion, Zehenpicken

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Conference Information

XIV European Symposium on the Quality of Eggs and Egg Products and

XX European Symposium on the Quality of Poultry Meat

Organized by working groups 4 'Eggs' and 5 'Poultry Meat' of WPSA European Federation will take place from 4-8 September, 2011 in Leipzig, Germany. For more details, please visit the official homepage www.eggmeat-2011.de. The scientific program with keynote presentation is displayed under page Program/Scientific Program.

Please consider that the <u>deadline for early-bird registration is 15 May, 2011</u>. Details can be found on the page Registration.

I would be happy to meeting many of you at the CCL in Leipzig!

Best regards, Michael Grashorn Chief Organizer EggMeat Symposia 2011