Dimethyl Anthranilate Based Repellents Affect Cage Pecking and Feather Condition of Laying Hens

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Summary

Knowledge on the usage of taste deterrents (i.e. repellents) and its association with feather pecking is limited and studies of reduction of feather pecking in commercial flocks of laying hens have not been performed previously. In this study we examined the effect of two dimethyl anthranilate (DA) based repellents on plumage condition and behaviour of 180 non-beak-trimmed laying hens housed in enriched cages (10 birds/cage) with an emphasis on feather pecking. Birds were divided into 3 groups of 60 birds each. From 20 to 40 weeks of age they were sprayed at two-week intervals with 300ml of distilled water (control group – group "C"), a water solution of DA (group "T") and a propylene glycol solution of DA (group "P"). Hens' behaviour was recorded by direct observation for 3 days (one, six and 13 days after spraying) in each of the two observation periods starting at hens' age of 26 and 38 weeks. Feather condition of individual hen was recorded at 20, 26 and 38 weeks of age. Both repellents reduced cage pecking significantly ($p<0.05$) compared to the group C. Even though there was no significant difference in feather pecking between groups, the plumage condition of the repellent-treated birds was poorer ($p<0.05$) than that of the group C. This study was the first to investigate the potential of repellents to discourage feather pecking in a commercial setting.

Key words

taste deterrent, feather pecking, behaviour, animal welfare, poultry

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Received: May 1, 2017 | Accepted: August 10, 2017

ACKNOWLEDGEMENTS
This study is a part of the national project (Animal welfare including health of poultry and pigs in conventional and alternative housing systems, V4-1604) that was financially supported by the Slovenian Research Agency and Ministry of Agriculture, Forestry and Food.

Introduction

Past studies have shown that feather pecking and cannibalism are two complex behaviours affected by numerous environmental and genetic factors. Incidence of feather pecking and aggression in general increases with increase of light intensity. Kjaer and Vestergaard (1999) documented death rates in laying hens of 30% and 6% for 30lux and 3lux lighting respectively. Diet composition and feed form affect feather pecking; feed with more raw fiber decreases feather pecking frequency (Hartini et al., 2002), while large particle diets are related to a higher risk of feather pecking (El-Lethey et al., 2000; Lindberg and Nicol, 1994). Microclimatic conditions also have an effect on occurrence of cannibalism. Drake et al. (2010) found that for hens between 24 and 30 weeks of age every increase of CO2 concentration for 200ppm stimulates feather pecking for 14.8%. The same study also found that every increase of NH3 for 15ppm stimulates feather pecking for 10.1% in hens between 15 and 17 weeks of age. The genetic factor is also important since Craig and Lee (1990) found that some breeds and lines are more susceptible to feather pecking than others. Heritability of feather pecking ranges between 0.12 and 0.38 depending on the method of calculations and the age of the laying hens (Rodenburg et al., 2003). Selective breeding for reduced aggression thus remains a promising approach for improved animal welfare in particular production systems. The biggest downsides of selective breeding are slow progress and the possibility of boosting undesirable traits that are positively correlated to the reduced aggression (Nordquist et al., 2011). Feather pecking has different levels of incidence in different production systems. The lowest incidence is in cage systems; considerably more aggression can be observed in single tier floor system, free range and aviary systems. Tauson (2005) argued that bigger groups of laying hens in alternative systems harder establish stable social structures. Despite having a good understanding of various contributing factors to feather pecking and cannibalism, beak trimming remains the most effective preventive method. Since this procedure causes acute and chronic pain in animals, researchers have tested multiple animal-friendly methods to prevent or decrease the incidence of feather pecking. A promising method mentioned is the use of repellents. Harlander-Matauschek and Rodenburg (2011) used natural substances (garlic, clove, and almond) as well as different concentrations of quinine and manganese chloride. Feathers of dead animals were soaked in listed substances and offered to the laying hens in cages. They found quinine in the concentrations of 2% and 4% as most repulsive. In order to gain wider acceptance of this approach, other non-toxic substances that could replace quinine have to be tested. In the present study we used two repellents with dimethyl anthranilate (DA) as the active ingredient to evaluate the repellents' effects on commercial laying hens' behaviour and feather condition. DA is used as a flavouring agent in the human food industry, but has been demonstrated to be aversive to numerous species of birds, e.g. starlings, quail, pigeons, jungle fowl etc. (Kare, 1971). However, to our knowledge it has not been tested as a substance for reduction of feather pecking. The aim of this study was to evaluate possible benefits of the repellents on hens' welfare. Our hypothesis was that the repellents' aversive properties reduce feather pecking and consequently feather damage.

Materials and Methods

Animals, housing and management

One hundred and eighty non-beak trimmed layers (*Gallus gallus domesticus*) of Slovenian provenance Prelux-R, were used in the study. Prelux-R is characterized with 1.7 kg at 18 weeks and 2.2 kg at 70 weeks of age and an average of 294 eggs until 70th week with an average of 65 g per egg.

Our birds were reared and kept in a deep litter system until 18 weeks of age. Then they were transferred to Facco's three-tiered enriched battery cages with 10 birds per cage. Each cage was equipped with feed trough in the front of the cage, separated nest area, 4 nipple drinkers in the back of the cage, litter pad for pecking and scratching, two parallel perches and claw shortening device. The birds were randomly divided into 3 test groups of 60 birds per group, i.e. 6 cages per group. Each group had 3 cages in a column and 2 in a row. One column of cages was left empty on both sides of each test group. All hens were housed in a windowless and fan-ventilated room. Red incandescent bulbs covered by glass jars were installed in a horizontal position at a service aisle. The lights were on from 3:00 to 18:15. Commercial feed for layers in a crumble form and water were supplied *ad libitum*. The average light intensity per cage measured at the trough level varied from 0.3 to 3.3lux.

Application of repellents

Each test group of birds was subjected to a spray treatment every 14 days from 20 weeks of age onward. The groups were sprayed with repellents named "P" and "T" while the control group (named "C") was sprayed with pure distilled water. Repellent P had the following chemical composition (Kare, 1961): 2.34ml DA, 37.5ml methyl phenylacetate and 260.1ml propylene glycol; a total of 300ml. The repellent T consisted of (Kare, 1961): 13.5ml DA, 1.5ml geraniol, 15.0ml polysorbate 80 (a.k.a. Tween 80) and 270.0 ml distilled water; a total of 300ml. It was found that a 300 ml can was sufficient to spray birds in a particular test group and that this amount of spray gave good coverage of areas needing protection. At the beginning of the study at 20 weeks of age, the average body weight (±SE) of the birds for groups C, P and T was 1763±20g, 1762±17g and 1790±19g, respectively.

Data collection

The behavioural observations and feather condition scores were performed in two periods, starting at the bird's age of 26 and 38 weeks. Each period lasted 14 days; starting with one application of the repellent and ending with the subsequent application. An additional feather score was performed at the start of the experiment at 20 weeks of age when all the birds still had the perfect plumage. Feather condition of an individual hen was assessed using the scoring system of Tauson et al. (2005). Six body parts (back, wings, tail, vent/cloaca, neck and breast) were scored separately with scores from one to four where higher scores represent better feather condition.

The following nine behavioural patterns were observed in the study: feeding, drinking, pecking to the head, feather pecking, feather peck, preening, comfort behaviour, cage pecking and air pecking. The definitions are given in the Table 1.

Table 1. Ethogram used for data recording

Behaviour	Definition
Feeding	Feed pecking
Drinking	Pecking of the drinking nipples or the trough under the nipples
Pecking to the head	Pecking to the head of another bird except of pecking another's beak
Feather pecking	Peking of another bird's feathers. At least 2 pecks in the same bout
Feather peck	Peck of another bird's feathers that happens exactly once in the same bout
Preening	Preening its own feathers
Comfort behaviour	Shaking of the whole body with feathers on the whole body getting bristled
Cage pecking	Pecking of any object in the cage except of the feed trough
Air pecking	Pecking that is not directed at any object or pecking of the dust in the air

The behaviour of the birds was observed by direct observation 3 days in each of the two measurement periods, namely the day after the application of the repellents, 6 days after the application and the day before the subsequent application (13 days after the last application). Three 1.5-hour recording sessions were allocated for the observations; in the morning (9:00 – 10:30), at noon (12:15 – 13:45) and in the afternoon (15:30 – 17:00). Out of 5min available for the observation of each cage, 3min were used for the recordings and 2min for the observer to move to the next cage. The order of the cages was randomized using 18x18 Latin squares. Each 3min sample interval was further divided into 12 sample points of 15s. Scan sampling every 15s was used to record whether at least one individual was feeding or not. For other behaviours, focal sampling, with one-zero recording within each 15s interval, was used. As an adaptation to the low intensity of lighting in the barn the behaviours, except of drinking, were recorded only for the animals that were in the area between the front of the cage and the first perch. Drinking was recorded for animals in the back of the cage where the nipples were positioned. This was possible as the light coming from the other side of the cage structure allowed the observer to have a clear overview of drinking activity.

Statistical analysis

The statistical analysis was performed with the SAS package, version 9.4 (SAS Institute, 2008). For the purpose of the analysis, the recordings of "pecking to the head", "feather pecking" and "feather peck" were merged into a new variable named "total pecking". For the variable "air pecking" not enough data was recorded to run the model. The data residuals for behaviour variables did not follow a normal distribution (UNIVARIATE procedure) so a non-parametric Generalized Linear Model procedure (proc GENMOD) was utilized taking into account the Binomial distribution while proc GLM using Gaussian distribution was used for feather condition scores. A cage or an individual bird nested within a cage was included as a repeated subject. For behaviour models, a significant difference of the tested effects; repellent (n=3; P, C and T), observation period (n=2), day within an observation period (n=3), part of a day (n=3; morning, noon, afternoon) and sample interval (n=12), was set at P=0.05 while tendencies towards significance at 0.10. The average of light intensity per cage was included as a covariate. For feather condition two models were developed, one containing sum of scores of all six body parts (named "total feather score") while the second sum of scores for back and vent/cloaca (named "back + vent/cloaca"). The effects of repellent and observation period (n=3) were tested. Although we did find a significant effect of part of a day on feeding, drinking, preening and total pecking; and of observation period on drinking and comfort behaviour in the results section only significant results of the effect of repellent are presented as estimates and standard errors. The reported P-values are 2-tailed. The procedure CORR was used to investigate Spearman correlation coefficients in order to assess the relationship between the behaviours and feather condition.

Results and discussion

Spraying laying hens with DA based repellents significantly influenced only cage pecking (Chi-Square=7.02, $p<0.05$; Fig. 1) and feather condition. Hens from treatment C (-2.160±0.286) performed more cage pecking compared to hens from T (-3.843±0.411; $p<0.05$) and P (-2.710±0.222; $p<0.07$) while hens from P showed more cage pecking compared to T ($p<0.05$). It is known that hens have high motivation to use their beak and spend much of the time investigating their environment by pecking (Shimmura et al., 2008a). As in other studies (Shimmura et al., 2008b) in our study too, hens directed their pecking towards the cage. A possible explanation for the cage pecking to be the most pronounced in the treatment C is the aversive taste of repellents to the hens. The repellents did not stick only to the birds but also to the cages. Significant difference in cage pecking between treatment P and T is probably the consequence of different DA concentration. The repellent T's concentration of DA was almost six times as high as that of repellent P.

Although repellents decreased cage pecking, they did not decrease the incidence of feather pecking. We found no significant difference in feather pecking between treatments, however the usage of repellents affected the cumulative plumage variable back+vent/cloaca (F-value=6.85, $p<0.05$; Fig. 2) as well as variable total feather score (F-value=10.49, $p<0.05$; Fig. 2). For the

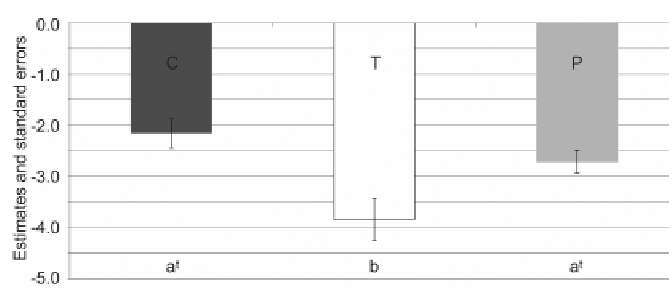

Figure 1. The cage pecking behaviour of hens by treatment (C - control, P - repellent P, T - repellent T). ^{a,b} a significant difference of $p<0.05$. ^{t} a tendency significance of $p<0.10$.

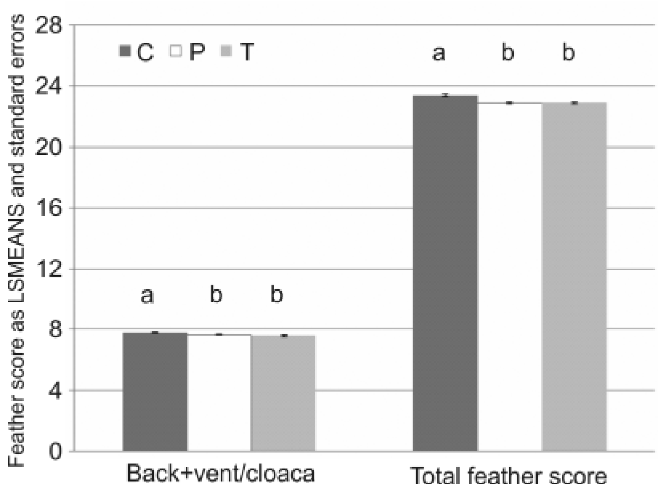

Figure 2. The feather condition by treatment (C - control, P - repellent P, T - repellent T). [a,b] a significant difference of $p<0.05$

variable back+vent/cloaca, hens from treatment C (7.81±0.05) had the highest feather score compared to hens from T (7.57±0.05; $p<0.05$) and P (7.66±0.05; $p<0.05$). Similar picture emerged for variable total feather score (Fig.2). Hens from treatment C (23.4±0.08) had the highest feather score compared to hens from P (22.9±0.08; $p<0.05$) and T (22.9±0.08; $p<0.05$). Perhaps hens from the treatment C directed their pecking behaviour mostly toward their environment and not to the cage mates; however, this is only a speculation. The results might become clearer by the end of this on-going study.

Correlation analysis of behaviours and feather condition regardless of the treatment gave two positive correlation coefficients with tendencies towards significance. These were feather score for vent/cloaca and preening ($r=0.45$, $p<0.10$) and total feather score and preening ($r=0.44$, $p<0.10$). The analysis gave also a positive significant correlation between feather score for back+vent/cloaca and feeding in treatment C ($r=0.81$, $p<0.05$). The latter result is in agreement with the results of Jordan et al. (2010). Longer time spent feeding decreased the risk of feather pecking and consequently increased the feather condition.

Conclusions

Spraying a distasteful substances based on the DA on Prelux-R laying hens feather cover under farm conditions decreased the occurrence of cage pecking. Further, the repellents affected hens' feather condition in the back and vent/cloaca region as well as on total six body regions with repellents causing poorer feather condition. Our hypothesis about a positive impact of repellents on birds' welfare was not supported.

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acs82_35