

Effects of edible environmental enrichments during the rearing and laying periods in a littered aviary—Part 1: integument condition in pullets and laying hens

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ABSTRACT Severe feather pecking is a damaging allopecking behavior in pullets and laying hens which was found to be associated with multiple factors. The aim of this study was to investigate whether the occurrence of feather pecking could be reduced by additional environmental enrichment materials (EM; pecking stones and hard-pressed alfalfa bales) in a littered housing system. In total 4,000 pullets (2000 Lohmann Brown classic [LB] and 2000 Lohmann Selected Leghorn classic [LSL]) were reared in an aviary system until week 18, and 1,320 remaining laying hens were kept thereafter, from week 19 to 48. During rearing, half of the compartments were permanently supplied with enrichment materials, while the other half did not receive any EM. After transferring to the laying hens' stable, we examined the hens under four treatment variants: V1 - no EM over the entire study period; V2 - rearing period with and laying period without EM;

V3 - rearing period without and laying period with EM; V4 - EM over the entire study period. The integument condition (i.e. beak protrusion, beak fissures, plumage condition, skin and toe injuries, and foot pad dermatitis) was scored in weeks 2, 4, 6, 10, 14, 18 (rearing) and 25, 30, 40, and 48 (laying period). At the end of the study (week 48), lower plumage damage was observed in the variants provided with EM during rearing (V2 and V4) compared to the other two variants (V1 and V3; $P < 0.001$). Skin injuries were less prevalent in week 40 in hens with access to EM during rearing (V2 and V4) than the other variants (V1 and V3) within LSL ($P < 0.001$), and in week 48 in the both strains ($P < 0.001$). The variants with access to EM during the laying period (V3 and V4) showed lesser protrusion of the beak tip in week 48 ($P < 0.001$). The observed beneficial effects of EM could be attributed to its impact on foraging behavior and beak-tip abrasion.

Key words: enrichment, pullet, laying hen, feather pecking, plumage

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INTRODUCTION

Feather pecking is a serious problem affecting laying hens' welfare and production performance (Appleby and Hughes, 1991; Niebuhr et al., 2006; Rodenburg et al., 2013). Feather pecking is a nonaggressively motivated damaging allopecking behavior, similar to

cannibalism, and can be distinguished into gentle feather pecking and severe feather pecking (SFP) (Savory, 1995). Gentle feather pecking is considered to be a normal explorative behavior, whereas SFP leads to feather damage, resulting in skin areas devoid of feathers, which can promote cannibalism and pecking-associated injuries (Savory, 1995; Rodenburg et al., 2013). Cannibalism, indicated by pecking and pulling on the skin and underlying tissues of conspecifics, can lead to severe bleeding injuries, especially in the cloacal region, finally resulting in death (Spindler et al., 2016). For a long time, trimming of the beak tip was commonly performed to reduce the occurrence of SFP and cannibalism (Damme, 1999; Spindler et al., 2016). However, routine beak trimming has garnered criticism as a

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noncurative intervention and has nowadays been abandoned in several European countries, such as Austria, Denmark, and Sweden (Spindler et al., 2016), as well as in Germany since August 2016, as a result of a voluntary agreement between the Federal Government and the poultry industry (BMEL, 2015).

SFP can occur in all types of laying hen husbandry systems (Sherwin et al., 2010). However, controlling this damaging allopecking behavior appears to be more difficult in alternative systems than in conventional cage systems because of the larger groups and the resulting higher number of potential victim birds in these systems (Niebuhr et al., 2006; Rodenburg et al., 2013). The causes of SFP and cannibalism are multifactorial and depend on genetics, feeding, husbandry, and management practices (van Krimpen et al., 2005; Kjaer and Bessei, 2013; Janczak and Riber, 2015; Bessei et al., 2018). SFP is regarded as a misdirected foraging and pecking behavior (Wennrich, 1975; Blokhuis, 1986), with phenotypic patterns resembling those of foraging and ground pecking (Dixon et al., 2008). Therefore, provision of litter and other changeable materials is particularly important for controlling SFP (Rodenburg et al., 2013). Studies on the influence of manipulable environmental enrichment material (EM) on the incidence of SFP have been conducted in different laying hen housing systems. These studies mostly focused on the comparison of manipulable EM effects between litter-free housing on perforated floors (e.g., cages or furnished cages) and housing on different litter substrates. Evidence supporting the positive effect of EM in low-stimulus cage systems has been found. However, the effects of additional EM in littered housing systems have not been examined exhaustively yet, although these are the most common in Northwestern Europe (reviewed by Schreiter et al., 2019). Therefore, the aim of this study was to examine the effects of EM on the incidence of integument lesions in pullets and laying hens kept in a littered housing system. In addition, the effect of EM supply during the rearing period and of a change in supply between the rearing and laying periods was investigated. The following hypotheses were tested: 1) Supply of EM throughout the study would reduce the prevalence of severe plumage damage at the end of the observation period (week 48), 2) supply of EM during at least one of both phases (i.e., rearing or laying period) would reduce integument lesions (i.e., plumage damage, skin injuries, toe injuries, and foot pad lesions), and 3) supply of EM would improve the abrasion of the beak tips and reduce beak fissures.

MATERIALS AND METHODS

The study was conducted at the Bavarian State Research Center for Agriculture, Department of Applied Research and Education in Poultry Kitzingen/DE, on non-beak-trimmed pullets and laying hens, from October 2017 to September 2018. The effect of additional EM on the prevalence of integument lesions, as well as on physical development and biological

performance, was investigated in white-egg and brown-egg layers in one run. The results of the study on physical development and biological performance are available in the study by Schreiter et al. (2020).

Housing and Management

The rearing stable was equipped with a two-floored aviary system (Natura Filia; Big Dutchman AG, Vechta-Calveslage/DE), divided into 16 identical compartments (12.9 m² of floor area, thereof 6.6 m² grids and 6.3 m² litter; including perches, feeder space, and nipple drinkers). On the first day of life, 250 chicks per compartment were placed in the lower aviary level, and on day 8, 125 chicks were transferred to the upper aviary level. Both aviary levels had equal floor areas and were equally equipped. In the aviary compartments, 80% of the grid area was covered with chick paper (roll corrugated board; REKA Wellpappenwerke, Kitzingen/DE), which was removed at the end of week 5. Chick starter feed (discussed in the following paragraphs) was provided on the chick paper, from days 1 to 8, to encourage chicks to eat. On day 35, the aviary compartments were opened, enabling access to the floor area littered with soft wood shavings (Premiumspan; Hobelspanverarbeitung GmbH, Dittersdorf/DE). In order to maintain an attractive, loose litter substrate, the littered areas were relittered with the same quantity of softwood shavings every 2 wk. No manure was removed from the littered area during rearing. In week 19, the birds were transferred to a laying stable divided into 44 identical compartments (4.07 m² of floor area each) and with thermostatically controlled vacuum ventilation and spray cooling (Big Dutchman AG, Vechta-Calveslage/DE). Each compartment housed 30 hens and was equipped with a storage feed trough (Big Dutchman AG, Vechta-Calveslage/DE), a nipple drinker line (Big Dutchman AG, Vechta-Calveslage/DE), a family nest with Astroturf mats (VencoTec GmbH, Holzheim/DE), and perches (self-made from wood, rectangular cross-section, 4 × 5 cm). One-third of the available area was littered with soft wood shavings (Premiumspan; Hobelspanverarbeitung GmbH, Dittersdorf/DE), while the remaining two-third consisted of perforated flooring (metal grids; Big Dutchman AG, Vechta-Calveslage/DE) with an underlying manure belt (Big Dutchman AG, Vechta-Calveslage/DE). The manure was removed once a week. High-frequency lightings (HF-Wannenleuchte; Big Dutchman AG, Vechta-Calveslage/DE), mounted on the ceiling of the central aisle, served to illuminate the stable. These fluorescent tubes with electronic ballast units operated in the range of over 2,000 Hz. The light intensity at animal height in the litter and trough area was 30 lux.

For demand-oriented feeding during the rearing period (i.e., phase feeding adapting the nutrient contents in the feed to the age-related changes in the nutrient requirements of the animals), a four-phase feeding program (from Deutsche Tiernahrung Cremer GmbH & Co. KG, Regensburg/DE), with mashed chick starter

feed (weeks 1–2, Bonimal GK Starter), complete chick feed (weeks 3–10, Bonimal GK KAM), and complete pullet feed (weeks 11–18, Bonimal GK JAM) in the rearing stable and a prelaying diet after moving to the laying stable (weeks 19–20, Bonimal GK Vorlegemehl), was provided. Then, birds were fed a complete feed for laying hens (weeks 21–48, Bonimal GK LAM 44) designed for phase-I until the end of the study period. The nutrient and active substance levels were based on current recommendations (Lohmann Tierzucht, 2017). Owing to the observed plumage damage, the supplementary feed VeyFo Jecuplex (Veyx-Pharma GmbH, Schwarzenborn/DE; declared as supplement feed and labelled for oral administration via drinking water) was mixed with drinking water (2 mL/L water resulting in a final concentration of 0.2%) and was administered to all birds over 6 d in week 12 after a veterinary consultation, for an additional vitamins and amino acids' supply. A regulated step-down-step-up light program, based on the current management recommendations (Lohmann Tierzucht, 2017), was used for targeted control of animal development and laying maturity. The window surfaces in the rearing and laying stables were completely darkened by wooden panels. In order to ensure stable animal health in the later stages of the laying period and to reduce animal losses, a vaccination program appropriate for the stock, region, and intended use was undertaken following the recommendations of Lohmann Tierzucht (2017).

Animals, Study Design, and Data Collection

For this study, 2,000 one-day-old chicks of the white-egg layer hybrid strain Lohmann Selected Leghorn classic (LSL; Lohmann Tierzucht, Cuxhaven/DE), and 2,000 chicks of the brown-egg layer hybrid strain Lohmann Brown classic (LB; Lohmann Tierzucht, Cuxhaven/DE) were purchased from the LSL Rhein Main hatchery (Dieburg/DE) and housed in the LSL rearing stable in alternating compartments and study blocks (Supplementary Figure 1A). Each compartment housed 250 chicks.

Birds of both strains were divided into 2 different variants. In the control group (CON), no EM was supplied apart from the chick paper and litter. In the experimental group (EXP), additional EM were available to the birds from the first day of life. Two pecking stones (Vilolith medium; Deutsche Vilomix Tierernährung GmbH, Neuenkirchen-Vörden/DE) and 4 hard-pressed alfalfa bales (Einstreuprofi, Seelingstädt/DE) were supplied in each compartment as EM during the entire rearing period. The EM were provided *ad libitum* and were replaced in the compartment shortly before complete consumption. The two EM were replenished independently of each other. Pecking stones and alfalfa bales were placed centrally in the aviary compartment (weeks 1–5) and in the litter area (from week 6) next to each other. From week 8 onwards, EM were offered in the littered area only. In the phase with closed aviary segments (weeks 1–5), pecking stones and alfalfa blocks were

placed in flat feed trays (Futterteller; Siepman GmbH, Herdecke/DE).

After 18 wk, the birds were transferred to the laying stable (Supplementary Figure 1B), and the CON and EXP groups from the rearing period were further split into 2 groups, with or without access to EM during the subsequent laying period, resulting in 4 different variants: variant 1 (V1), no EM over the entire study period (LB: n = 150, LSL: n = 180); variant 2 (V2), rearing period with and laying period without EM (LB: n = 180, LSL: n = 150); variant 3 (V3), rearing period without and laying period with EM (LB: n = 180, LSL: n = 150); and variant 4 (V4), EM over the entire study period (LB: n = 150, LSL: n = 180).

For this, 660 LB and 660 LSL hens were placed in 44 compartments of the laying stable. The compartments of 30 hens each were assigned alternatively for the strains and blocked for the study variants. Only hens from the same rearing compartment were housed in one laying compartment. The V3 and V4 study variants had access to EM in the scratching area, from the first day of housing till the end of the study period. Hens in each compartment were supplied with a pecking stone (Vilolith hart; Deutsche Vilomix Tierernährung GmbH, Neuenkirchen-Vörden/DE) and a hard-pressed alfalfa bale (same as before), renewed shortly before complete consumption. The two EM were replenished independently of each other. The pecking stones, weighing 10 kg each, were divided into 4 equal parts by an angle grinder (GWS 13–125; Robert Bosch GmbH, Leinfelden-Echterdingen/DE), and one of these parts was placed in each compartment. These parts of the pecking stone were consumed more quickly by the hens, thus reducing the contamination with excrements.

In this observational longitudinal study, characteristics of integument condition and body mass, biological performance, egg quality, mortality, feed consumption, EM' consumption, and carcass composition were assessed (Figure 1).

For indirect determination of the occurrence of SFP and cannibalism, a scoring of the integument was performed on predecided dates (Figure 1). For this, individual birds were regarded as experimental units, and the required sample size was calculated from our preliminary investigations (data not shown), using a Web-based tool (<http://imsieweb.uni-koeln.de/beratung/rechner/b2.html>). In order to prove differences of 17% in the proportion of birds with integument damages, with a statistical power of 0.80 and a significance level sets at $\alpha = 0.05$, a sample size of a minimum of 110 birds per variant was necessary. During rearing, in weeks 2, 4, 6, 10, 14, and 18, a subset of 30 pullets per compartment (i.e., for each n = 7 from the lower aviary level, n = 7 from the upper aviary level, n = 8 from the middle scratching area, and n = 8 from the outer scratching area), from 4 compartments per strain, was therefore scored (i.e., n = 120 per strain). During the laying period, hens were assessed in weeks 25, 30, 40, and 48. As for the rearing period, a subset of up to 30 hens per compartment, from 4 compartments per treatment variant and for

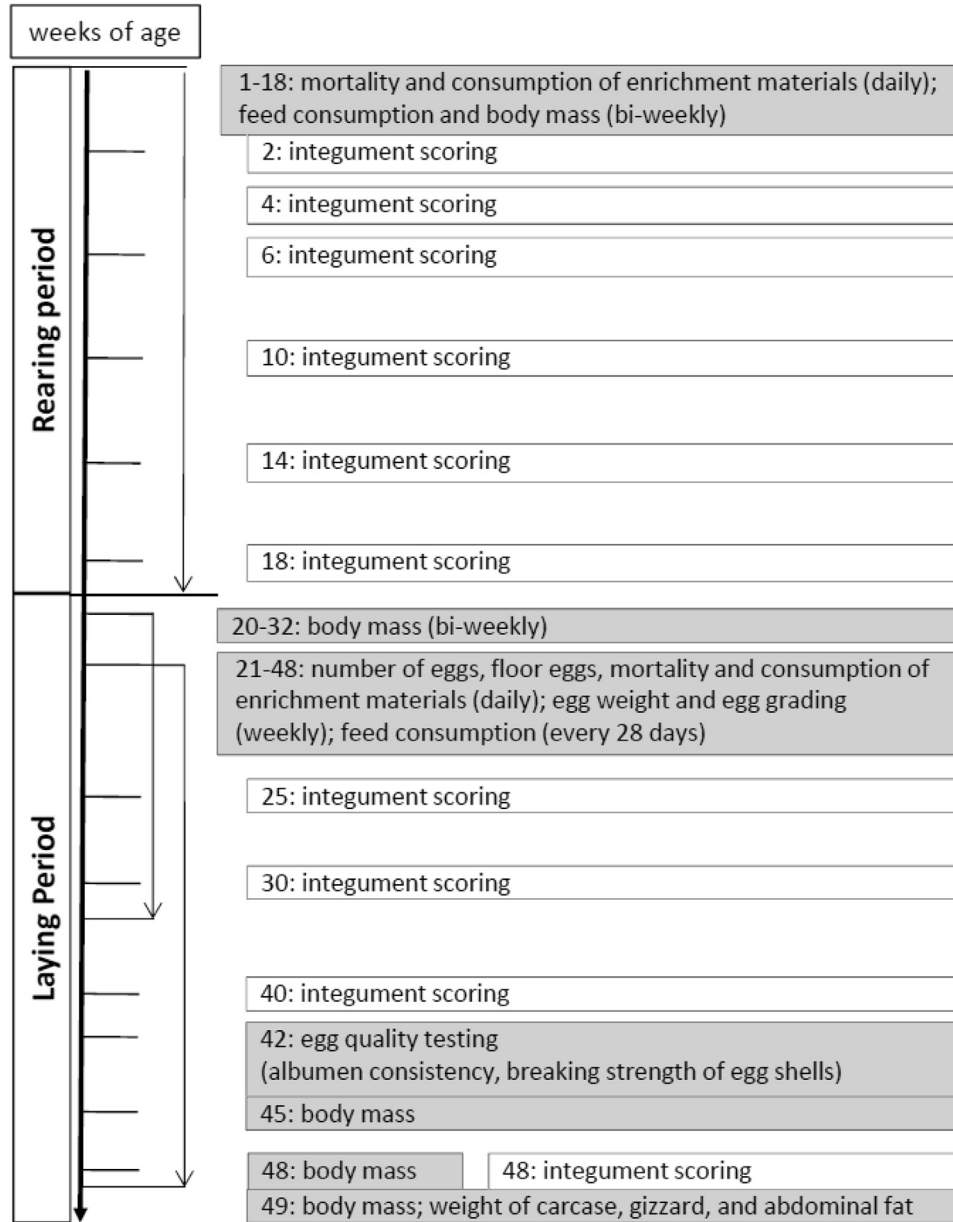


Figure 1. Study design—times of scorings and data collection (grey boxes—for results, see part 2 of the article).

each strain (i.e., 32 compartments per scoring date), was scored. All the scoring were performed by the same person, that had experience in applied scoring systems and had passed a preliminary training program with a total of 2,000 hens.

In pullets (weeks 1–18), the scoring system of [Keppler \(2017a, Supplementary Table 1\)](#) was used, whereas in laying hens (weeks 25–48), the scoring was based on the [Welfare Quality \(2009\)](#) protocol modified by [Keppler \(2017b, Supplementary Table 2\)](#). The application of a scoring system during rearing that differed from the scheme in the laying period was necessary because of different threshold values for the classification into the individual scores. The smaller animal size in chicks and pullets in comparison to laying hens was an indication to use a published system for this age group. Individual scores were assigned for

back plumage, abdominal plumage (including cloacal region and underside of rump), and wing feathers in pullets and for back plumage, belly plumage (including cloacal region and underside of rump), and dorsal neck plumage in laying hens. These three individual scores were then additionally summed to obtain a total plumage score. The feathers of the front of the neck and the breast were not included in the scoring, as feather damage in these areas due to mechanical stress from the feeding trough does not provide strong evidence for SFP ([Bilcik and Keeling, 1999](#)). All body regions, except the head and feet with toes, were considered for pecking injuries of the skin, in addition to the blood-filled feather follicles in pullets. In the scoring of toe injuries and foot pad dermatitis, toes or foot pads with more severe lesions were considered while assigning the score.

Statistical Analyses

Microsoft Excel (version 2013; Microsoft Corporation, Redmond, WA) was used for data collection, processing, and creation of selected diagrams. For further descriptive and inferential statistical analyses, the Standard SAS program package (version 9.4.; SAS Institute Inc., Cary, NC) and the IBM SPSS Statistics program (version 23; SPSS Inc., Chicago, IL) were used.

In a first step, the ordinally scaled integument characteristics (i.e., beak protrusion, beak fissures, total plumage score, skin injuries, toe injuries, and foot pad dermatitis) were analyzed univariately for differences between the study variants, using the Mann-Whitney U test (rearing period, 2 variants) or the Kruskal-Wallis test (laying period, 4 variants) (du Prel et al., 2010). For this purpose, all birds were considered independently of strain (designated as LB/LSL) and subsequently separated for LB and LSL hens. Mann-Whitney U test was used to test the integument characteristics for differences between the 2 strains. A post-hoc pairwise comparison was performed using the Mann-Whitney U test, if necessary, while testing the integument characteristics during the laying period between the variants (du Prel et al., 2010). Results are expressed in grouped medians for integument traits. When scoring the integument traits, the observations were summarized in classes (i.e., scores). Thus, the grouped median was the most appropriate measure of location.

In a second step, multiple logistic regression models with integument characteristics as dependent variables, strain and variants independent variables, and age as covariate, were fitted to the data using binary logistic regression models (Baltés-Götz, 2012). For multiple logistic regressions, the ordinal data scaling (as defined by Keppler, 2017a,b) was transformed into a nominal scaling: 0 for score 0 and 1 for score ≥ 1 , excepted for total plumage score where 0 for score 0 & 1 and 1 for score ≥ 2 were used, and for beak fissures where 0 for score 0 and 1 for score 1 were kept. Absence of multicollinearity was ensured by calculation of Pearson's correlation coefficient and by a collinearity diagnosis with a variance inflation factor and condition index (Menard, 1995; Field, 2013). Nagelkerke's R^2 values, which give an indication of the extent of the variation of the dependent variables explained by the model, were calculated. Nagelkerke's R^2 values ≥ 0.5 were considered as high, reflecting a good explanatory power of the final model (Backhaus et al., 2008). Differences were considered statistically significant for $P \leq 0.05$ and tended to be significant if $0.05 < P \leq 0.1$. To control for false discovery rate due to multiple testing, the Benjamini-Hochberg procedure was used (Victor et al., 2010).

RESULTS

Rearing Period

The results of the univariate analyses revealed a significant strain effect in all evaluated integument traits,

except for the beak tip. LB pullets exhibited less plumage damage than LSL pullets in weeks 2 ($P < 0.001$), 4 ($P < 0.001$), and 6 ($P = 0.007$); however, in weeks 10, 14, and 18, plumage damage was significantly more severe in LB pullets ($P < 0.001$; data not shown). The strains differed significantly in the course of plumage damage during rearing. Maximum plumage damage in the rearing period was recorded in week 4 in the LSL pullets (score 1—EXP: 6.7%, CON: 14.2%; score 2—EXP: 0.6%, CON: 3.6%) and in week 14 in the LB pullets (score 1—EXP: 23.9%, CON: 30.8%; score 2—EXP: 4.4%, CON: 9.2%). In case of skin injuries, the strains differed in weeks 4, 14, and 18 ($P < 0.001$), where the most severe injuries were observed in week 4 in LSL and in weeks 14 and 18 in LB pullets, similar to the results of the total plumage score. Toe injuries were not observed during rearing in the LB pullets. However, in the LSL pullets, toe injuries were recorded in weeks 4, 6, and 10. The effect of strain regarding toe injuries was significant in week 4 ($P = 0.004$; data not shown).

EM supply was observed to influence beak tip from week 6 onwards, with smaller protrusions recorded in EXP than in CON (Supplementary Table 3A; $P \leq 0.038$). The extent of beak protrusion decreased with increasing age, and the largest differences between the variants were observed in week 18 in both strains (Figure 2). No influence of EM was observed in the occurrence of beak fissures (data not shown). The provision of EM significantly reduced plumage damage in weeks 2 ($P = 0.006$), 4 ($P = 0.001$), and 14 ($P = 0.028$) and tendentially in week 10 ($P = 0.063$), over both strains (Table 1). When the 2 strains were evaluated separately, the effect of the variant was significant in week 4 only in LSL and in weeks 10 and 14 only in LB pullets.

An effect of the variant on the occurrence of skin injuries was observed in week 2 in LSL pullets ($P = 0.027$) and week 14 in LB pullets ($P = 0.029$), with lower damages recorded in EXP (Supplementary Table 3B). Severe skin injuries (score 2) were observed in LB from week 14 (EXP: 1.7%, CON: 6.7%) and were still prevalent in week 18 (EXP: 0.8%, CON: 0.8%). Toe injuries were not affected by the variant (data not shown).

The logistic regression models showed significant effects of the variant on beak protrusion ($P < 0.001$), beak fissures ($P = 0.006$), skin injuries ($P = 0.008$), and the total plumage score ($P < 0.001$; Table 2A). Furthermore, the models revealed an influence of the strain with more beak fissures ($P < 0.001$), more severe plumage damages ($P < 0.001$), and more skin injuries ($P = 0.021$; Table 2A) in the LB than in the LSL hens. However, the calculated Nagelkerke's R^2 values were low (Table 2A; $R^2 < 0.20$).

Laying Period

The univariate analyses showed an effect of the strain in all investigated integument characteristics, at least for certain weeks of age. The beak protrusion of LSL hens

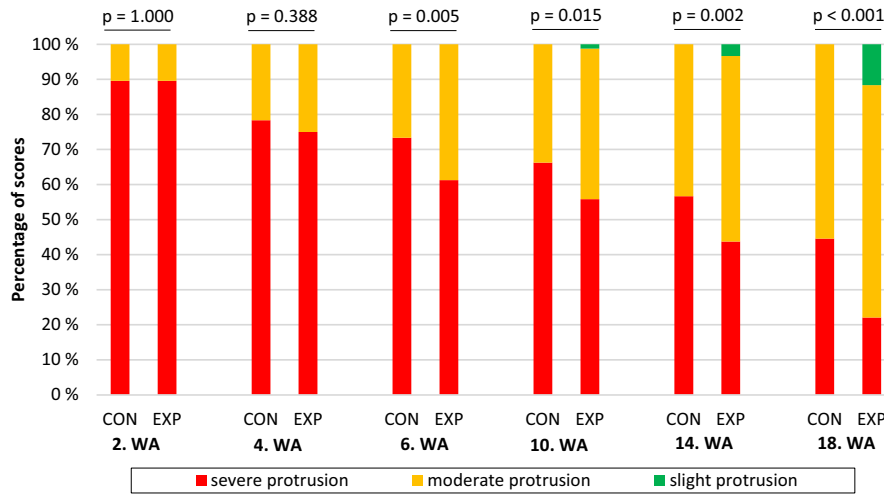


Figure 2. Distribution of the scores for protruding keratin of the beak tip during the rearing period summarized for depending on the supply of enrichment materials. CON, control group (without additional enrichment materials); EXP, enrichment group (supply of additional enrichment materials). Abbreviation: WA, week of age.

was less pronounced than the one of LB hens in weeks 25 ($P = 0.003$), 30 ($P < 0.001$), and 40 ($P = 0.030$; data not shown). Throughout the laying period, LSL had better total plumage scores ($P < 0.001$) and fewer pecking injuries ($P < 0.001$; data not shown) than LB hens, with pecking injuries observed only at week 40 in LSL hens (Supplementary Table 4B). Toe injuries were observed in LSL hens only, at week 40 and in all the variants (data not shown), with a significant effect of strain ($P < 0.001$). The strain had a significant effect on the foot pad condition at all scoring times, with a higher prevalence of foot pad dermatitis recorded in LSL hens ($P < 0.001$).

Table 1. Effect of enrichment materials on total plumage scores during the rearing period.

Age of chicks/pullets (weeks)	Strain	Total plumage scores (grouped medians)		<i>P</i>
		CON	EXP	
2	LB/LSL	0.15	0.09	0.006
	LB	0.07	0.06	0.687
	LSL	0.23	0.12	<0.001
4	LB/LSL	0.29	0.14	0.001
	LB	0.13	0.10	0.596
	LSL	0.49	0.18	<0.001
6	LB/LSL	0.17	0.13	0.247
	LB	0.09	0.10	0.947
	LSL	0.26	0.16	0.124
10	LB/LSL	0.37	0.27	0.063
	LB	0.64	0.43	0.026
	LSL	0.15	0.13	0.317
14	LB/LSL	0.50	0.36	0.028
	LB	1.34	0.80	0.001
	LSL	0.03	0.04	0.473
18	LB/LSL	0.29	0.26	0.565
	LB	0.61	0.50	0.437
	LSL	0.08	0.08	1.000

Bold values indicate significant differences between study groups. Abbreviations: CON, control group (without additional enrichment materials); EXP, enrichment group (supply of additional enrichment materials); LB, Lohmann Brown classic; LSL, Lohmann Selected Leghorn classic.

During the laying period, variants with access to EM (i.e., V3 and V4) showed lesser protrusion of the beak tip in week 48 ($P < 0.001$; Supplementary Table 4A), with a desirable slight beak protrusion (score 2) observed only in weeks 40 and 48 in V3 and V4. Similarly, in weeks 40 and 48, lesser protrusion was observed in variants provided with EM during the laying period (V3 and V4) than those not supplemented with EM in this period (V1 and V2). In week 48, in the variants deprived of EM during the laying period, score 2 (only slight beak protrusion) was not assigned to any of the birds. At week 48, no difference on beak protrusion was found between V1 and V2 (i.e., variants without EM during the laying period; $P = 0.905$).

Plumage damages increased with increasing age during the laying period (Table 3). On combined evaluation of the 2 strains, significant differences were observed for V4, which had less severe feather damage in week 30 than the three other variants and in week 40 than V2. In LB hens, less plumage damages were found in weeks 30 and 48 in V4 than in the other variants and in week 40 compared to V2. In week 48, less plumage damages were observed in V4 in both strains, where EM were provided throughout (i.e., the rearing and laying periods). In LSL hens, more severe plumage damages were recorded in week 48 in V1, where no EM were provided during the entire study period.

The prevalence of skin injuries increased throughout during the laying period in all the variants ($P \leq 0.05$; Figure 3, Supplementary Table 4B). The grouped medians of the skin injury scores showed an effect of the variant in weeks 30, 40, and 48 ($P \leq 0.009$; Supplementary Table 4B). In week 30, LB hens had the highest skin injuries' score in V1, which differed significantly from V2 (lowest score; $P < 0.001$) and V3 ($P = 0.044$), but not from V4 ($P = 0.171$). Skin injuries were less prevalent in hens with access to EM during rearing (V2 and V4) than the other variants (V1 and V3) in week 40 in LSL only ($P \leq 0.002$) and in week 48 in both strains ($P \leq 0.014$). In week 48 in LB hens,

Table 2. Effect of study groups and genetic strains on the integument traits in pullets and laying hens—results of the logistic regression models.

Trait	Score 1 (%)	Coefficients (SE)	Odds ratio (95% CI)	Individual P value	Overall P value	Nagelkerkes R ²
A, rearing period						
Beak tip						
CON	31.9	Ref.	baseline			
EXP	42.1	0.51 (0.08)	1.67 (1.42–1.97)	<0.001		
LSL	38.5	Ref.	baseline		<0.001	0.193
LB	35.4	−0.157 (0.08)	0.85 (0.73–10.01)	0.060		
Intercept		−2.10 (0.11)				
Beak fissures						
CON	0.8	Ref.	baseline			
EXP	1.3	0.95 (0.35)	2.59 (1.31–5.11)	0.006		
LSL	0.1	Ref.	baseline		<0.001	0.168
LB	2.8	3.04 (0.73)	20.97 (5.05–87.01)	<0.001		
Intercept		−8.49 (0.84)				
Total plumage score						
CON	23.4	Ref.	baseline			
EXP	17.9	−0.37 (0.09)	0.69 (0.57–0.84)	<0.001		
LSL	12.6	Ref.	baseline		<0.001	0.123
LB	28.7	1.07 (0.10)	2.91 (2.39–3.54)	<0.001		
Intercept		−2.63 (0.13)				
Skin injuries						
CON	4.1	Ref.	Baseline			
EXP	3.2	−0.51 (0.19)	0.60 (0.41–0.87)	0.008		
LSL	3.3	Ref.	Baseline		0.001	0.020
LB	5.1	0.44 (0.19)	1.55 (1.07–2.25)	0.021		
Intercept		−3.46 (0.23)				
Toe injuries						
CON	0.1	Ref.	Baseline			
EXP	0.4	0.00 (0.58)	1.00 (0.32–3.12)	1.000		
LSL	0.8	Ref.	Baseline		<0.001	0.141
LB	0.0	−21.55 (0.00)	0.00 (0.00–0.00)	0.987		
Intercept						
B, laying period						
Beak tip						
V1 (−/−)	48.3	Ref.	Baseline			
V2 (+/−)	51.3	0.12 (0.09)	0.89 (0.74–1.06)	0.195		
V3 (−/+)	61.5	0.54 (0.09)	0.58 (0.48–0.69)	<0.001		
V4 (+/+)	66.1	0.74 (0.10)	0.48 (0.39–0.57)	<0.001	<0.001	0.040
LSL	59.2	Ref.	Baseline			
LB	52.3	−0.38 (0.07)	1.47 (1.29–1.67)	<0.001		
Intercept		0.14 (0.15)				
Beak fissures						
V1 (−/−)	1.0	Ref.	Baseline			
V2 (+/−)	2.3	0.93 (0.40)	0.39 (0.18–0.87)	0.021		
V3 (−/+)	3.1	1.22 (0.39)	0.29 (0.14–0.63)	0.002		
V4 (+/+)	3.6	1.38 (0.38)	0.25 (0.12–0.53)	<0.001	<0.001	0.185
LSL	0.0	Ref.	Baseline			
LB	5.0	18.69 (0.00)	0.00 (0.00–0.00)	<0.001		
Intercept		22.83 (0.55)				
Total plumage score						
V1 (−/−)	61.6	Ref.	Baseline			
V2 (+/−)	60.1	−0.17 (0.16)	1.19 (0.86–1.64)	0.299		
V3 (−/+)	59.5	−0.24 (0.16)	1.28 (0.92–1.76)	0.143		
V4 (+/+)	55.1	−0.82 (0.16)	2.28 (1.65–3.15)	<0.001	<0.001	0.774
LSL	42.9	Ref.	Baseline			
LB	75.2	4.50 (0.22)		0.011		
Intercept		15.97				
Skin injuries						
V1 (−/−)	15.5	Ref.	Baseline			
V2 (+/−)	8.6	−0.76 (0.16)	2.14 (1.57–2.91)	<0.001		
V3 (−/+)	17.0	0.13 (0.14)	0.89 (0.69–1.14)	0.329		
V4 (+/+)	10.7	−0.49 (0.15)	1.63 (1.22–2.18)	0.001	<0.001	0.223
LSL	5.0	Ref.	Baseline			
LB	20.9	1.75 (0.13)	0.17 (0.14–0.22)	<0.001		
Intercept		6.47 (0.30)				
Toe injuries						
V1 (−/−)	0.8	Ref.	Baseline			
V2 (+/−)	2.1	0.97 (0.43)	0.38 (0.16–0.88)	0.023		
V3 (−/+)	1.3	0.44 (0.47)	0.64 (0.26–1.61)	0.346		
V4 (+/+)	0.8	−0.02 (0.51)	1.02 (0.38–2.78)	0.964	<0.001	0.235
LSL	2.5	Ref.	Baseline			
LB	0.0	−23.15 (0.00)	0.00 (0.00–0.00)	<0.001		
Intercept		9.35 (1.07)				

(continued on next page)

Table 2. (continued)

Trait	Score 1 (%)	Coefficients (SE)	Odds ratio (95% CI)	Individual <i>P</i> value	Overall <i>P</i> value	Nagelkerkes R ²
Foot pad dermatitis						
V1 (-/-)	36.2	Ref.	Baseline			
V2 (+/-)	37.7	0.09 (0.11)	0.91 (0.72–1.14)	0.393		
V3 (-/+)	33.0	-0.19 (0.12)	1.21 (0.97–1.53)	0.096		
V4 (+/+)	34.3	-0.13 (0.12)	1.14 (0.91–1.43)	0.264	<0.001	0.390
LSL	44.3	Ref.	Baseline			
LB	26.3	-1.12 (0.09)	3.08 (2.60–3.63)	<0.001		
Intercept		5.59 (0.21)				

Abbreviations: CI, confidence interval; LB, Lohmann Brown classic; LSL, Lohmann Selected Leghorn classic; Ref., reference; rearing period: CON, control group (without additional enrichment materials); EXP, enrichment group (supply of additional enrichment materials); laying period: V1 (-/-), variant 1 (no additional enrichment materials supplied over the whole study period); V2 (+/-), variant 2 (rearing period with and laying period without supply of additional enrichment materials); V3 (-/+), variant 3 (rearing period without and laying period with supply of additional enrichment materials); V4 (+/+), variant 4 (supply of additional enrichment materials over the whole study period).

V2 and V4 were found to be different ($P = 0.005$), with less pecking injuries being recorded in V4.

The treatment variant had no effect on the occurrence of toe injuries ($P \leq 1.000$; data not shown). Severe foot pad dermatitis was only observed in the LSL groups, in weeks 40 (in V1) and 48 (in V1–V4). A significant effect of the variant was observed in week 40 in LB hens only (Supplementary Table 4C). Foot pad dermatitis were less pronounced in V3 and V4 (i.e., hens with access to EM during the laying period) than in V1 and V2.

The logistic regression models revealed a significant effect of the strain in all evaluated integument traits during the laying period. Remarkably, the total plumage score was influenced by the variants ($P < 0.001$), with the model showing a high Nagelkerke's R² value (0.774; Table 2B) for this trait.

DISCUSSION

The aim of this study was to examine whether the supply of edible EM during the rearing and/or laying periods in 2 hen strains could reduce the occurrence of

damaging allopecking behaviors and, in particular, plumage damage. So far, there is no consistent knowledge about the effects of EM on integument condition in littered systems, in particular longitudinal observations over both the rearing and laying periods are lacking. Alfalfa bales and pecking stones were chosen because EM with the possibility of oral intake are considered particularly suitable (Huber-Eicher and Wechsler, 1997; Dixon et al., 2010), and pecking stones also promote abrasion of the beak tip (Icken et al., 2017). Zepp et al. (2018) demonstrated that the combined use of pecking stones and alfalfa bales is a promising approach to reduce plumage damage. Moreover, these 2 materials are the most frequently used EM options in practice, which are usually offered in combination by laying hen farmers (Spindler and Gaio, 2019). To obtain results with high practical relevance, we decided to supply these 2 materials simultaneously. Therefore, statements on the effects of the single substrates cannot be made within this study and should be investigated in further studies. In comparison to the laying period, pecking stones with a lower degree of hardness were used during rearing to

Table 3. Effect of enrichment materials on total plumage scores during the laying period.

Age of hens (weeks)	Strain	Total plumage scores (grouped medians)				<i>P</i>
		V1 (-/-)	V2 (+/-)	V3 (-/+)	V4 (+/+)	
25	LB/LSL	0.16	0.14	0.12	0.11	0.600
	LB	0.32	0.28	0.26	0.22	0.507
	LSL	0.00	0.01	0.00	0.01	0.572
30	LB/LSL	0.67^b	0.59^b	0.67^b	0.35^a	<0.001
	LB	1.18^b	1.55^b	1.81^b	0.99^a	<0.001
	LSL	0.00	0.03	0.00	0.00	0.070
40	LB/LSL	2.48^{a,b}	2.70^b	2.73^{a,b}	2.15^a	0.039
	LB	2.45^{a,b}	3.79^b	3.57^{a,b}	3.14^a	0.041
	LSL	1.65	1.69	1.76	1.34	0.182
48	LB/LSL	3.90^b	3.59^a	4.01^b	3.03^a	<0.001
	LB	4.39^b	4.48^b	4.60^b	3.90^a	<0.001
	LSL	3.49^c	2.74^b	2.89^b	2.43^a	<0.001

Bold values and different indices (a, b, c) indicate statistically significant differences between study groups.

Abbreviations: LB, Lohmann Brown classic; LSL, Lohmann Selected Leghorn classic; V1 (-/-), variant 1 (no additional enrichment materials supplied over the whole study period); V2 (+/-), variant 2 (rearing period with and laying period without supply of additional enrichment materials); V3 (-/+), variant 3 (rearing period without and laying period with supply of additional enrichment materials); V4 (+/+), variant 4 (supply of additional enrichment materials over the whole study period).

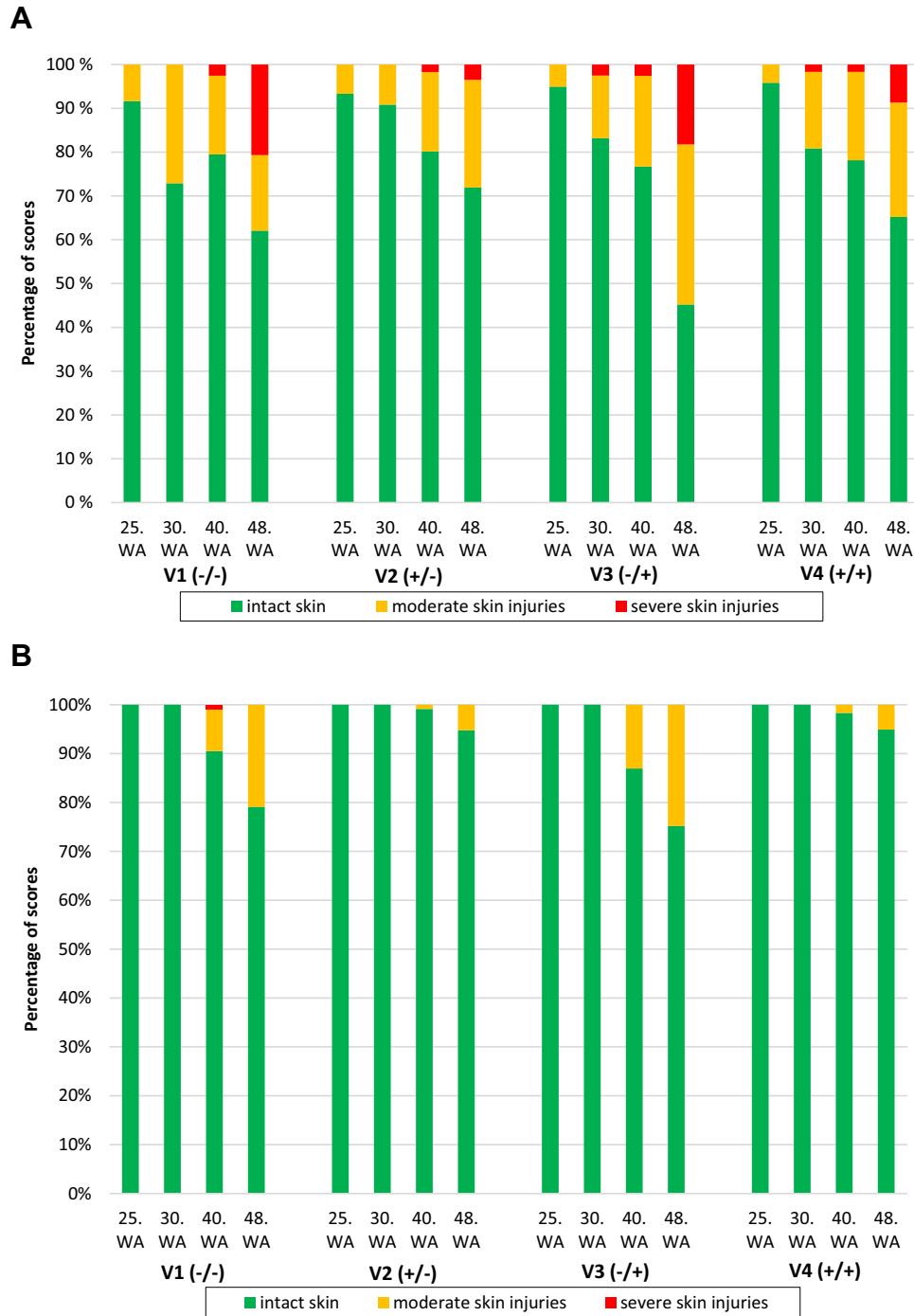


Figure 3. Distribution of the skin injury scores during the laying period in (A) brown-egg layers and (B) white-egg layers over time, as well as in (C) both genetic strains in their 48th week of age, depending on the supply of enrichment materials (alfalfa bales and pecking stones). Different indices (a, b) indicate statistically significant differences between the study groups within a genetic strain. V1 (-/-), variant 1 (no additional enrichment materials supplied over the whole study period); V2 (+/-), variant 2 (rearing period with and laying period without supply of additional enrichment materials); V3 (-/+), variant 3 (rearing period without and laying period with supply of additional enrichment materials); V4 (+/+), variant 4 (supply of additional enrichment materials over the whole study period). Abbreviation: WA, week of age.

ensure the attractiveness to the pullets. However, the raw materials for the pecking stones and the manufacturer were the same in the rearing and laying periods.

We quantified SFP indirectly by scoring the plumage damage of different body regions in both strains throughout the study (Bilcik and Keeling, 1999). The plumage condition was affected by the strain, variant, and weeks of age. In particular, the plumage condition of LSL birds was worse than that of LB birds until

week 6, after which LSL birds' scores improved until week 40. From week 10 until the end of the study, LB birds had on average worse plumage scores than LSL birds, with a peak in week 14 and another from week 30 onwards. Our results confirm that laying hens are at greater risk of plumage damage when damaging allo-pecking behavior already occurred during the rearing period (de Haas et al., 2014). Moreover, the insufficient body mass increases are also known to be a risk factor

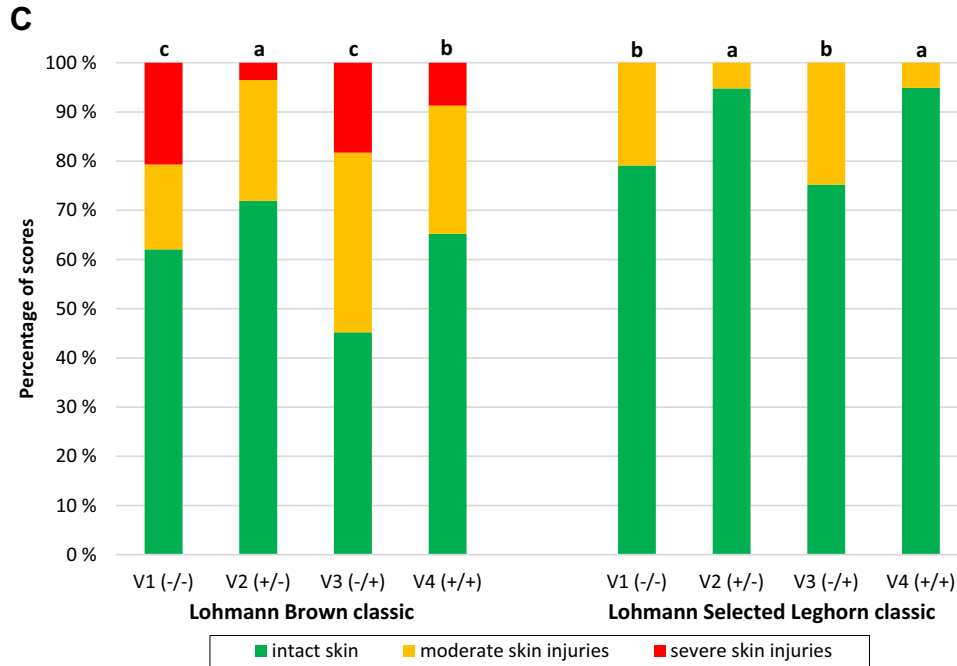


Figure 3. Continued

for plumage damage (Garrelfs et al., 2016; Pottgüter et al., 2018), as it has been seen in LB layers during the laying period from week 26 onwards (see the study by Schreiter et al., 2020).

Our results highlight the positive effect of EM on plumage condition at different weeks of age. During the rearing period, we observed that EM reduced plumage damage until week 4 in LSL pullets and between weeks 10 and 14 in LB in the present study. In these age groups, the use of EM reduced plumage damage, but could not prevent it completely. McAdie et al. (2005) accordingly reported a greater reduction of plumage damage when EM were provided from the first day of life. With our study, it remains unclear whether a permanent supply of EM throughout the 2 studied periods is necessary to limit plumage damage. During the laying period, LB hens that had permanent access to EM (i.e., V4) showed better plumage scores than other variants (including V2, i.e., EM provided during the rearing period only) from week 30, while in LSL hens, no differences on plumage score was seen between V2 and V4 before week 48. Previous studies conducted in littered housing systems have shown a reduction in plumage damage by the provision of EM (Blokhuis and van der Haar, 1992; Norgaard-Nielsen et al., 1993; McAdie et al., 2005; Steinfeldt et al., 2007; Zepp et al., 2018). However, other studies did not find such a benefit (Lugmair, 2009; Lambton et al., 2010; Hartcher et al., 2015; Freytag et al., 2016; Cronin et al., 2018). Differing results may be partially explained by the supply of different EM and their characteristics. Although edible EM such as pecking stones and alfalfa bales were identified particularly suitable to reduce the occurrence of damaging allopecking behaviors (e.g., Huber-Eicher and Wechsler, 1997; Dixon et al., 2010; Zepp

et al., 2018), not all studies found such a benefit with regard to plumage condition (Freytag et al., 2016).

Blokhuis and van der Haar (1992), Norgaard-Nielsen et al. (1993), and McAdie et al. (2005) also reported a reduction in plumage damage in the laying period in hens provided with EM during the rearing period, which has been attributed to an early realization of adequate exploratory and foraging behavior. In contrast, Cronin et al. (2018) and Hartcher et al. (2015) did not observe any improvement in plumage condition due to environmental enrichment during the rearing period. However, Cronin et al. (2018) started the EM provision in the sixth week of life, which might have been too late to induce an improvement in the plumage score during the laying period. The importance of a very early supply of EM was shown by Huber-Eicher and Wechsler (1998), who observed a higher SFP prevalence in chicks that had access to a sand bath from day 10, compared to those that were provided with a sand bath from the first day of life.

The assumption that withdrawal of EM after rearing with additional enrichment promotes SFP (Spindler, 2019) could not be confirmed in the present study. In both strains, hens provided with EM during rearing but not during the laying period (V2) exhibited less severe plumage damage than hens without access to EM over the entire study period (V1) and hens provided with EM during the laying period but not during rearing (V3).

There are three possible reasons for the reduction of plumage damage achieved by providing pecking stones and alfalfa bales in this study. First, both materials can promote adequate foraging and feeding behavior, which is crucial for preventing SFP (Huber-Eicher and Wechsler, 1997; Rodenburg et al., 2013). Second, the use of EM, particularly of the pecking stone, resulted in a desired abrasion of the upper beak keratin. The

less protruding and rounded beak tip is less effective for precise pecking of feathers (Icken et al., 2017). However, it must be noted that greater beak abrasion does not necessarily lead to lesser plumage damage (Morrissey et al., 2016). Third, with the consumption of pecking stones and alfalfa bales, nutrients and active substances were also ingested. Assuming that hens did consume both supplied edible EM, the intake of sodium and crude fiber was 22 and 6% higher, respectively, than the sole consumption of laying hens' complete feed. A reducing effect on observed plumage damage of these 2 nutrients was indeed previously described (Cooke, 1992; van Krimpen et al., 2008). Furthermore, the gizzard muscle mass was found to be higher in the EM-supplemented groups (Schreiter et al., 2020), which may have led to improved nutrient digestion and availability (Amerah et al., 2009; Svihus, 2014). Thus, nutrient imbalances promoting plumage damage (Kjaer and Bessei, 2013) may have been compensated, at least in part.

To generalize the beneficial effect of EM on plumage condition in laying hens' husbandry, the group size has to be considered as a deviating condition. In contrast to our study, in which hens were kept in groups of 30 in a one-floored laying stable, commercial stocks are usually kept in large groups (i.e., up to 6,000 hens per compartment) in multi-floored aviaries. The higher group size also results in a reduced availability of EM for individual hens. According to a practical survey by Spindler (2019), one additional enrichment element is used for 500 to 1,500 hens, which is considerably lower than the ratio of 30 hens per enrichment element used in our study. Hence, a validation of our results under practical conditions is recommended.

Moreover, in our study, skin lesion observations throughout the 2 studied periods were distributed in a similar way to total plumage scores. Rearing with EM significantly reduced pecking injuries of the skin in the following laying period. Our results also highlighted that EM provision during the laying period solely did not have any beneficial effect on the skin injuries. This was also true in week 48, indicating that a lower protrusion of the beak tip is not necessarily associated with a reduction in skin injuries.

Toe injuries, as a possible consequence of toe cannibalism, were observed exclusively in LSL birds, regardless of EM supply, whereby other studies also found higher prevalence of toe injuries in white-egg layers than in brown-egg layers (Niebuhr et al., 2006; Damme et al., 2018).

The improved foot pad condition in week 40 in the variants with access to EM during the laying period (V3, V4) may be explained by a greater use of the littered area (Freytag et al., 2016; Cronin et al., 2018) and a higher zinc intake due to pecking stone consumption (Hess et al., 2001; Kamphues et al., 2014).

In the absence of specific measures to ensure beak tip abrasion in laying hens with untrimmed beaks, pronounced upper beak protrusion and sharp-edged beak tips are common. This is, however, undesirable because

of the increased risk of injury (van de Weerd et al., 2006; Icken et al., 2017).

In our study, we found that EM provision seem to reduce beak protrusion in both strains from week 6 during the rearing period and from week 40 during the laying period. Thus, pecking stones and alfalfa bales might be suitable for beak tip abrasion. Remarkably, Iqbal et al. (2018) did not observe such a reduction in the beak length in groups provided with pecking stones, although pecking stone intake was found to be negatively correlated with beak length.

CONCLUSIONS

Pecking stones and alfalfa bales supplied as additional edible EM during pullet rearing in littered housing systems had a positive effect on plumage condition and skin injuries during the rearing period. Furthermore, EM provision during rearing has also a beneficial effect on plumage condition in the subsequent laying period. In both the genetic strains studied, the occurrence of severe plumage damage could generally be minimized by EM provision, when available permanently or solely during the rearing period. However, EM provision during the laying period only did not exert such a beneficial effect. The risk of cannibalistic pecking during the laying period has been found to be considerably reduced by EM supply during rearing. Permanent EM provision has also been found to have a beneficial abrasive effect on the beak tip.

However, further studies remain to be conducted to examine whether a permanent preventive supply of EM during the rearing and/or laying periods should be recommended to preserve hens' integument condition or whether EM could be provided only once damaging allopecking behavior. Finally, the suitability of different enrichment substrates, and in particular the individual use of edible EM, to reduce plumage damage in larger animal groups should be further investigated.

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Ethical statement: The work described in this article with research on live animals was conducted in accordance with the principles and specific guidelines presented in the Guide for the Care and Use of Agricultural Animals in Research and Teaching, third edition, 2010.

Conflict of Interest Statement: The authors did not provide a conflict of interest statement.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.07.013>.

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