

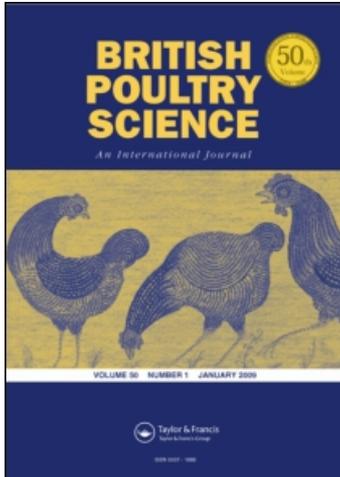
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Reducing stress during welfare inspection: validation of a non-intrusive version of the LayWel plumage scoring system for laying hens

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Abstract 1. The objectives of the present study were to validate a reduced, non-intrusive version (RLS) of the LayWel plumage scoring system in domestic laying hens with reference to complete, intrusive scoring (CLS) and to investigate the effect of these two scoring methods on corticosterone metabolite concentrations.

2. A total of 312 medium-heavy laying hens from 4 commercial hybrids kept in 24 floor pens were scored by two experienced teams. Another 150 hens from two hybrids kept in 6 pens were used for estimating scoring treatment effects on corticosterone metabolites in droppings.

3. Plumage scores were in general higher using the RLS method compared to the CLS method. The agreement between teams for plumage scores (CLS) were on a high (total score) to an excellent (single body part except breast and cloaca) level.

4. Birds subjected to CLS tended to have higher concentrations of corticosterone metabolites in droppings 2 h after scoring compared with birds in the control treatment (not scored). Birds subjected to RLS had intermediate concentrations.

5. It was concluded that a reduced version of the LayWel scoring system is a valid and reliable scoring method which tends to induce less stress to the subjects than the original procedure.

INTRODUCTION

Animal welfare inspection of laying hen flocks often includes the evaluation of plumage condition which is considered to be an indirect measure of the amount of feather pecking in the flock (Hughes, 1982). Such scoring systems are important since recording the incidence of feather pecking in a flock by direct observation is difficult and very time consuming. Several methods of plumage scoring have been used, however, for practical reasons, subjective scoring is by far the most common method. The methods are classified as (1) application of a general score for the plumage of the whole body (Hughes and

Duncan, 1972; Hill, 1980) and (2) application of a specific score for individual parts of the body (Tauson *et al.*, 1984; Gunnarsson *et al.*, 1995; Abrahamsson, 1996; Gunnarsson, 2000; Kjaer, 2000; Tauson and Holm, 2003). In the LayWel EU-project (LayWel, 2005) it was concluded that meta-analysis of plumage data was difficult due to the use of several different scoring systems. Hence a new practical system based on the scoring of several body parts was proposed as a standard to use in future research (Tauson *et al.*, 2005, 2007).

This new system involves the capture and handling of individual birds, which are scored for plumage condition on 6 parts of the body (neck,

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breast, back, wings, cloaca/vent and tail) along with scores for pecking damage to the rear of the body and comb, keel bone deviations and bumble foot lesions. Scores of 1–4 are awarded with higher scores indicating better condition of the integument. The system can be used to compare the incidence of scores 1–4 for individual body parts, or the scores can be pooled to give a whole body score ranging from 6–24. In the present study, we considered only the plumage condition element of the scoring system, which we refer to as the ‘complete LayWel plumage scoring system’ or CLS.

Scoring systems should be (1) simple to apply and allow for good repeatability (Tauson *et al.*, 1984), (2) impose as little disturbance to the birds as possible, and (3) be time-effective, enabling the user to score a large number of birds under commercial conditions in a short period of time, and without stressing the birds. Capture and handling are stressful procedures which can raise plasma corticosterone concentrations within minutes (Kannan and Mench, 1996). In addition, capturing a representative sample in floor or free range systems can be difficult and there are potential risks of sampling errors. CLS fulfils the requirements of the first criterion, but not the second and third. We therefore developed a reduced version of the LayWel scoring system (RLS) by introducing two major changes: (1) scoring only 4 body parts (neck, back, wings and tail) and (2) scoring birds on the ground without catching and handling birds at any point. To our knowledge, no results from objective recording of physiological stress variables after a combination of catching, handling and plumage scoring have yet been published. We therefore assessed adrenocortical activity non-invasively (by quantifying corticosterone metabolites in the droppings) after scoring, comparing values obtained with these two scoring methods with concentrations of un-scored controls.

Thus, the objectives of the present study were to (1) validate the reduced, non-intrusive version of the LayWel plumage scoring system in domestic laying hens (*Gallus gallus domesticus*) with reference to the complete, intrusive scoring, and (2) to investigate the effect of these two scoring methods on corticosterone metabolite concentrations.

MATERIALS AND METHODS

Animals, housing and management

A total of 1100 non-beak-trimmed chickens of 4 hybrid strains (ISA Warren (IW), Lohmann Brown (LB), Lohmann Tradition (LT) and Tetra SL (TS)) were reared on litter floor and housed at

16 weeks in 44 (11 per breed) partly (2/3) slatted floor pens (1.15 m wide × 4.10 m long) at a stocking density of 6 hens per m² (25 hens per pen) and with a communal nest at the rear of the pen. Layer mash (200 g CP/kg, 11.2 MJ ME/kg feed) and water were supplied *ad libitum*. A 14L:10D light programme was applied with an approximate light intensity of 15 lux. The hens were part of an experiment investigating the design of ‘on farm’ performance tests of laying hen hybrids for organic egg production.

Data collection

Plumage scoring

Plumage condition of 312 hens was scored at the age of 73 weeks by two teams, each consisting of two scorers and one assistant. Both teams scored the same 13 hens from each of 22 randomly chosen pens (6 pens per breed of LB and LT, 5 pens per breed of ISA and TB). The practical scoring procedure consisted of the following steps:

- (1) All hens were shepherded gently to one half of the pen and kept there by dividing it with a catching frame made of steel and wire netting.
- (2) Hens were separated by an assistant and individually guided through an opening to the other half of the pen, while the scorers were standing nearby (2–3 m) scoring (blind to the other scoring team) and recording the scores on paper (RLS method, see below).
- (3) The assistant then caught, marked and crated the hen that had just been scored. Blue dye marks on the legs enabled the second team to identify and score the same birds previously scored by the first team, in order to produce independent scores by both methods on each bird. The second team thus produced a CLS score blind to the RLS score given by the first team.
- (4) After scoring, birds were immediately released back into the pen, thus avoiding the risk of recapture.

The LayWel integument scoring system (CLS) is described in the Introduction. Supplementary material with colour illustrations is available at the LayWel homepage (LayWel, 2005) and also in Tauson *et al.* (2007). In the present experiment, only that part of the integument representing plumage quality was used and validated and this system will be called the ‘complete LayWel plumage scoring system’ (CLS).

The reduced LayWel plumage scoring system (RLS) introduced two major changes: (1)

scoring only 4 body parts, neck, back, wings and tail, and (2) scoring birds on the ground without catching and handling birds at all. The scorer stood outside the pen during the scoring, thus minimising disturbance.

No scoring was carried out in the control treatment (CON) and no humans stood in front of the pen. Only normal animal care, as practised in the other pens in the house, was permitted.

Measurement of corticosterone metabolites

Three pens of LB and three pens of LT hens, not previously scored for plumage condition, were used to investigate the effect of scoring method and genotype on stress hormone metabolites. One of three scoring methods was applied to each genotype: CLS, RLS or CON. In CLS, all birds of a pen were caught and crated before scoring 30 min later. Birds were scored while handled and released into the pen again. In RLS, the scorer went to the front of the pen, opened the door and scored the birds individually from a distance; scoring time was approximately 5 min per pen. In CON, there was no scoring or crating and no humans stood in front of the pen. Excreta samples (consisting of faeces and urates together) were taken from all hens in a pen beginning 120 min after initiation of the treatment. Excreta sampling lasted approximately 20 min per pen. Only samples of adequate size (>0.1 g) from 86 out of a total of 133 sampled birds were analysed. Samples were frozen at -21°C until later analysis for 3,11-dione glucocorticoid metabolites, as described by Rettenbacher *et al.* (2004) using a cortisone enzyme immunoassay previously validated for chicken excreta (Rettenbacher *et al.*, 2009). Data were expressed as ng hormone equivalent per g excreta.

Statistical analysis

Plumage scoring

For each combination of method (CLS, RLS) and scoring team (A, B), a total feather score for each hen was calculated by summing the scores for each body region and then calculating the average score per pen. This score had a minimum of 4 points (1 p per body part \times 4 body parts) and a maximum of 16 points (4×4) for the RLS method, summing scores for neck, back, wings and tail. For the CLS method, two sums were calculated; a partial sum (CLSP) used for comparing methods was calculated exactly like for the RLS method, summing neck, back, wings and tail. Further, a complete sum was calculated for testing repeatability of teams within this method, summing neck, back, tail, wings, breast and

cloaca, thus making the maximum possible sum 24 points per hen.

Pen average plumage score was the statistical unit. Method (CLS, RLS), team (A, B), the interaction between method and team, and genotype (LT, LB, TS, IW) were included as fixed factors into a mixed model procedure in SAS (SAS Inst. Inc., Cary, IL, USA). Method and team were included as repeated measures (Littell *et al.*, 1996), and normal distribution of residuals was accepted after visual inspection.

Reliability of methods and teams

The between-method and between-team reliability were estimated using several measures of agreement, namely kappa, weighted kappa and prevalence-adjusted bias-adjusted kappa (PABAK), since there is controversy as to the best statistic. The kappa statistic alone is appropriate if the marginal totals for the 2×2 table are relatively balanced, but if the prevalence of a given response is very high or low, the value of kappa may indicate poor agreement even when the observed proportion of agreement is quite high. Therefore we also present the prevalence-adjusted bias-adjusted kappa (PABAK) to more fully characterise the extent of the agreement between the two methods and teams respectively (Byrt *et al.*, 1993). The procedure FREQ of SAS was used to calculate kappa, asymptotic standard error, standardised test statistic, the *P* value for testing the two-sided hypothesis $\text{Pr} > |T|$, and weighted kappa. A custom-written SAS-program was used to calculate PABAK, following Cunningham (2009) and using the formula $\text{PABAK} = ([k + p] - 1) / (k - 1)$, where *k* is the number of categories and *p* the proportion of agreement. According to Fleiss *et al.* (1993), a PABAK score larger than 0.40 can be interpreted as good agreement and a score larger than 0.75 as excellent agreement.

Hormone data

Glucocorticoid metabolite concentrations, with pen mean as statistical units, were subjected to one-way analysis of variance using the GLM procedure of SAS. *Post hoc* inspection of the data revealed no effects of hybrid and the model included treatment only (CLS, RLS, CON).

RESULTS

Plumage scoring

Effects of method, team and genotype

Plumage scores were significantly higher for the wings and tail with the RLS method and for the

Table 1. Plumage score (least squares means) for methods (CLS = complete LayWel scoring; RLS = reduced LayWel scoring), teams and genotypes (IW = ISA Warren, LB = Lohmann Brown, LT = Lohmann Tradition and TS = Tetra SL)

Body part	Method		Team		Genotype				P values ¹			
	CLS	RLS	A	B	IW	LB	LT	TS	Method	Team	M*T	Genotype
Neck	2.28	2.29	2.36	2.21	2.35	2.22	2.36	2.21	ns	**	*	* ²
Back	3.68	3.77	3.71	3.74	3.62	3.68	3.76	3.84	ns	ns	ns	ns
Wings	3.58	3.78	3.71	3.65	3.74	3.62	3.71	3.65	***	ns	*	ns
Tail	3.62	3.84	3.76	3.70	3.65	3.71	3.86	3.71	***	*	**	ns
Total (CLSP)	13.2	13.7	13.5	13.3	13.4	13.2	13.7	13.4	***	*	ns	ns

¹*, ** or *** indicates a significant effect with $P < 0.05$, $P < 0.01$ or $P < 0.001$ respectively.

²After Tukey-Kramer adjustment for multiple tests, no pair-wise differences between genotypes were significant.

Table 2. Measures of agreement between methods (CLS = complete LayWel scoring; RLS = reduced LayWel scoring) within teams (A and B) and between-teams within methods. SE(kappa), Z and $P < |Z|$ are the asymptotic standard error, the standardised test statistic and the P value of the two sided test of the simple kappa, respectively

Comparison	Variable	Neck	Breast	Back	Wings	Tail	Cloaca	Total ¹
Method CLS vs. RLS								
<i>Within team A</i>								
	Kappa	0.59	–	0.62	0.51	0.23	–	0.28
	SE(kappa)	0.05	–	0.05	0.05	0.05	–	0.04
	Z	11	–	13	10	5	–	9
	$P < Z $	***	–	***	***	***	–	***
	Weighted Kappa	0.63	–	0.70	0.54	0.26	–	0.52
	PABAK	0.84	–	0.90	0.84	0.75	–	0.47
<i>Within team B</i>								
	Kappa	0.69	–	0.74	0.45	0.32	–	0.34
	SE(kappa)	0.06	–	0.05	0.05	0.05	–	0.04
	Z	13	–	16	9	6	–	11
	$P < Z $	***	–	***	***	***	–	***
	Weighted Kappa	0.73	–	0.79	0.53	0.40	–	0.61
	PABAK	0.94	–	0.95	0.78	0.77	–	0.53
Team A vs. B								
<i>Within method CLS</i>								
	Kappa	0.60	0.18	0.78	0.72	0.64	0.38	0.25
	SE(kappa)	0.05	0.04	0.05	0.05	0.05	0.06	0.03
	Z	11	5	15	13	12	6	11
	$P < Z $	***	*	***	***	***	***	***
	Weighted Kappa	0.64	0.41	0.83	0.76	0.69	0.42	0.61
	PABAK	0.87	0.40	0.96	0.90	0.87	0.73	0.35
<i>Within method RLS</i>								
	Kappa	0.44	–	0.78	0.59	0.34	–	0.33
	SE(kappa)	0.06	–	0.05	0.06	0.07	–	0.04
	Z	9	–	15	10	6	–	9
	$P < Z $	***	–	***	***	***	–	***
	Weighted Kappa	0.51	–	0.82	0.62	0.42	–	0.59
	PABAK	0.79	–	0.97	0.90	0.88	–	0.55

¹When comparing CLS with RLS the total score includes 4 body parts only (neck + back + wings + tail).

neck and tail by team A. Total scores were subsequently significantly higher for the RLS method and team A (see Table 1). Significant interactions were found between method and team for neck, wings and tail score (Table 1). Only neck score showed a significant main effect of genotype, with LB and TS having slightly lower scores even though pair-wise differences were not significant (Table 1).

Agreement between methods and teams

In general, kappa and weighted kappa values between methods and teams for the total plumage score ranged from 0.25 to 0.34 and all were significantly larger than zero (Table 2). PABAK values for total scores were higher (0.35 to 0.55). Scoring the single body parts gave excellent PABAK scores ranging from 0.75 to 0.97, with

a few (cloaca and breast) being somewhat lower (0.73 and 0.40 respectively) (Table 2). Kappa values for these body parts were also low (0.38 and 0.18 respectively).

Corticosterone metabolites

Treatment had no overall significant effect on concentration of corticosterone metabolites, but birds subjected to CLS tended to excrete more corticosterone metabolites 2 h after scoring compared with birds in CON (158 ± 25 ng/mL *vs.* 88 ± 25 ng/mL, $P=0.19$). Concentrations were intermediate after the RLS method (137 ± 36 ng/mL).

DISCUSSION

The results showed that it is possible to obtain good precision by scoring birds at a distance compared with catching them and scoring while handling. Furthermore, we found that adrenocortical activity tended to be highest when birds were scored after catching and handling, intermediate when scored at a distance, and lowest when not scored at all.

RLS scored significantly higher on all body parts than CLS. The same finding (RLS scoring lower damage than CLS) was reported by Bright *et al.* (2006). It makes sense that more damage is recorded when (1) birds are closely inspected (handled) and (2) when the number of body parts scored increase. In addition, teams scored significantly differently. This is a well known risk and the effect is normally balanced and included in the statistical models used.

Agreement between teams using the CLS method, based mainly on PABAK scores, were high for total plumage scores and excellent for single body regions, except for the breast and cloaca. These findings are comparable to those found when validating the original version of the LayWel scoring system (Tauson *et al.*, 1984) and other plumage scoring systems (Adams *et al.*, 1978; Bright *et al.*, 2006). Interestingly, the somewhat lower agreement between teams within CLS for the body parts breast and cloaca indicates that these body parts are more difficult to score and special attention should be given here during the introduction and training phase for scoring teams. Agreement was very high for the back score, which is one of the most important areas for estimating the extent of feather pecking in the flock.

Our data support and extend those of Bright *et al.* (2006), who compared handling *vs.* scoring at a distance using a scoring method (Bilcık and Keeling, 1999) only slightly different to the one used in the present study. The two methods

(handling *vs.* distance) used by Bright *et al.* (2006) were found to be significantly correlated. However, there was a potential risk of recapture in the investigation of Bright *et al.* (2006), thus possibly inflating the correlation. In the present study this problem was overcome by the experimental design.

To our knowledge, this is the first study to investigate the potential of plumage scoring to elicit physiological stress responses. The differences (45–65 ng/g) in corticosterone metabolite concentrations between controls and treated birds were smaller than those reported by Janczak *et al.* (2007), who found elevations of between 100 to 250 ng/g faecal corticosteroid metabolites when unpredictable feeding schedules were applied to hens. Baseline levels were very similar in the present experiment to those of Janczak *et al.* (2006), even though, among other things, genotypes differed (White Leghorns *vs.* medium-heavy strains). This is in accordance with findings of Fraisse and Cockrem (2006), who found no differences in baseline concentrations of plasma corticosterone, but different stress response patterns between two layer strains. Contrary to Janczak *et al.* (2006), severe feather pecking in young pullets did not induce any significant change in the concentration of corticosterone metabolites in excreta (Riber *et al.*, 2006).

In conclusion, a reduced version of the LayWel scoring system is a valid and reliable scoring method which tends to induce less stress to the subjects than the original procedure. The scores cannot, however, be expected to equal those obtained with the complete method.

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