

The development of fault bars in domestic chickens (*Gallus gallus domesticus*) increases with acute stressors and individual propensity: implications for animal welfare

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Abstract

Negative experiences during feather growth can result in fault bar formation. Fault bars are malformations perpendicular to the rachis of the feather caused by stressful experiences during feather growth. However, there are little data on the causal effect of psychological stress on the formation of fault bars in chickens. The objective of this study was to examine the effect of acute stress in domestic chickens (*Gallus gallus domesticus*) on fault bar formation to validate this measure as a welfare indicator. Thirty broiler breeder pullets were housed in six cages at 21 days of age. Three cages were exposed to an acute stress protocol while the other three were the unstressed control. Feathers were marked as close as possible to the growing follicle at 21 (wing feathers) and 60 (all feathers) days of age. Acute stress came in the form of three procedures (unpredictable feed delivery, induction of tonic immobility, and crowding) repeated twice, 3–8 days apart and randomly, from 28 to 60 days of age. Wing, tail, and cover feathers were removed and measured at 60 days of age for weight, length, and number of fault bars. Exposure to acute, unpredictable stress increased the number of fault bars in wing feathers of chicks with a high number of initial fault bars. Feather growth decreased for the stressed group compared to the control. These results suggest that feather traits, including fault bars and feather growth, can be used as indicators of negative welfare in chickens.

Keywords: animal welfare, feather growth, *Gallus gallus domesticus*, psychological stress, stress response, welfare indicator

Introduction

Fault bars are translucent malformations perpendicular to the rachis in the feather caused by negative experiences that last for less than 24 h during feather growth (Jovani & Diaz-Real 2012). Fault bars develop due to a lag in protein deposition in the follicle collar as the feather grows, probably as a result of changes in blood pressure (Riddle 1908) and muscle contraction in the follicle (Murphy *et al* 1989), in response to stressful events (Jovani & Rohwer 2017). For this reason, the experience of negative acute stressful events (short-term) rather than chronic stressful states (long-term) is likely to induce formation of fault bars. The presence of a high number of feather fault bars in wild birds has been associated with environmental stressors and low fitness (Jovani & Rohwer 2017). Certainly, previous studies have reported a positive correlation between the development of the fault bars and low survival (Bortolotti *et al* 2002), handling (Murphy *et al* 1988), feed restriction, food unpredictability (Whitmore & Marzluff 1998) and the severity of parasitic infection (Møller *et al* 1996). Therefore, the number of feather

fault bars has the potential to be a welfare indicator, pointing out individual susceptibility to challenging experiences. However, very few studies have examined whether psychological stress induces the formation of fault bars (Jovani & Rohwer 2017) and a validation test is needed to assess whether the experience of negative acute stress induces the formation of fault bars.

Feather growth is an indirect measure of nutritional status and body condition (Riddle 1908; Murphy *et al* 1988), but also an indicator of stress (Romero *et al* 2005; Strohlic & Romero 2008; DesRoches *et al* 2009). Strohlic and Romero (2008) noted that the combination of psychological stress and physical stress (ie feed restriction) in European starlings (*Sturnus vulgaris*) delayed daily feather growth in wing and tail feathers, resulting in a reduced number of fully grown feathers than the unstressed group. DesRoches *et al* (2009) examined the effect of stress on feather traits in European starlings, and authors noted that starlings implanted with (exogenous) corticosterone had lighter feathers (including wing [primaries and secondaries] and tail feathers) with a lower resilience to breakage (hooking strength) compared to the feathers of control starlings. For

Table 1 Experimental design and chronology of procedures applied to pullets under the control treatment and the acute unpredictable stress protocol.

Age (days)	Treatments	
	Control	Acute unpredictable stress
21	Onset: BW, initial line drawn on wing feathers	Onset: BW, initial line drawn on wing feathers
28		Tonic immobility
34		Crowding
40		Tonic immobility
46		Crowding
49		Feeding delay
57		Feeding delay
60	End: BW, line drawn on all feathers	End: BW, line drawn on all feathers
62	Feather collection	Feather collection

this reason, feather traits (including feather growth and other intrinsic characteristics of feather structure) can provide useful information regarding the birds' experience as the feather develops. Indeed, the use of feather traits as welfare indicators has not been considered prior to now, and the combination of both fault bars and feather growth can be reliable indicators of negative experiences in poultry.

Most of the studies on the effect of stressful conditions on fault bars and feather growth have focused on wild birds (Jovani & Rohwer 2017). Yet, very few of these studies considered the potential of these feather traits as welfare indicators and there is a paucity of information available on the development of fault bars under acute stress. Jovani and Rohwer (2017) suggested a mechanistic model in which the effect of the feather type on the formation of fault bars depended on the individual propensity and severity of the stressor, although this hypothesis has not been tested. Therefore, the objective of this experiment was to assess the effect of unpredictable acute stress on the formation of fault bars and feather growth in different feathers of domestic chickens (*Gallus gallus domesticus*). Pullets exposed to acute stressful events were predicted to develop more fault bars and to have lower feather growth compared to unstressed pullets.

Materials and methods

A total of 30 Ross 308 broiler breeder female chicks were donated for this experiment at one day of age, courtesy of Aviagen (via Horizon Poultry, Hanover, Ontario, Canada). The sample size was calculated based on power calculations according to Strohlic and Romero (2008). All the procedures used in this experiment were approved by the University of Guelph's Animal Care Committee (AUP# 3141) and were in accordance with the guidelines outlined by the Canadian Council for Animal Care.

Housing and management

At the hatchery, chicks were infra-red beak treated and vaccinated based on local recommendations and the health programme in the research facility. Chicks were raised under broiler breeder management (see below) at the OMAFRA Arkeel Poultry Research Station (Guelph, ON, Canada) from February to May 2018. Upon arrival, chicks were housed in three cages (ten chicks per cage at 24.8 chicks per m²), before being relocated at 21 days of age to six empty cages (five chicks per cage at 12.9 chicks per m²) so that average bodyweight and uniformity were close to their targets for bodyweight (Aviagen 2016b). Cages (51 × 76 × 56 cm; depth × width × height) provided water *ad libitum* from a nipple drinker line with two nipples per cage and an independent trough feeder per cage (70 × 8.5 × 9 cm; length × width × depth) at 14-cm feeder space per chick with 11-cm height visual partitions at both sides. Cages were laid out in two tiers and two rows, and each cage tier was lit independently.

Pullets were managed based on breeding company guidelines for broiler breeders (Aviagen 2013) and management practices remained consistent across treatments. Room temperature started at 32°C at one day of age, and gradually decreased to 22°C by 42 days of age. Relative humidity remained around 25% during rearing. The light programme was 23L:1D at 100 lux on day 1–3, 12L:12D at 30 lux on day 4–13, and 8L:16D at 30 lux on day 14–60 (when the experiment ended). Lights came on at 0730h and pullets were fed 30 min later. Chicks were fed *ad libitum* for the first week and daily feed restriction started at seven days of age (Aviagen 2016a). Chicks were manually fed with a Starter diet until 41 days of age and a Grower diet from 42 to 64 days of age. Feed allotment was provided based on recommendations for Ross 308 (Aviagen 2016b). Chicks were individually identified with wing tags at seven days of age (Ketchum Mfg Co Inc, Lake Luzerne, NY, USA), and individual bodyweights recorded at the start and end of the experiment. Pullets were checked twice daily and mortality recorded as it occurred.

Experimental design

Cages were organised into a randomised block design with three replicates per treatment and two treatments: control and an unpredictable acute stress protocol. Control cages were adjacent to experimental cages and treatments applied to balance for site of cage within the room (tier, level and location).

Chicks in three cages received the unpredictable acute stress while chicks in another three served as the control group. The unpredictable acute stress protocol involved three events repeated twice across the experiment. The unpredictable acute stressor protocol included unpredictable feed delivery, induction of tonic immobility, and crowding. This protocol was refined from previous publications to induce acute rather than chronic stress (Strohlic & Romero 2008; Gualtieri *et al* 2016). Feed was delivered 2 or 3 h after the typical feeding time (0800h) on the two days of unpredictable feed delivery. On another two random days, pullets were physically restricted to induce tonic immobility beside their home cage for 20 min by the same researcher in accordance with Forkman *et al* (2007). On two further random days, all pullets

from the same cage were placed into a novel empty crate with solid floor (24 pullets per m²) located within the home room for 2 h. The stressful procedures were randomly distributed over a 29-day period, 3–8 days apart, and any such procedure was not repeated within two consecutive days (Table 1).

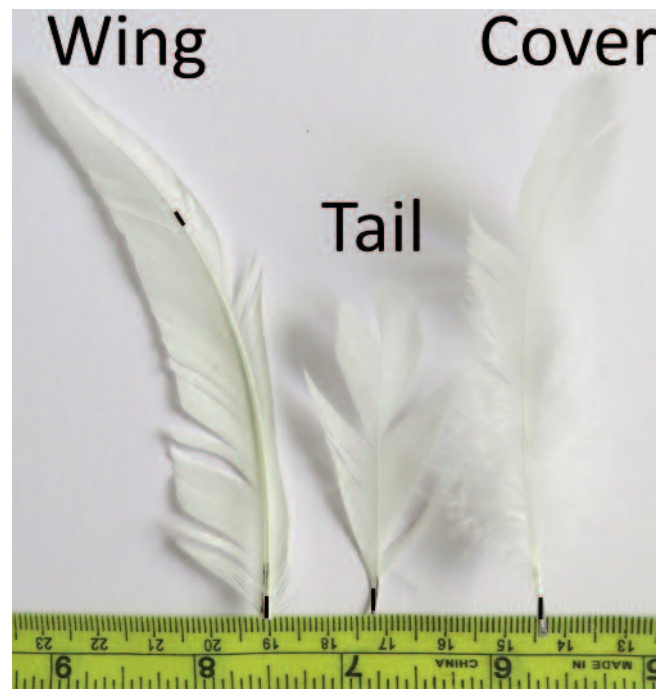
Methodology

Bodyweights were recorded for all pullets after consumption of their daily feed ration in both treatments at 21 and 60 days of age to examine growth rate. The mean (\pm SD) bodyweight was the same between treatments at the onset of the experiment (control treatment: 360.7 [\pm 32.2] g and stress treatment: 345.47 [\pm 43.4] g), and pullets were on target for bodyweight at 21 days of age (Aviagen 2016b).

A total of six feathers were collected from each pullet at 60 days of age: two wing feathers (P8, the third of the outer wing feathers), two cover feathers (SC1, the longest scapular feather), and two tail feathers (R1, mid of the tail feathers). Figure 1 illustrates the three selected feathers, and feathers were collected from the left and right side. Wing feathers started growing at hatch, and a first line was drawn on the rachis of the wing feathers at 21 days of age to account for initial feather growth prior to the start of treatment. This line on the rachis served as a chronological reference and was drawn with permanent black marker as close to the growing follicle as possible. A second line was drawn at 60 days of age for the six focal feathers per pullet. As feathers grew, lines divided the wing feather in three sections: before 21 days of age; from 21 to 60 days of age; and after 60 days of age (calamus) and in two sections for the cover and tail feathers: before and after 60 days of age (Figure 1). The onset of feather growth was estimated to be at ten days old for cover feathers and 15 days of age for tail feathers (R1), but initial growth rate was not estimated for these feathers due to minor development prior to treatments starting.

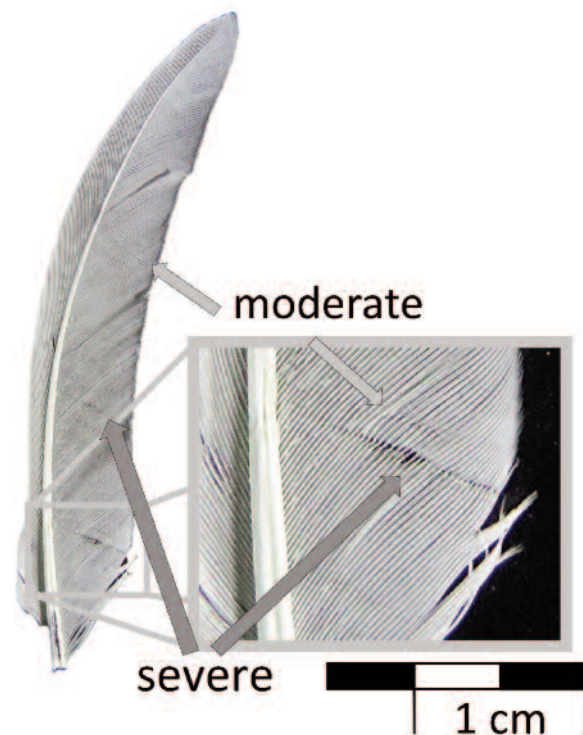
Feathers were cut above the growing follicle at the end of the experiment at 60 days of age. Each type of feather was blindly labelled with the pullets' wing tag number. Feather fault bars were defined as a translucent line perpendicular to the feather rachis visible during observation of the feather against the light. Fault bars were macroscopically classified according to length as moderate (< 5 mm) or severe (\geq 5 mm), as illustrated in Figure 2. A milligram scale (Ohaus E01140, nearest at 0.1 mg) was used to weigh feathers and a digital caliper (Mitutoyo Absolute Digimatic calipers, Mitutoyo Corp, Japan; nearest at 0.01 mm) for feather length. Daily feather growth was estimated by dividing feather length or mass by the growth period in days. Broken and very dirty feathers were removed from the dataset and data were pooled by feather and pullet. The number of fault bars was estimated per feather and counted twice on two independent events to calculate intra-observer reliability. Overall intra-observer reliability was above 95% (see Appendix 1 in the supplementary material to papers published in *Animal Welfare*: <https://www.ufaw.org.uk/the-ufaw-journal/supplementary-material>).

Figure 1



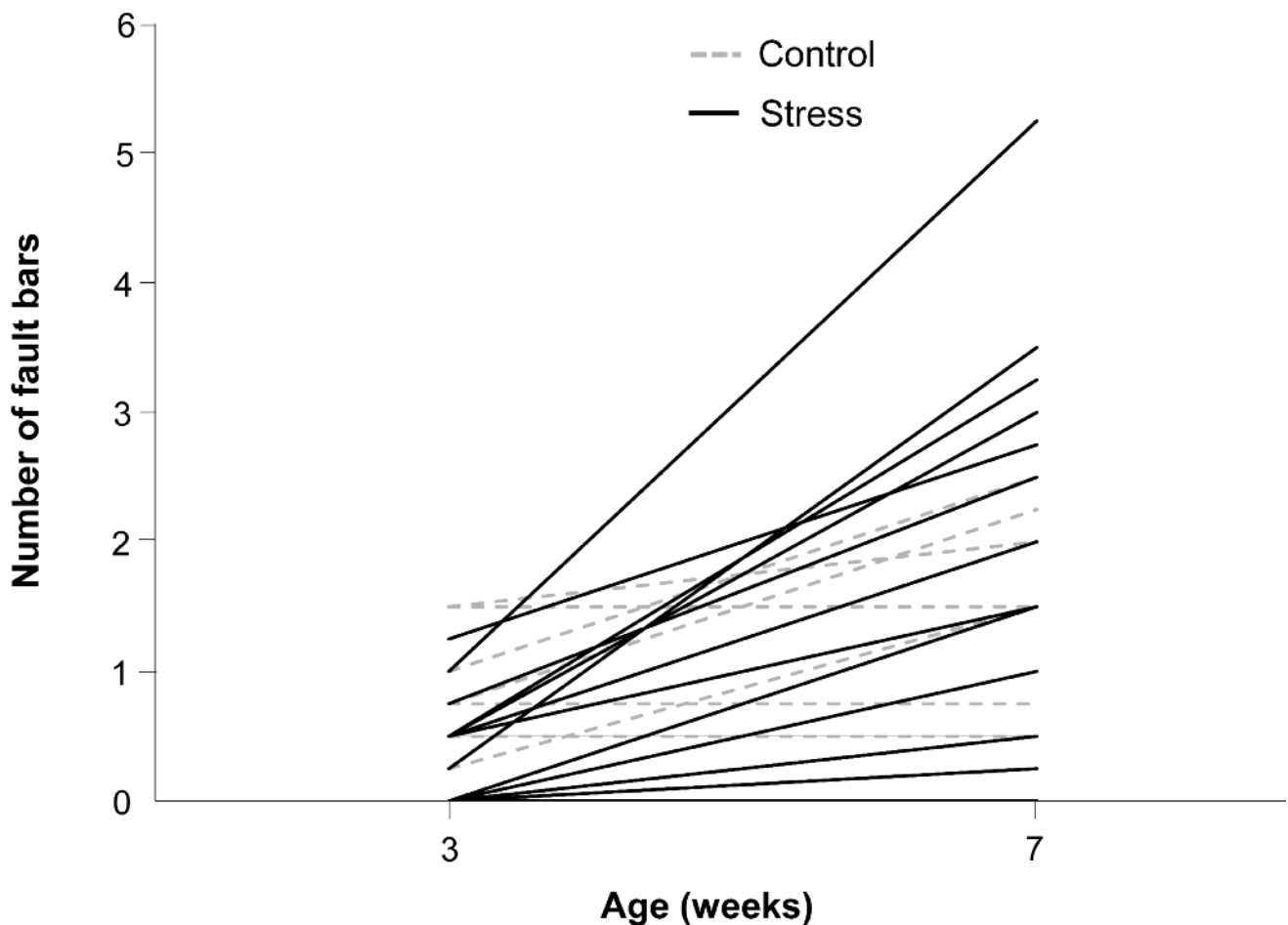
Feathers (wing [P8], tail [R1] and cover [Sc1]) in domestic chickens (*Gallus gallus domesticus*) at 60 days of age. Lines were drawn on the rachis (vertical black line) of the three feathers at 60 days of age in all feathers, and at 21 days of age for wing feathers.

Figure 2



Fault bars in the wing feather (P8) of a broiler breeder pullet showing two moderate (< 5 mm) and two severe fault bars (\geq 5 mm) indicated by arrows. Fault bars were categorised as moderate and severe based on length. Scale belongs to the zoomed-in picture.

Figure 3



Number of fault bars in wing feathers of domestic chickens exposed to acute stress for four weeks (solid line, total of 15 pullets) compared to a control group within the same room (dashed line, total of 14 pullets). Baseline measures were performed at 21 days of age, followed by four weeks of acute stressors and the final measure at 60 days of age. The exposure of acute unpredictable stress increased the number of fault bars in wing feathers of chicks with a high number of fault bars before treatments started ($F_{1,6} = 6.54$; $P = 0.04$).

Statistical analysis

The effect of acute stress on the number of fault bars and on feather growth was analysed using a generalised linear mixed model, with cage nested in the model as the independent experimental unit. Statistical analyses were performed using SAS Version 9.4 (SAS Institute, Cary, NC, USA) with a Glimmix procedure and the degree of significance was set for P -values lower than 0.05.

Treatment, feather type and their interactions were included as fixed effects for each model. The effect of treatments on the number of fault bars was analysed by feather type due to heteroscedasticity when data are pooled (high number of fault bars in tail feathers). The initial number of fault bars before treatments started were included as a covariate for wing feathers. Bodyweight was included as a covariate in the model for feather weight and length. Individual identity, tier and cage location within the room were included in the covariance structure as random effects. The covariance included feather type as a repeated structure (for feather

growth), cage as the subject, and treatment as the group. Significance differences between multiple mean comparisons were corrected using Tukey-test adjustment. Outliers were defined as observations with absolute studentised residuals higher than 3.4 and excluded from the model.

Results

Data are presented using estimated mean values followed by the standard error of the mean. One pullet from the control group was an outlier and excluded from the dataset.

Bodyweight

Pullets were on target bodyweight until 60 days of age (control treatment: 741.1 [\pm 28.4] g, and stress treatment: 745.5 [\pm 40.2] g) without treatment effect on body growth rate ($F_{1,12} = 0.16$; $P = 0.69$). Bodyweight gain was similar between the stressed (10.3 [\pm 0.4] g) and the control treatment (9.8 [\pm 0.5] g per day; $F_{1,6} = 0.24$; $P = 0.64$). Higher pullets' bodyweight gain was associated with a higher feather weight gain ($F_{1,18} = 5.96$; $P = 0.03$) without

Table 2 The effect of stress on the number of fault bars in domestic chicken feathers by feather type based on the length of the fault bar (moderate or severe) (mean \pm SEM; $n = 18$).

Feather	Number of fault bars					
	Moderate (< 5 mm)		Severe (\geq 5 mm)		Total ^z	
	Control	Acute stress	Control	Acute stress	Control	Acute stress
Wing	0.6 (\pm 0.1)	0.5 (\pm 0.1)	0.6 (\pm 0.1) ^b	1.3 (\pm 0.2) ^a	1.1 (\pm 0.1) ^b	1.9 (\pm 0.2) ^a
Cover	0.3 (\pm 0.1)	0.3 (\pm 0.1)	0.1 (\pm 0.1)	0.3 (\pm 0.1)	0.4 (\pm 0.1)	0.6 (\pm 0.1)
Tail	0.9 (\pm 0.1)	0.7 (\pm 0.1)	11.7 (\pm 0.9)	11.7 (\pm 0.9)	12.7 (\pm 0.6)	12.3 (\pm 0.6)
Average	0.6 (\pm 0.1)	0.5 (\pm 0.1)	4.1 (\pm 0.4)	4.4 (\pm 0.3)	4.9 (\pm 0.4)	5.1 (\pm 0.3)

^{a,b} Different superscripts indicate significant mean differences ($P < 0.05$);

^z Combination of moderate and severe fault bars.

Table 3 The effect of stress on feather growth in domestic chickens by feather type and feather growth (length and mass) (mean \pm SEM; $n = 18$).

Feather	Feather growth			
	Length (mm per day)		Mass (mg per day)	
	Control	Acute stress	Control	Acute stress
Wing ^z	2.71 (\pm 0.06)	2.68 (\pm 0.06)	2.03 (\pm 0.06)	1.95 (\pm 0.07)
Cover ^y	1.62 (\pm 0.05)	1.64 (\pm 0.05)	0.42 (\pm 0.02)	0.40 (\pm 0.02)
Tail ^x	1.31 (\pm 0.06)	1.11 (\pm 0.06)	0.44 (\pm 0.07)	0.27 (\pm 0.02)
Average	1.85 (\pm 0.07)	1.79 (\pm 0.08)	0.89 (\pm 0.03) ^a	0.81 (\pm 0.03) ^b

^{a,b} Different superscripts indicate significant mean differences ($P < 0.05$);

^z From 21 to 60 days of age;

^y From 10 to 60 days of age;

^x From 15 to 60 days of age.

effect on length gain ($F_{1,18} = 0.03$; $P = 0.86$). Similarly, stress did not influence the bodyweight uniformity of pullets ($F_{1,12} = 0.03$; $P = 0.87$).

Feather traits: fault bars

The number of fault bars varied among feather type ($F_{2,18} = 102.71$; $P < 0.0001$). Tail feathers had more fault bars (14.5 [\pm 0.6] fault bars) than wing (1.3 [\pm 0.6] fault bars; $t_{18} = 25.91$; $P < 0.0001$) and cover feathers (0.5 [\pm 0.6] fault bars; $t_{18} = 28.29$; $P < 0.0001$). Table 2 indicates the number of fault bars by category, feather and treatment. The percentage of severe fault bars in tail, wing and cover feathers was 94.0, 63.3 and 35.3%, respectively. Figure 3 represents the effect of acute stress on the development of fault bars in wing feathers based on the initial number of fault bars prior to treatment onset. Acute unpredictable stress increased the number of fault bars in wing feathers of pullets with a high initial number of fault bars ($F_{1,6} = 6.54$; $P = 0.04$). Pullets with a low initial number of fault bars were unaffected by stress treatment. Initial number of fault bars accounted for 28.4% of the variation in fault bars in

wing feathers after growing for four weeks ($F_{1,6} = 28.29$; $P = 0.002$). Overall, the slope of the regression curve was 2.31 for the stress treatment ($t_6 = 5.99$; $P = 0.001$) compared to 1.13 for the regression in the control group ($t_6 = 2.28$; $P = 0.06$). The number of fault bars did not differ between the stress and control pullets for the cover ($F_{1,6} = 0.53$; $P = 0.49$) and tail feathers ($F_{1,6} = 0.59$; $P = 0.47$).

Feather traits: growth

Table 3 summarises feather growth as impacted by treatment and feather. Daily feather growth varied among feathers in length ($F_{2,18} = 265.69$; $P < 0.0001$) and weight ($F_{2,18} = 543.46$; $P < 0.0001$). Wing feathers grew heavier and longer compared to cover ($t_{18} = 32.71$; $P < 0.0001$ and $t_{18} = 11.21$; $P < 0.0001$, respectively) and tail feathers ($t_{18} = 25.97$; $P < 0.0001$ and $t_{18} = 23.05$; $P < 0.0001$, correspondingly). There was a tendency for feather length to be shorter depending on stress treatment and feather type ($F_{2,18} = 2.84$; $P = 0.08$). Pullets on the stress treatment had tail feathers 0.20 (\pm 0.07) mm per day shorter than pullets in the control treatment (Table 3). However, this interaction

was not significant for feather weight gain ($F_{2,18} = 2.38$; $P = 0.12$). Unpredictable acute stress reduced feather weight gain consistently across the three types of feathers ($F_{1,18} = 5.29$; $P < 0.05$). Pullets under the unpredictable acute stress protocol grew feathers that were $0.09 (\pm 0.03)$ mg per day lighter than feathers from the control pullets ($t_{18} = 2.30$; $P = 0.03$).

Discussion

The objective of this study was to validate feather fault bars as indicators of welfare, and to determine the effect of unpredictable acute stress on feather traits in domestic chickens. Multiple and unpredictable acute stress was predicted to induce the development of fault bars in plumage and decrease feather growth compared to a control group, as suggested by Romero *et al* (2005), Strohlic and Romero (2008), DesRoches *et al* (2009) and Jovani and Rohwer (2017). Acute stressful procedures increased the prevalence of fault bars, at least in the wing feathers, particularly in individuals predisposed to develop more fault bars before stressful procedures started, and decreased feather growth.

Our results suggest that the number of fault bars increases under multiple acute stressful events based on individual propensity. Stressful negative experiences during early-life stages have been associated with the formation of fault bars in feathers (Jovani & Rohwer 2017). The presence of fault bars has been previously associated with negative events, such as feed restriction (Riddle 1908; Murphy *et al* 1988; Strohlic & Romero 2008), low survival (Bortolotti *et al* 2002), handling in wild birds (Murphy *et al* 1988), and severe parasitic infection (Møller *et al* 1996). Additionally, The individual variation in the formation of fault bars, between and within treatments, can be explained by the subjective perception of the stressful event (Veissier & Boissy 2007). For this reason, the formation of fault bars may be a reflection of how the bird perceives a potentially threatening stimulus (Jovani & Rohwer 2017). Alternatively, the inter-individual variability in our results may suggest an individual genetic predisposition for the formation of fault bars. However, there is a lack of research examining this hypothesis. The number of fault bars also varied among the type of feathers. Tail feathers were the most likely feather to have fault bars, in accordance with previous studies (Jovani & Blas 2004), and the number of fault bars in tail feathers was high even in the control (unstressed) pullets. Such elevated numbers of fault bars in tail feathers may represent the chronic stress that broiler breeders experience during rearing due to feed restriction (Arrazola *et al* 2019). Modern broiler breeders are feed-restricted throughout the entire production cycle to avoid the negative consequences of obesity, such as elevated mortality and poor reproductive performance (Hocking *et al* 2002a; Heck *et al* 2004; D'Eath *et al* 2009). In the current study, the number of fault bars in tail feathers was similar to broiler breeder pullets in Arrazola (2018), to which no stressful procedure was applied other than feed restriction. Jovani and Rohwer (2017) indicated that the propensity for fault bar formation (and its severity) can be feather-specific

according to the severity of the stressor. Therefore, some feathers might be more likely to develop more fault bars (and with a higher severity) compared to others for the same stimulus perception (fault bars allocation hypothesis). Our results corroborate this hypothesis and indicate that tail feathers were more likely to develop severe fault bars compared to cover feathers (few fault bars and moderate), whereas wing feathers were a sensible indicator of negative acute stress under feed restriction. Under current stressful conditions, the tail feathers of chickens might have shown a saturated response to the formation of fault bars but cover feathers presented very few fault bars. Conversely, Jovani and Blas (2004) indicated that in white storks (*Ciconia ciconia*) cover feathers were more likely to present fault bars than wing feathers. Both studies support the fault bars allocation hypothesis although the propensity of fault bar formation can be feather species-specific, probably as a result of different selective pressures (Jovani & Rohwer 2017). For example, the propensity for fault bar formation may be downregulated in flight feathers of soaring species (eg white storks: Jovani & Blas 2004) although there might be an evolutionary benefit to downregulate the propensity of fault bar formation in cover feathers of ground species (eg chickens). Nevertheless, the fault bars allocation hypothesis is yet to be tested across multiple taxon species. The propensity of fault bar formation is also phylogeny and ontogeny dependent. The findings of Møller *et al* (2009) were suggestive of pheasants and partridges (family: *Phasianidae*) being more prone to the development of fault bars in their plumage than nocturnal birds of prey (family: *Strigidae* and *Tytonidae*). Jovani and Diaz-Real (2012) observed the feathers of nestling storks to be more likely to develop a higher number of fault bars and longer bars than those of adult storks. As regards feather growth, Jovani and Diaz-Real (2012) also concluded that fault bars could be formed as a result of stressful events lasting, on average, 7 h for juveniles and 4 h for adult white storks. However, it is unclear whether this difference relates to feather characteristics alone, to causal mechanisms involved in the formation of fault bars or to previous experiences during ontogeny. In line with this idea, our results indicate that acute stress induces the formation of fault bars, dependent, perhaps, on individual perception of the stimulus. These results provide experimental validation that fault bars indicate the experience of stressful negative events during feather growth in chickens. Nevertheless, the effect of chronic stress on the formation of fault bars and the use of fault bars as an indicator of chronic stress, such as depression-like states or prolonged fasting is unknown.

The size of the effect between treatments for the number of fault bars was lighter than expected. This may have been as a result of our crowding procedure not being particularly negatively stressful. Pullets' crate restraint occurred at a stocking density higher than in their home cage, however, individuals were able to perform behaviours, such as dust-bathing and scratching (behaviours restricted in their home cage). Such behaviours are reinforcing for chickens (Duncan 1998) and pullets displayed them during the

crowding procedure (which may indicate a rebound effect). Therefore, the perception of the crowding procedure as being a negative stressful event is doubtful within the context of this experiment and it may have lacked the severity to induce formation of fault bars even in individuals with a high propensity (Jovani & Rohwer 2017). Additionally, the small effect size between treatments may be due to housing, as the control group was located next to the cage receiving the stress treatment. Further research assessing the effect of chronic and acute psychological stress on feather traits, such as fault bars and feather growth, can provide additional information about the potential role of these indicators in birds' welfare.

Our results indicate that exposure to stressful events reduced feather growth (overall weight and tendency for length in tail feathers). Specifically, pullets in this study were chronically physically stressed (ie feed-restricted) and a number were acutely psychologically stressed, and psychologically acute stress reduced feather growth (resulting in overall lighter feather weight and shorter tail feathers). Previous research into the effect of the stress response on feather growth highlighted that endogenous and exogenous increases in corticosterone decreased feather growth (Romero *et al* 2005; Strohlic & Romer 2008; DesRoches *et al* 2009). Romero and colleagues (2005) examined the effect of exogenous corticosterone (implants) on feather growth (wing [primaries and secondaries] and tail feathers) in white-crowned sparrows (*Zonotrichia leucophrys gambelii*). Plasma corticosterone was four times higher in the implanted sparrows compared to the control and this surge in corticosterone levels was coupled with a decrease in feather growth. Similarly, Strohlic and Romero (2008) assessed the effect of endogenous corticosterone (acute and chronic stress) on feather growth (length of wing [primaries and secondaries] and tail feathers) in European starlings (*Sturnus vulgaris*). Here, the authors observed that the feather growth of starlings under chronic stress and feed restriction was lower and lasted for longer compared to control (unstressed) starlings. This effect remained consistent across wing and tail feathers, wing feathers (P8, same as in our study) were lighter in stressed starlings compared to controls (Strohlic & Romero 2008). This lead us to conclude that in chickens acute stress decreases overall feather weight and tail feather length, as has been noted in previous research with other avian species; mediated, potentially, by a surge of plasma corticosterone triggered via activation of the HPA axis under stressful conditions.

Animal welfare implications

Our results showed that the number of fault bars increased under negative psychological stress (in a feather-dependant fashion) and that the number of fault bars can indicate individual propensity. Whether the individual propensity for fault bar formation indicates an individual genetic predisposition to develop fault bars or an individual susceptibility to stress (ie subjective perception of the stimuli) is not straightforward. However, the number of fault bars in feathers is an objective, reliable and minimally invasive indicator to assess the extent of negative experiences, in addition to other

welfare indicators. Tail feathers in chickens had the largest number of fault bars, but the number did not differ between treatments, probably due to chronic feed restriction in all broiler breeders and/or the control pullets being housed near those being stressed (saturated response). In chickens, wing or tail feathers can be sensitive indicators of negative experiences depending on the severity of the stressor (tail feathers for moderate acute stress and wing feathers for severe acute stress). There seems to be a correlation between the number of fault bars and the experience of negative stress in chickens as presented in this study. However, further research is needed to unravel the meaning of the variation in number of fault bars within the treatments.

Conclusion

Results here indicated that psychological stress in feed-restricted broiler breeders reduces feather growth and induces the formation of fault bars in individuals with a greater number of fault bars prior to the onset of treatment, probably as a result of a greater susceptibility to stress. Our results imply that feather traits, such as fault bars and feather growth, can be used as additional welfare indicators in chickens to assess the experience of negative short-term events.

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