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How to see stress in chickens: On the way to a Stressed Chicken Scale

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ABSTRACT For many species, scales are used to classify discomfort and stress (e.g., facial expression/pain scales). Although a significant number of vertebrates used for scientific purposes are chickens, a corresponding scale for birds has not yet been established. We developed a Stressed Chicken Scale (***SCS***) to investigate whether it is possible to assess discomfort in a chicken by its body posture. A selective review with additional handsearch was conducted to find suitable parameters for visual stress assessment. Seven potential body signals were identified: Tail and head position, eye closure, beak opening, leg and wing position, and plumage fullness (ruffled or fluffed up feathers). The SCS was evaluated for interobserver reliability with veterinary students (n = 20), using randomized pictures of stressed and unstressed chickens in lateral view (n = 80). Observers were able to identify the body signals on the pictures after a brief training session. Agreement scores for interobserver agreement ranged from $\kappa = 0.31$ (fair agreement) for eye closure to $\kappa = 0.78$ (substantial agreement) for beak opening. We found that the number of body signals displayed in a stressed expression had an impact on observers' overall assessment of the chickens, for example, chickens were more likely to be rated as stressed if more than 4 signals indicative of stress were present. We conclude that the 7 individual body signals can be used to identify discomfort in chickens.

Key words: animal welfare, laboratory animal, chicken, poultry, noninvasive stress assessment

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INTRODUCTION

Chickens (*Gallus gallus domesticus*) are well known for their use as farm animals for meat and egg production. In addition, they are commonly used for scientific purposes. In 2020, nearly 8 million animals were used as laboratory animals in the EU and Norway, of which 5.3% were domestic fowl (live animals, first-time use). This makes domestic fowl the third most commonly used species after and mice (48.9%) and rats (8.4%), and considerably more frequently used than other farm animals such as pigs (0.9%), sheep (0.2%) or cattle (0.3%) (European-Commission, 2023b). In total, 513,762 birds were used for research, testing, routine production, education, and training purposes in the EU and Norway in 2020. Of these, 83.1% were domestic fowl (European-Commission, 2023a). Poultry was mainly used for basic research, translational and applied research, and regulatory purposes (European-Commission, 2023b).

Chickens are smart (Marino, 2017) and social animals (Bestman, et al., 2013) with a strong flight instinct. As a prey species, chickens, like many other birds, tend to hide signs of illness (Christen, 2011), weakness (Kostka and Bürkle, 2010; Pollock, 2011a; Scope, 2011; Doneley, 2016; Pees, 2018) or pain (Paul-Murphy, 2006) to avoid drawing attention to themselves or the flock for as long as possible (Scope, 2011; Doneley, 2015; Doneley, 2016; Pees, 2018). Severely sick birds, however, are usually too weak to hide their symptoms (Scope, 2011). Thus, if clinical signs are seen in a bird, its general condition is often very poor (Pollock, 2011a; PoultryDVM, 2021).

Birds are capable of nociception (Paul-Murphy, 2006; Gentle, 2011) and can experience pain (Gentle, 2011; Powers, 2015). Because a bird's painful state can be overlooked (Paul-Murphy, 2006), medical treatment may begin too late (Powers, 2015) and result in undertreated patients (Mikoni et al., 2022). Either way, unrecognized suffering and pain is a serious animal welfare issue.

Generally, sick birds show reduced activity (PoultryDVM, 2021) to the point of apathy (Christen, 2011; Siegmann and Neumann, 2011; Rautenschlein and Ryll,

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2014; Doneley, 2015; Gartner, et al., 2018; Pees, 2018), sitting motionlessly and sleeping more than usual (Doneley, 2015). They show reduced attention to their environment (Scope, 2011; Bestman, et al., 2018), fluffed plumage (Korbel, et al., 2001) as well as range of unspecific symptoms (Korbel, et al., 2001; Pollock, 2011a; Scope, 2011; Siegmann and Neumann, 2011). In ornamental and pet birds a decreased escape reflex (often misinterpreted as "sudden tameness") can be observed during illness (Korbel, et al., 2001; Kostka and Bürkle, 2010). Chickens separate themselves from the flock (PoultryDVM, 2021) and attempt to hide when unwell (Bestman, et al., 2013; Bestman, et al., 2018).

Unwell birds are often described as showing characteristic "sick bird signs" (Powers, 2015), also cumulatively described as the "sick bird look" (Doneley, 2015; Doneley, 2016) or, specifically in chickens, the "depressed bird look" (Okinda et al., 2019; PoultryDVM, 2021). Chickens commonly exhibit similar body signals after pecking order attacks (Kolb, 1980). The position of the tail has been described as an anatomically reliable behavioral parameter (Bessei, 1972).

The "sick bird look" refers to a bird with a dropped tail (Damerow, 2015), a tucked-in head (Damerow, 2015; Doneley, 2016), partially or fully closed eyes (Damerow, 2015; Doneley, 2015; Doneley, 2016), an open beak for breathing (Damerow, 2015), dropped wings (Damerow, 2015; PoultryDVM, 2021), and expressing a hunched posture (Damerow, 2015) with fluffed feathers (Damerow, 2015; Doneley, 2015; Doneley, 2016). The ruffling and fluffing of feathers begins in the head and neck area (Kostka and Bürkle, 2010) and is particularly prevalent in the back area (ITIS, 2014).

A chicken showing the "depressed bird look" is described having a bent tail (Okinda et al., 2019; PoultryDVM, 2021), a tucked-in head (Okinda et al., 2019; PoultryDVM, 2021), partially or completely closed eyes (PoultryDVM, 2021), dropped wings (Damerow, 2015; PoultryDVM, 2021), a hunched posture (PoultryDVM, 2021), and ruffled plumage (PoultryDVM, 2021). In addition, the "depressed bird look" refers to a chicken whose shape tends to be more circular and convex compared to that of a healthy animal (Okinda, et al., 2019).

Pain and stress assessment in laboratory animals is of great importance and can be particularly challenging in prey animals such as chickens, and observers must be sensitive to body signals that may indicate a state of pain or discomfort.

Facial Expression Scores or Grimace Scales are used for pain assessment in non-verbal humans (Hadjistavropoulos et al., 2001). In a similar approach, "facial action units" (**FAUs**) have been defined for a large number of animal species. These are recognizable changes in predefined regions of an animal's face when the animal is confronted with a negative stimulus. They can be used to distinguish between pain-free and painful animals (for a review see: Evangelista et al. (2022), Fischer-Tenhagen et al. (2022)). While applicability varies between species, it has been shown that these scales can help to identify suffering animals more quickly.

So far, such scores have been described for mice (Langford et al., 2010), rats (Sotocinal et al., 2011), rabbits (Keating et al., 2012), horses (Dalla Costa et al., 2014), cats (Holden et al., 2014), cattle (Gleerup, et al., 2015; Müller, et al., 2019), pigs (Di Giminiani et al., 2016), ferrets (Reijgwart et al., 2017), seals (MacRae et al., 2018), sheep (McLennan and Mahmoud, 2019), donkeys (Orth et al., 2020) and several primates. However, at this point in time, research regarding facial expression in birds is limited to 3 studies dealing with the recognition of positive emotional states: In blue-and-yellow macaws, ruffled head feathers (crown, nape, and cheek) and blushing on the bare skin of the cheek were recorded in response to positive events (Bertin, et al., 2018a), in Japanese quails, the position of the crown and throat feathers and pupil dilation were found to indicate positive emotions (Bertin, et al., 2018b), and in Sulphur-crested Cockatoos, calmness was associated with ruffling of the cheek and nape feathers (Bertin, et al., 2020).

Results from Mikoni, et al. (2023b) suggest that birds have a reduced ability to express pain through facial expression. Since their anatomy differs from mammals, some of the typical FAUs used in mammals cannot be applied to birds in the same way.

While welfare assessment systems such as the M-Tool (Keppler, et al., 2017) and the Welfare Quality Assessment protocol are in place to assess management, maintenance, and housing conditions of poultry (Welfare-Quality-consortium, 2009) and laying hens (Welfare-Quality®-consortium, 2019), there appears to be a lack of information on stress and pain assessment when it comes to individual chickens and birds with only a handful of validated methods in use today (Mikoni et al., 2022). Currently existing systems have been summarized and reviewed by Gentle (2011) and Mikoni et al. (2022).

Current avian discomfort assessment systems include 1) pain scales for pigeons (Desmarchelier et al., 2012), birds in general (Mikoni et al., 2023b), and cockatiels (Mikoni et al., 2023a), 2) ethograms for Hispaniolan parrots (Paul-Murphy, et al., 2009), pigeons (Desmarchelier, et al., 2012), laying hens (Casey-Trott and Widowski, 2016), and for cockatiels (Turpen et al., 2019), 3) info brochures for pet birds (ITIS, 2014), and 4) infographics for turkeys (Mailyan, 2019) and for chickens (PoultryDVM, 2021) (Table 1). Pictorial scales can be helpful in assessing specific body signals on an individual level.

Automated systems have been developed to detect individual sick birds in a flock (Okinda et al., 2019; Zhuang and Zhang, 2019). However, these birds tend to only be identified at a very late stage of illness, often only when all movement has ceased or the bird is already dead (Zhuang and Zhang, 2019).

Existing assessment tools, such as ethograms, primarily focus on behavior, which differs from the focus of our study. Automated systems and welfare guides for chickens prioritize flock health, whereas our aim is to assess individual chickens. While some studies discuss birds' facial expressions, they focus on positive emotions rather

Table 1. Comprehensive overview of previously described assessment systems in birds.

Bird species	Category	Reference (year)
Hispaniolan Parrots	Ethogram	Paul-Murphy et al. (2009)
Pigeons	Pain Scales/ Ethogram	Desmarchelier et al. (2012)
Pet Birds	Infographic	ITIS (2014)
Laying hens	Ethogram	Casey-Trott and Widowski (2016)
Blue-and-Yellow Macaws	Facial Expression	Bertin et al. (2018a)
Japanese Quails	Facial Expression	Bertin et al. (2018b)
Turkeys	Infographic	Mailyan (2019)
Broiler	Automated System	Okinda et al. (2019)
Cockatiels	Ethogram	Turpen et al. (2019)
Broiler	Automated System	Zhuang and Zhang (2019)
Chickens	Infographic	PoultryDVM, 2021
Cockatiels	Pain Scale	Mikoni, et al. (2023a)
Birds in general	Pain Scale	Mikoni, et al. (2023b)

than discomfort. In view of the absence of facial expression scales for chickens, this study aims to explore the use of posture and habitus of chickens to assess stress levels on a "Stressed Chicken Scale" (**SCS**). "Stress" was defined as any physical discomfort such as pain or illness (Bernatzky, 1997), as well as emotional distress caused by "all feelings of displeasure not covered by the exact concept of pain" (Bernatzky, 1997).

MATERIAL AND METHODS

Defining Parameters for a Stressed Chicken Scale

A selective literature review was conducted to identify appropriate parameters for stress assessment in chickens using the online database PubMed® and the university library of the Free University Berlin including the online literature selection available via the University-based platform "Primo," which also includes unpublished dissertations, i.e., monographs. Relevant information regarding commercial poultry, backyard poultry and pet birds was selected from books, journal publications and other sources such as online data (e.g., veterinary websites).

Firstly, the keywords "pain," "pain detection," "sick bird look/signs/posture," "depressed bird look/signs/posture," and "grieving posture" were used to identify relevant chapters in textbooks (n = 7) on poultry health care. Particular attention was paid to chapters on animal observation, flock health, clinical examination of avian patients and disease detection. The search was then expanded by using the same keywords to identify relevant information in textbooks from the domain of backyard poultry as well as pet birds and ornamental birds such as parakeets and parrots. Particular attention was paid to chapters on pain and pain management in avian patients (n = 8). The following keywords were then used individually and/or in various combinations to identify relevant literature on the platforms PubMed and "Primo": "chicken(s)," "bird(s)," "poultry," "laboratory animals," "facial expression," "grimace scale," "welfare," "animal welfare," "pain," "pain assessment," "pain evaluation," "pain face," "pain scale."

The results (body signals exhibited by chickens in different scenarios) were summarized (Table 2) and used for the development of a Stressed Chicken Scale.

Animals

This study is not an animal experiment according to German Animal Protection Act, since the conducted interventions are in first-level interventions of common veterinary practice and a part of standard livestock management. Ethical review and approval were not required for this study because no additional experimental interventions, which would not have been done during routine veterinary practice, were conducted. The filming process did not affect the chickens or the clinic staff. All chickens used in this study came to the Institute for Poultry Diseases, Faculty of Veterinary Medicine, Free University of Berlin, Berlin, Germany for regular vaccination. None of the chickens were handled or treated differently from the normal procedure during the vaccination events. Therefore, the procedure can be considered as a routine non-experimental clinical veterinary practice.

A total of 430 adult pet (backyard) chickens of various breeds and unknown age from private smallholdings presented for routine vaccination at the Institute of Poultry Diseases, between December 1, 2020, and September 7, 2021, were filmed for this study. This period was assumed to be long enough to generate a sufficiently large convenience sample because all appointments were scheduled, allowing an estimate of the number of patients who would be present during this period. All owners had made an appointment for vaccination in advance so the maximum waiting time before entering the treatment room was 10 min. Chickens were transported using cat transport boxes or cardboard boxes with 1 to 10 chickens per box. No inclusion or exclusion criteria were defined for either husbandry or origin, however, dwarf breeds, feather-footed breeds, frizzle and silkie chickens as well as tailless breeds (e.g., Araucana) were excluded from the study due to their breed-specific characteristics and anatomical features (Table 3).

Of the 430 chickens mentioned above, 81 hens and young roosters (up to 5 mo) from laying lines (n = 37, including brown and white layers) and dual-purpose breeds (n = 44, including Amrock, Bielefelder Chicken, Bovans Black, German Empire Chicken, Rhode Island Red, German Cuckoo, Sussex, and Vorwerk Chicken) were used to create and evaluate a Stressed Chicken Scale.

Data Collection

Upon presentation of each animal, a video camera (SONY HDR-PJ 650 Handycam) was set up to monitor

Table 2. Body signals indicative of stress (including discomfort, distress, pain, signs of disease) in different species of bird; results of the selective literature review.

Body signal	Expression in an unstressed animal	Expression in a stressed animal	Reference (year)
Tail position	Tail carried high	Tail drooping	Christen (2011)[2], Damerow (2015)[4], Gentle (2011)[3], Kostka and Bürkle (2010)[2], Mailyan (2019)[5], Okinda et al. (2019)[4], PoultryDVM, 2021[4], Scope (2011)[2]
Head position	Head raised high, outstretched neck	Head tucked in	Bestman, et al. (2013)[4], Damerow (2015)[4], Doneley (2016)[1], Okinda et al. (2019)[4], Paul-Murphy (2006)[1], PoultryDVM, 2021[4]
Eye closure	Eyes wide open	Eyes fully or partially closed	Bestman, et al. (2013)[4], Bestman, et al. (2018)[4], Christen (2011)[2], Damerow (2015)[4], Doneley (2015)[2], ITIS (2014)[2], Kostka and Bürkle (2010)[2], Mailyan (2019)[5], Pollock (2011b)[2], PoultryDVM, 2021[4]
Beak opening	Beak closed while breathing	Beak fully or partially opened, panting	Bestman, et al. (2013)[4], Bestman, et al. (2018)[4], Damerow (2015)[4], Kostka and Bürkle (2010)[2], Mailyan (2019)[5], Pees (2018)[2], Rautenschlein and Ryll (2014)[3], Siegmann and Neumann (2011)[3]
Wing position	Wings carried high, held close to the body	Wings drooping	Damerow (2015)[4], Doneley (2016)[1], ITIS (2014)[2], Kostka and Bürkle (2010)[2], Mailyan (2019)[5], Pees (2018)[2], PoultryDVM, 2021[4], Powers (2015)[1]
Leg posture	Upright standing position	Hunched posture, bent legs, crouching or lying down	Bestman, et al. (2013)[4], Bestman, et al. (2018)[4], Christen (2011)[2], Damerow (2015)[4], Gartner, et al. (2018)[4], Gentle (2011)[3], Kostka and Bürkle (2010)[2], Mailyan (2019)[5], Okinda et al. (2019)[4], Paul-Murphy (2006)[1], Pollock (2011b)[2], PoultryDVM, 2021[4], Powers (2015)[1], Scope (2011)[2], Swayne, et al. (2020)[3], Zhuang and Zhang (2019)[4]
Fullness of plumage	Streamlined plumage	Ruffled / fluffed feathers	Bestman, et al. (2013)[4], Bestman, et al. (2018)[4], Butcher, et al. (2018)[3], Christen (2011)[2], Damerow (2015)[4], Doneley (2015)[2], Doneley (2016)[1], Gartner, et al. (2018)[4], ITIS (2014)[2], Korbel, et al. (2001)[3], Kostka and Bürkle (2010)[2], Mailyan (2019)[5], Pees (2018)[2], Pollock (2011b)[2], PoultryDVM, 2021[4], Powers (2015)[1], Rautenschlein and Ryll (2014)[3], Scope (2011)[2], Siegmann and Neumann (2011)[3], Swayne, et al. (2020)[3], Zhuang and Zhang (2019)[4]

[1]Birds in general.
[2]Ornameltal/pet birds.
[3]Poultry/fowl.
[4]Chickens.
[5]Turkeys.

the ensuing examination. A white screen was placed behind the examination table. The camera was set up 157 cm from the table and aimed at the chicken at eye level.

Each chicken was taken out of the transport box and placed on the examination table where it was left to adjust to its surroundings for a period of approximately 10 to 20 s. During this time, a superficial adspection of the animal was performed. Next, each chicken underwent a physical examination that lasted approximately 2 min, modified according to Kummerfeld (2015). The examination included adspection of the plumage, eyes, nose, and beak, as well as the vent area, legs and feet, palpation of the breast muscles to determine the animal's body condition and palpation of the coelom (including assessment of laying activity). It also included inspection of the oral cavity, glottis and trachea as well as auscultation of the heart and lungs using a stethoscope and weighing of the animal. Clinically healthy animals then received 0.5 mL of an inactivated vaccine against Newcastle Disease and Infectious Bronchitis (NOBILIS ND + IB, Intervet Deutschland GmbH, a company of MSD Tiergesundheit, Unterschleißheim, Germany), which was administered subcutaneously in the neck. After inoculation, the chicken was returned to the examination table and filmed for a further 10 to 20 s before being returned to the transport box, while the next chicken was taken out of the box. Chickens exhibiting symptoms of illness did not receive the vaccine.

All chickens included in this study are used to being handled. They were therefore classified as "unstressed" or "slightly stressed" (due to the transport) upon

Table 3. Selection of potentially problematic breed characteristics in stress assessment.

Body signal	Breed characteristic (selection)	Affected chicken breeds (selection)
Tail position	No tail	Rumpless Araucana
		Rumpless Araucana Bantam
		Rumpless Game
		Ruhlaer Bantam
	Tail carried in a straight position	German Reichshuhn
		German Reichshuhn Bantam
		Dominique
		Hamburg
		Phoenix
		Phoenix Bantam
		Sumatra
		Sumatra Bantam
		Old English Game and similar
	Tail carried in a lowered position	Cubalayas
		Shamo
Eye closure	Crest / tuff	Crève Coeurs
		Crevecoeur Bantam
		Houdan
		Houdan Bantam
		Padovana Chicken
		Poland Bantam
		Sultan
		Other crested chickens
	Head plumage	Rumpless Araucana
		Rumpless Araucana Bantam
		Brabanter Chicken
		Faverolles
		Faverolle Bantam
		Orloff
		Orloff Bantam
		Sulmtaler
		Sulmtaler Bantam
		Silkie and bearded chickens
Wing position	Wings worn down	Chabos
		Okina-Chabos
		Ohiki
		Sebright Chicken
		Sultan
		Sundanese Gamefowl
		Tuzo
		Some Bantams and dwarf breeds
Leg posture	Low stand / short legs	Krüper
		Zwerg-Krüper
		Ohiki
		Okina-Chabos
		Frizzle
		Some Bantams and dwarf breeds
Fullness of plumage	Special plumage / feather structure	Cochin
		Pekin Bantam
		Frizzle
		Silkies

In some chicken breeds, some of the body signals mentioned cannot be used, or can only be used to a limited extent, due to outstanding breed characteristics (see Schmidt and Proll [2011]).

presentation. After vaccination, we expected the chickens to develop an increased stress level, as signs of increased stress are to be expected after intensive handling or injections, according to current literature (Balcombe et al., 2004).

Video Preprocessing and Creation of a Stressed Chicken Scale

An individual video clip of each chicken was created form the raw video material using iMovie for iOS and macOS (APPLE Inc., Cupertino, CA). On average, video clips were 28 s long and all audio was removed. Each clip consisted of a shot of the chicken before vaccination followed by a black screen and a shot of the chicken after vaccination. The video clip was only included in the study if the chickens were from laying lines or dual-purpose breeds and if they were fully visible on screen for the entire duration of the clip ($n = 80$).

Videos clips were reviewed by the first author, who has been involved in the veterinary care of backyard poultry for several years and has studied chickens' behavior and body signals extensively as part of her scientific work. Screenshots were taken whenever the chicken displayed one of the predefined indicators of discomfort mentioned in the literature in stressed expression (Table 2), and the chicken was visible in its entirety and from a lateral viewpoint. The screenshots, which the first author considered to have particularly good visibility of the body signals, were used to create an image catalog - the Stressed Chicken Scale (Figure 1).

Screenshot Generation for Reliability Testing

For each video clip, a screenshot was taken 1 s after vaccination if the chicken was seen from the side or 2 s after vaccination if the chicken was in motion. The screenshots were randomly divided into 4 sets of 20 pictures, using the RAND function in MICROSOFT Excel (Microsoft Office 365).

The screenshots included chickens showing none of the body signals in stressed expression as well as some chickens showing several signals in a stressed expression. The number of body signals displayed in stressed expression was variable, as pictures were randomly assigned to the picture sets. Chickens can be expected to be mildly stressed due to handling, restraint and vaccination (see Balcombe, et al. (2004)), so the screenshots consist only of pictures of potentially stressed chickens.

Reliability Testing

There were 5 groups of 4 observers (veterinary students). Each student rated a set of 20 screenshots.

As part of their clinical rotation in livestock medicine, veterinary medicine students in their final year of education at the Free University Berlin ($n = 20$) were recruited as observers for reliability testing. The observers were split into groups of 4 and received a 20-min training session on how to use the Stressed Chicken Scale, delivered by the first author of this manuscript. First, the observers watched a MICROSOFT PowerPoint presentation (Microsoft Office 365) on the 7 body signals indicative of discomfort in chickens. Then, the observers were presented with 10 pictures of chickens

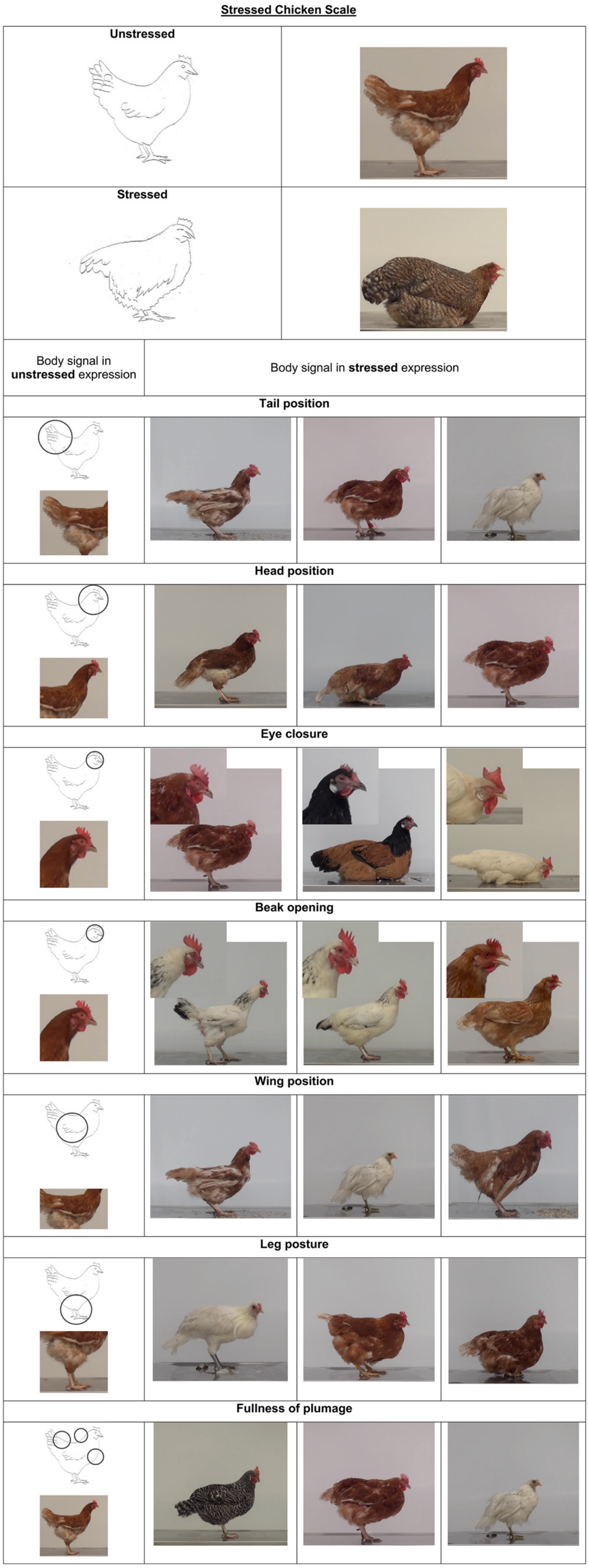

Figure 1. Stressed Chicken Scale **(SCS).** An image catalog was created showing chickens from laying and dual-purpose breeds in lateral view. It consists of 3 example pictures for each of the 7 body signals in a stressed chicken, as well as an example of the expression in an unstressed animal.

showing varying degrees of expression of discomfort. These were screenshots from video material that had previously been excluded because they were not clear enough to be included in the SCS.

Immediately after the training session, each observer was then presented with a set of 20 screenshots for independent evaluation. For each screenshot, they were asked to assign a score (signal "visible" = stressed expression (including all 3 sample pictures on the scale), "not visible" = unstressed expression, "I don't know" = uncertain to score or unable to interpret/recognize) for each of the following body signals: Position of the tail, position of the head, eye closure, beak opening, wing position, leg posture and fullness of plumage. In addition, they were asked to rate the overall appearance of the animal as either "stressed" or "unstressed." For guidance, observers were given a printed version of the SCS.

Statistical Analysis

Score sets were recorded in MICROSOFT Excel (Microsoft Office 365) worksheets. For each picture, the frequency of assigned scores was derived. The frequency of "I don't know" responses indicated whether the observers had difficulties to assess the respective body signal after the short training session on the use of the SCS. If they chose the "I don't know" option, we assumed they couldn't identify the body signal on the individual screenshot. Images with particularly high "I don't know" ratings were reevaluated by the first author after the survey to determine potential bias.

Statistical analyses were performed using SPSS Statistics, version: 28.0.1.0 (142) (IBM, IBM Deutschland GmbH, Germany, 2021). The observers' assessments on average were compared with an expert standard (= first author's assessment, taken as the truth of reference) and used to determine the interobserver agreement between the multiple observers. In accordance with Landis and Koch (1977) the following cut-off values were used to assess kappa values (κ): <0.00 = lass than chance (poor) agreement, 0.00-0.20 = slight, 0.21−0.40 = fair, 0.41 −0.60 = moderate, 0.61−0.80 = substantial, 0.81 −1.00 = almost perfect. The variability of agreement (kappa values) due to having several student ratings being compared to the expert assessment was visualized using box-and-whisker plots.

Positive and Negative Predictive Values (**PPV, NPV**) and Spearman Rank correlations were used to compare the strength of the association between each of the body signals and the overall rating of the chickens as stressed or unstressed. The Spearman correlation coefficient ($\mathbf{r_s}$) was interpreted as follows: 0.0 < 0.1 = no correlation, 0.1 < 0.3 = low correlation, 0.3 < 0.5 = moderate correlation, 0.5 < 0.7 = high correlation, 0.7 < 1 = very high correlation (Bühl and Zofel, 2005). *P*-values <0.05 were considered significant.

Finally, it was assessed whether the number of individual positive scores was associated with the observers' overall rating of the chicken as stressed or unstressed using the SUM function in MICROSOFT Excel (Microsoft Office 365).

RESULTS

Selective Review The selective literature review yielded 33 publications and 25 textbooks, which were searched for suitable information on the posture of a bird in discomfort. In total, 15 books, 4 journal articles and 5 website entries contained explicit descriptions of posture in unwell birds. Of these, 3 referred to birds in general, 6 to poultry in general, 7 to pet and ornamental birds, 1 was specific to turkeys and 7 were specific to chickens (Table 2).

Especially ruffled and fluffed feathers, a crouched posture with hanging wings, a bent tail and a tucked in head were mentioned as early signs of a possible discomfort of the "stressed chicken." Closed eyes and/or an open beak when breathing are further indications of a disturbed general condition and/or distress/pain.

Body signals associated with distress in birds in the literature reviewed: fullness of plumage (n = 21), leg posture (n = 16), eye closure (n = 11), beak opening (n = 8), tail position (n = 8), wing position (n = 8), head position (n = 7) (Table 2, Figure 2).

The "depressed/sick bird" look is described differently by different authors and contains the body signals in different combinations. Moreover, the stressed expression of the body signals is sometimes described slightly

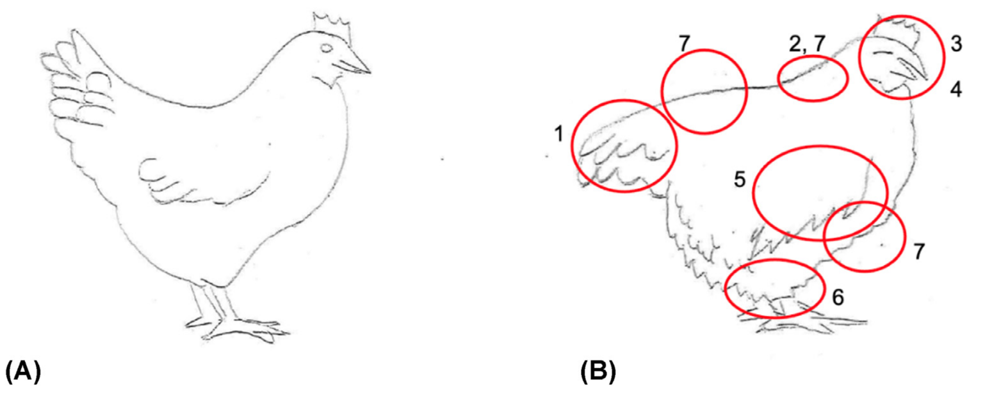

Figure 2. Schematic representation of the 7 body signals; tail position, head position, eye closure, beak opening, wing position, leg posture and fullness of plumage: (A) Unstressed chicken standing upright with tail and head raised high, eyes open, beak closed while breathing, wings in physiological position held close to the body, and streamlined plumage. (B) Stressed chicken showing signs of discomfort, distress, pain, or illness, with a drooped tail (1), a tucked-in head (2), (partially) closed eyes (3), a (partially) open beak for breathing (4), wings drooping (5), expressing a hunched posture with legs bent (e.g., sitting or lying down) (6), with ruffled and fluffed plumage beginning in the neck, lower back, and lower breast area (7).

differently from the summary shown in Table 2 for some bird species.

Poultry, like chickens, in pain shows a lowered head (Gentle, 2011; PoultryDVM, 2021), while a bent or stretched neck or a under-one-wing-tucked head can be seen in unhealthy turkeys (Mailyan, 2019). Some birds will have their heads tucked over their shoulders when showing signs of illness (Doneley, 2016).

An open beak with tail bobbing while breathing is mentioned as a common symptom in sick (ornamental) birds (Pees, 2018), sick turkeys may exhibit wheezy breathing (Mailyan, 2019) and having hanging, upwardly drawn wings (Mailyan, 2019). In ornamental and pet birds periods of illness can cause spread wings (Pees, 2018).

Poultry in pain tends to sit down (Gentle, 2011) and generally exhibits a crouched posture (Swayne, et al., 2020). Chickens in prolonging pain, stress or fear, too, show a crouched posture (Paul-Murphy, 2006). A hunched posture has been described for sick chickens (Bestman et al., 2013; Damerow, 2015; Bestman et al., 2018; Gartner et al., 2018). They may be sitting (Bestman, et al., 2013; Bestman, et al., 2018), slumped (Bestman, et al., 2018), or even lying down (Zhuang and Zhang, 2019), or can be seen sitting on their hocks (PoultryDVM, 2021). An unhealthy turkey may have drawn in legs (Mailyan, 2019).

Generally, a bird in pain shows an altered, loose sitting posture (Powers, 2015). An ornamental bird sitting on the ground may be sick (Kostka and Bürkle, 2010; Pollock, 2011b) or in poor general condition (Christen, 2011; Scope, 2011). It may have its legs spread apart (Christen, 2011; Scope, 2011), showing a hunched posture (Kostka and Bürkle, 2010) or unevenly loaded feet (Pees, 2018).

In contrast to that, a healthy/unstressed chicken has been described in the literature as standing upright, with head and tail raised high, streamlined plumage and clear eyes open. The beak is closed when breathing, the wings are held close to the body in physiological position (see Pees (2018) and Mikoni, et al. (2023b)) (Figure 2).

Stressed Chicken Scale The study utilized a selective review to identify potential body signals that may indicate pain, stress, discomfort, or disease in chickens. From these, 7 body signals indicative of a distressed or sick bird were identified: A dropped tail, a tucked head, (partially) closed eyes, an open beak to breathe, drooping wings, a crouched posture with legs bent and ruffled plumage (Table 2). These signals were used to create the Stressed Chicken Scale (Figure 1). Varying degrees and combinations of expression of these body signals are possible in stressed chickens. Fullness of plumage can be seen especially in the neck, lower back, and lower breast area. The primary feathers are the first to become visible when a wing drop begins.

Recognition of Body Signals. For the body signals head position, leg posture, tail position, fullness of plumage, wing position, and beak opening observers opted for the rating "I don't know" in under 5% of cases (head position 0.5%, leg posture 1%, tail position 1.5%, fullness of plumage 2.5%, wing position 3.25%, beak opening 3.5%). For eye closure, the "I don't know" option was selected in 5.75% of cases, with the option being used 23 times (Figure 3).

Reliability Testing. There was substantial agreement between observer overall rating and expert rating ($\kappa = 0.66$). When looking at individual signals, agreement again was substantial for beak opening ($\kappa = 0.8$), tail position ($\kappa = 0.75$), head position ($\kappa = 0.72$), general appearance ($\kappa = 0.68$), and leg posture ($\kappa = 0.67$), and moderate for wing position ($\kappa = 0.58$), eye closure ($\kappa = 0.51$) and fullness of plumage ($\kappa = 0.54$). As depicted in Figure 4, eye closure in addition to having the lowest average kappa showed a rather wide value range.

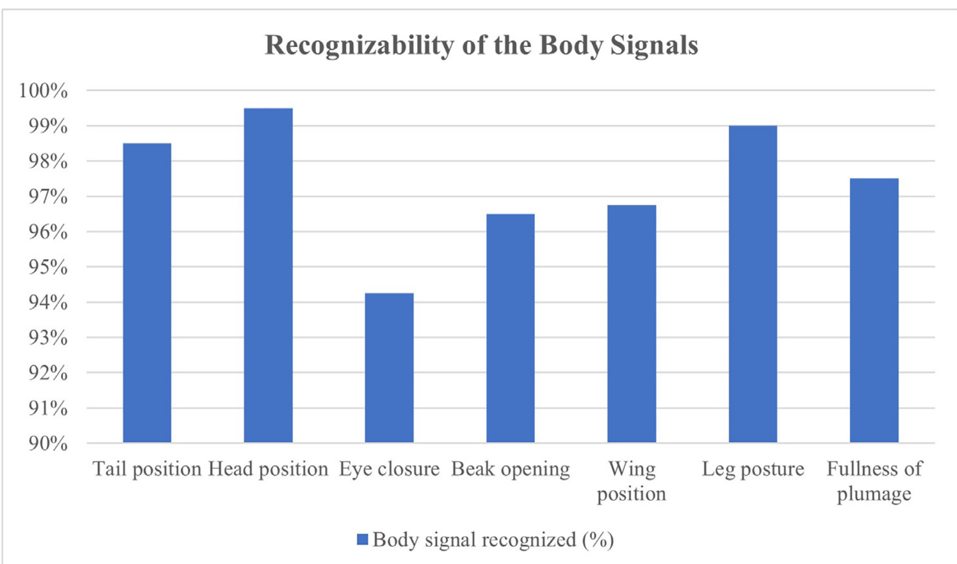

Figure 3. Body signal recognizability in percent. An "I don't know" option was used to determine how confident observers were in recognizing the body signals in the pictures shown. Both response options, parameter "visible" (= stressed expression, including body expressions as seen in the 3 sample pictures on the scale) and "not visible" (= unstressed expression), were counted as recognizing the body signal.

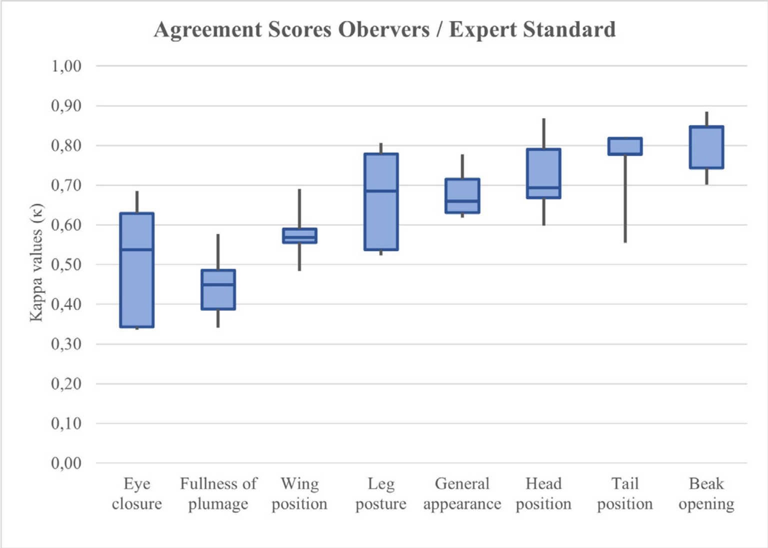

Figure 4. Agreement scores between observers and expert standard. Distribution of agreement scores of Cohen's Kappa values between each observer and expert standard/reference. Boxes represent the difference between the 25th and 75th percentiles of the data (interquartile range **IQR**) while feathers indicate the spread of the data (minimum – maximum).

Overall, interobserver reliability was moderate ($\kappa = 0.53$). The highest interobserver reliability was found with substantial agreement for the body signals: Beak opening ($\kappa = 0.78$, ranging from 0.66 to 0.95) and tail position ($\kappa = 0.76$, ranging from 0.66 to 0.87). This was followed by moderate agreement for the body signals: Head position ($\kappa = 0.59$, ranging from 0.41 to 0.71), general appearance ($\kappa = 0.54$, ranging from 0.42 to 0.69), leg posture ($\kappa = 0.51$, ranging from 0.29 to 0.73), and wing position ($\kappa = 0.44$, ranging from 0.29 to 0.61) and fair agreement for the body signals: Fullness of plumage ($\kappa = 0.33$, ranging from 0.17 to 0.59) and eye closure ($\kappa = 0.31$, ranging from -0.01 to 0.46). Again, eye closure showed the widest range of kappa values (Figure 5).

Parameter Effects. The parameter "closed eyes" had the highest positive predictive value (90.16%) for the final outcome "stressed". This was followed by "tail drooping" (PPV: 85.4%), "wing drooping" (PPV: 84.4%), "open beak" (PPV: 83.82%), "ruffled plumage" (PPV: 82.44%), "head tucked in" (PPV: 79.84%) and "bent legs" (PPV: 72.38%) (Figure 6).

The parameter "tail dropping" had the highest negative predictive value (64.34%), so a chicken not showing this body signal was most likely to be labeled "unstressed" for the final outcome. This was followed by "bent legs" (NPV: 63.88%), "closed eyes" (NPV: 60.26%), "head tucked in" (NPV: 59.44%), "wings dropping" (NPV: 53.5%), "open beak" (NPV: 40.38%) and "ruffled plumage" (NPV: 40.36%) (Figure 6).

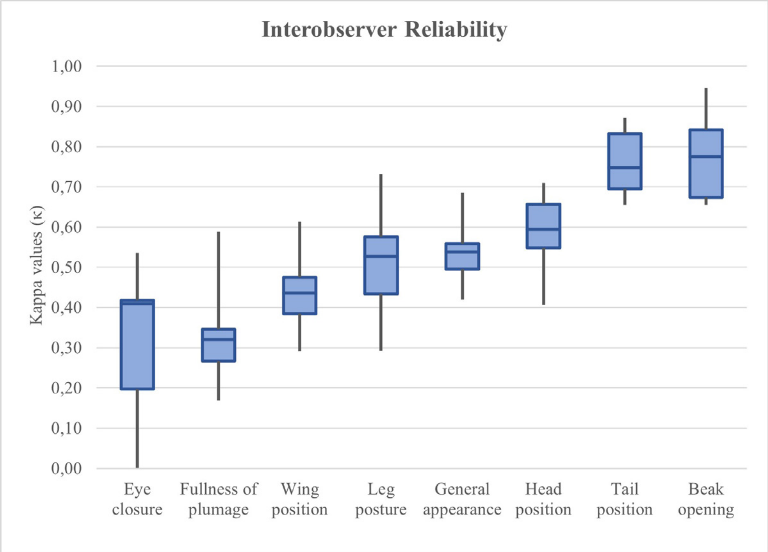

Figure 5. Interobserver reliability. Distribution of agreement scores of Cohen's Kappa values between pairs of individual observers. Boxes represent the difference between the 25th and 75th percentiles of the data (interquartile range IQR) while feathers indicate the spread of the data (minimum – maximum).

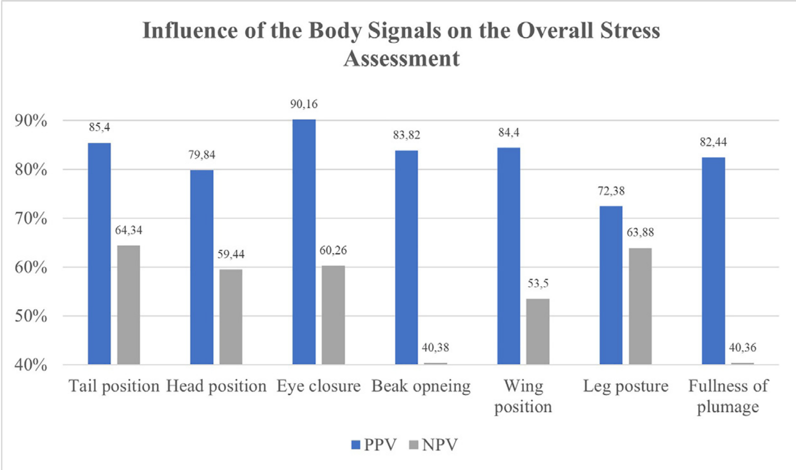

Figure 6. Influence of the body signals on the overall stress assessment of chickens. Positive predictive value (**PPV**) and negative predictive value (**NPV**) were used to show the probability that a chicken displaying a particular body signal in its stressed/unstressed expression would be classified as stressed/unstressed. A chicken that meets the "eyes closed" parameter will be classified as stressed with a probability of more than 90%, while there is a 64.34% probability that a chicken that does not meet the "tail down" parameter will be classified as unstressed.

There was a high correlation between the overall appearance and the tail position ($r_s = 0.51$) and a moderate correlation between the overall appearance and the body signals: Fullness of plumage ($r_s = 0.43$), head position ($r_s = 0.4$), wing position ($r_s = 0.37$) and leg posture ($r_s = 0.32$). These correlations were found to be significant for the body signals: Tail position, head position, fullness of plumage, leg posture, and wing position with $p = <0.001$, $p = <0.001$, $p = 0.01$, $p = 0.01$, $p = 0.02$, respectively.

Correlation was low for the body signals: Eye closure ($r_s = 0.19$) and beak opening ($r_s = 0.17$), and not significant ($p = 0.17$, $p = 0.21$).

In addition, we found that the number of body signals (equally weighted) shown in either stressed or unstressed expression by a chicken affected the overall rating of the chicken (≥ 4 body signals in stressed expression led to an overall rating as stressed, pictures with ≤ 2 parameters in stressed expression were rated as unstressed) (Figure 7).

DISCUSSION

Despite the fact that avian species are commonly kept as companion and laboratory animals (Paul-Murphy et al., 2009), information regarding pain (Paul-Murphy et al., 2009) or stress (Mikoni et al., 2022) assessment in these species is scarce. Grimace scales and facial expression scales are excellent tools to assess pain in other species and can be used in laboratory assessments and as educational tools for study and training (Mikoni et al., 2022). However, at this point in time, a scale comparable to grimace/facial expression scales does not exist for chickens. Hence, the aim of this study was to develop a non-invasive tool for stress

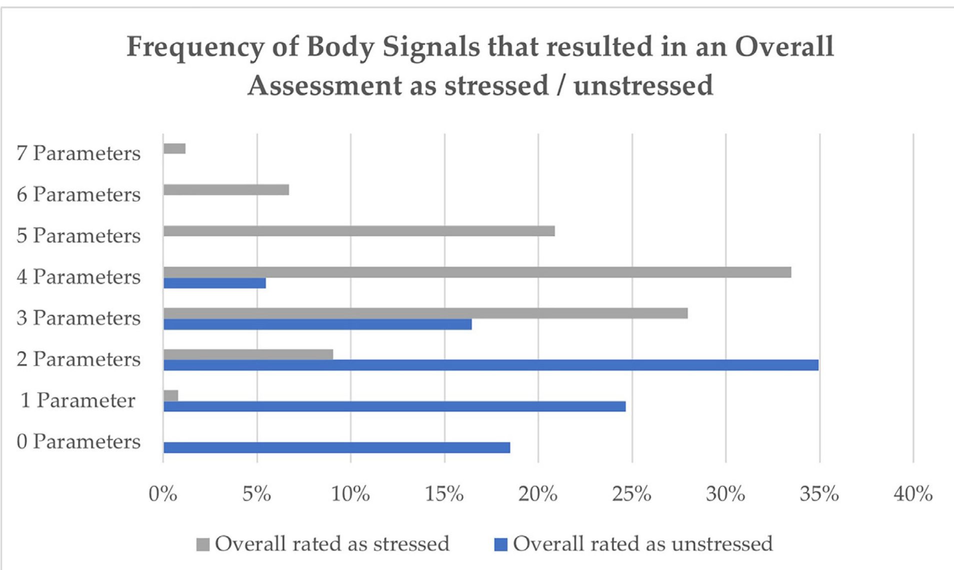

Figure 7. Frequency of body signals in either stressed or unstressed expression that resulted in an overall assessment as stressed or unstressed. Frequency (%) of the number of body signals rated as "parameter visible" for the overall rating as stressed and frequency (%) of the number of body signals rated as "parameter not visible" for the overall rating as unstressed.

assessment in chickens. More specifically, we set out to compile common body signals indicative of discomfort in chickens and create a scoring system based on these body signals. A selective literature review revealed that the most common body signals indicative of discomfort in chickens are: A dropped tail, a tucked head, (partially) closed eyes, an open beak to breathe, drooping wings, a crouched posture with bent legs, and ruffled plumage. For a good assessment of pain or stress, one should use validated indicators that are appropriate for the target species (Prunier et al., 2013). Therefore, the selective review was initially focused on chickens. In order not to miss a potentially suitable body signal, the selective review was subsequently extended to other avian species (Table 2).

The scale developed in this study was based on dual-purpose and laying lines as these are the most common breeds in animal testing and poultry farming (egg production). For this purpose, the SCS was invented and evaluated using pictures of hens and young roosters from fitting breeds and excludes breeds with outstanding features (Table 3). Further research is needed to determine the applicability to other breeds or any necessary modifications to the scale itself.

When presented with images of chickens exhibiting signs of distress, observers were able to recognize the 7 most common indicators of discomfort, after a short training session. However, some of the signals seemed to be easier to recognize than others. For the body signal head position, the answer "I don't know" was only chosen twice. In contrast, in 23 cases, observers were unsure whether or not the chicken had its eyes closed. This is in line with findings regarding facial expression scores with up to 21% of observers unable to recognize "tension above the eye" in horses by Dalla Costa et al. (2014). Possible reasons include varying picture quality and variations in the coat color of the horses, with darker coat colors proving to be more difficult for observers to rate and, unsurprisingly, a picture of a dark-coated horse against a dark background also seemed to be problematic (Dalla Costa et al., 2014). In our study, all chickens were filmed in front of a uniform screen, which rules out the background as a factor that could cause problems. Nevertheless, we looked at the 10 screenshots with either the best or worst overall observer agreement compared to the expert standard, as well as the 10 ones with the most used "I don't know" options and were unable to identify any breed or plumage color that was particularly bad or good.

When testing the applicability of a grimace scale for ferrets, the parameters "orbital tightening" and "ears" were undistinguishable for 4% and 9% of observers, respectively with a larger percentage of uncertainty among images of ferrets with longer fur (Reijgwart et al., 2017). Di Giminiani et al. (2016) showed that, when using a grimace scale on pigs, only 2% of observers were unable to identify "orbital tightening" whereas 72% were unable to identify "nostril dilatation". In the study by Di Giminiani, et al. (2016), a lamp suspended above the observation area may have caused unwanted shadows and led to these results. Overall, poor lighting, poor picture quality as well as the fast movements of the piglets could have resulted in these scores (Di Giminiani, et al., 2016). The images used in our study were screenshots from video material, which may have affected the quality and resolution. Keating et al. (2012) showed in the rabbit grimace scale that variable picture quality influenced the overall accuracy of global pain assessment.

In line with our findings for participants using the "I don't know" option, the body signal eye closure showed rather wide value ranges for the agreement scores. However, there were good, respectively moderate, agreement scores for eye closure and fullness of plumage, while the body signals beak opening and tail position, were rated similar to each other. This indicates a certain degree of subjectivity when interpreting body signals. Similar observations have been made for other scoring systems such as facial expression scores for farm animals with high levels of interobserver reliability for the ear region and greater degrees of variance for the orbital and eye region (Fischer-Tenhagen et al., 2022). Intraclass Correlation Coefficient (**ICC**) ranged from 0.58 for "flattening of the profile" and "strained nostrils" to 0.97 for "stiffly backward ears" in horses (Dalla Costa et al., 2014), while the interobserver reliability for the ferret grimace scale ranged from ICC 0.85 for "nose bulging" to ICC 0.97 for "orbital tightening" (Reijgwart et al., 2017). The overall prevalence of individual symptoms as well as the image quality may have biased the results in our study. Further studies are needed to test whether chickens with higher levels of stress are more likely to express certain body signals that may have been underrepresented in our current findings.

When assessing the chicken as whole ("stressed" or "unstressed"), agreement between observer ratings and expert ratings was moderate ($\kappa = 0.54$; ranging from 0.42 to 0.69). In a study conducted on donkeys, Orth et al. (2020) found that observers who have previous experience handling donkeys are more likely to choose the same score as an expert than observers who have no experience with donkeys. Thus, the number of false negative scores for each parameter tends to be lower when working with experienced observers, who are able to recognize more subtle signs. The observers in our study were veterinary students with little experience in handling chickens, which may explain this finding.

We found that, when assessing the chicken as whole ("stressed" or "unstressed"), certain body signals appear to be weighted more heavily than others. In addition, Chickens' leg posture, plumage and its eyes are the most commonly described body parts for health assessment in the literature. This is in line with findings from facial expression studies, suggesting that assessment of the orbital region appears to have a greater impact on overall wellbeing assessment outcomes than other factors (see Reijgwart, et al. (2017)).

The effect of different body signals on the overall assessment of chickens as either stressed or unstressed, were measured using the positive predictive value (**PPV**) and negative predictive value (**NPV**). In our study, the parameters “eyes closed”, “tail drooping” and “wings drooping” had the highest positive predictive values. In addition, the observers showed the greatest degree of uncertainty when rating whether or not the chicken had its eyes closed. “Wings drooping” was found least often in the literature reviewed (Table 2) but seems to have a great impact on the judgement by an observer, according to the results of this study.

The body signals tail position and leg posture had the highest negative predictive values for overall wellbeing of a chicken in our study. In line with this, the body signal tail position showed a high correlation with the overall impression of a chicken.

Although Spearman’s correlation coefficient is considered more accurate to assess overall correlation (irrespective of direction), PPV/NPV that are based on sensitivity (**SE**), specificity (**SP**) and prevalence of the respective (positive) signal in the sample, show the diagnostic reliability (correctness) of a positive or negative observer classification. Comparison of PPV and NPV values between the assessed signals may provide an opportunity to assign weights to body signals. More research is needed to assess that option and the resulting performance improvement of the scoring system.

The number of indicators of discomfort seen in one image also affected the outcome of the overall stress assessment. If a chicken showed 4 or more indicators of discomfort, it was considerably more likely to be rated as “stressed” overall. If the observer saw 2 or fewer indicators of discomfort, they were considerably more likely to opt for the answer “unstressed” in the overall assessment. Similar results have been recorded for the Horse Grimace Scale by Dalla Costa et al. (2014) as well as composite pain scales for rabbits (Banchi et al., 2020) and cats (Reid, et al., 2017).

In the screenshots used in our study, certain body signals were seen less often in a stressed expression than others. Further research is warranted to determine if this is because they are only expressed at high levels of distress or if they are generally uncommon in chickens. Additionally, not all chickens showed all body signals at the same time or in a stressed expression at all. As shown in the study by Orth et al. (2020), the gender of the observers and their experience with the animal species in question can influence their perception of pain states in that animal. We hypothesize that date or mood effects among observers may have also influenced the results in our study and possibly led to a cognitive bias, as different groups of observers rated the screenshots at different times. However, we did not pursue this matter any further.

We can assume that the chickens used in our study experienced mild to moderate stress levels at most (see Balcombe et al. (2004)). It is important to note that some animals who are not in pain still exhibit symptoms of a Grimace Scale and may be falsely classified as being in pain (Dalla Costa et al., 2014). With the expression of pain and discomfort in chickens not fully understood and the fact that no highly stressed animals were included in this study, the results must be viewed with some caution.

Other existing assessment tools for birds, such as ethograms (Desmarchelier et al., 2012; Casey-Trott and Widowski, 2016; Turpen, et al., 2019) and pain scales (Desmarchelier et al., 2012; Mikoni et al., 2023a; Mikoni et al., 2023b) often relate to animal behavior, which was not the main focus of this study. Automated systems (Okinda et al., 2019; Zhuang and Zhang, 2019) and animal welfare guides for chickens (Welfare-Quality-consortium, 2009; Keppler et al., 2017; Welfare-Quality®-consortium, 2019) focus on the health of the flock, whereas the SCS focuses on one chicken at a time. Even if there are studies describing facial expressions in birds (Bertin et al., 2018a; Bertin, et al., 2018b; Bertin et al., 2020), they focus on positive emotions rather than discomfort. Infographics (ITIS, 2014; Mailyan, 2019; PoultryDVM, 2021) mostly show an image of a bird with multiple body signals present simultaneously. In our dataset, body signals were present in different combinations. We conclude that all body signals can appear together, alone, and in different combinations.

Our results suggest that the SCS may serve as a useful tool in training students and laboratory animal technicians to identify stressed or sick chickens more easily. Despite the fact that our training was only 20 min long, interobserver agreement between the observers and the expert standard was substantial. This is in contrast to the results of Dai et al. (2020), who showed that 30 min of training may not be sufficient to increase interobserver reliability for the Horse Grimace Scale in observers without experience with horses. Some of the observers in our study also had little or no experience with chickens. This may indicate that the SCS is an easy-to-learn tool with good usability. It provides the ability to look at a chicken on an individual level, which could help caregivers to focus on explicit body signals. This may be helpful in identifying unwell birds earlier than other systems, which sometimes only detect dead animals on a flock basis (Zhuang and Zhang, 2019).

CONCLUSIONS

Standardized Scales can help to recognize and assess signals of distress and pain in animals. They are particularly useful in a laboratory context and for prey species. Observers in this study were able to recognize body signals suitable for classifying chickens as stressed after a short training session with moderate interobserver reliability and substantial agreement compared to the expert standard. Thus, scales such as the SCS may be an excellent tool for educational and training purposes.

Further research and field trials are needed to test the applicability and accuracy of the Stressed Chicken Scale under real-life conditions and with a larger number of chickens. In its current state, the SCS is an image catalog of sample pictures of stressed and unstressed chickens, which allows only one individual to be scored at a time. This is time consuming and carries the risk of human error. Nevertheless, this scale could help to make a statement about a chickens’ discomfort and therefore potentially find use not only in laboratory animal husbandry, but also as part of precision livestock farming in the commercial poultry industry.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2024.103875.

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