Physiology and Reproduction

Effects of putative stressors and adrenocorticotropic hormone on plasma concentrations of corticosterone in market-weight male turkeys

Colin G. Scanes,*† Kayla Hurst,‡ Yvonne Thaxton,* Gregory S. Archer,¶ and Alice Johnson‡

*Department of Poultry Science, University of Arkansas, Fayetteville 72701, USA; †Butterball LLC, Garner, NC 27529, USA; and ¶Department of Poultry Science, Texas A&M AgriLife Research, College Station, TX 77843, USA

ABSTRACT There is limited information on the effects of stress and/or physiological manipulation on plasma concentrations of corticosterone (CORT) in turkeys. Under basal conditions, there was evidence for episodic release of CORT in turkeys. The present studies determine the effects of handling, herding, endotoxin challenge, and challenge with turkey adrenocorticotropic hormone (ACTH) on plasma concentrations of CORT in market-weight male turkeys. Plasma concentrations of CORT were increased after challenge with turkey ACTH, handling together with saline injection or herding (moving birds from one pen to another). There were no effects on plasma concentrations of CORT of the following putative stressors: handling per se, endotoxin challenge, or of placing in an inverted position on simulated shackles.

Key words: turkey, shackling, herding, corticosterone, stress

INTRODUCTION The avian hypothalamic–pituitary–adrenocortical (HPA) axis consists of corticotropin-releasing hormone together with arginine vasotocin from the hypothalamus promoting release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland which, in turn, stimulates production and release of corticosterone (CORT) from the adrenocortical cells (Carsia, 2016; Nagarajan et al., 2017). It is presumed that the HPA axis will be similar in turkeys to that of other species. However, that is not necessarily the case as there is limited information available on the effects of production and other stressors in turkeys (Erasmus, 2017). In chickens, plasma concentrations of CORT are increased 30 min after intravenous (i.v.) ACTH challenge in young birds, irrespective of age, with similar responses being observed between 1 and 20 IU kg⁻¹ (Radke et al., 1985; Webb and Mashaly, 1985). In turkeys, the situation is less clear-cut. There was no increase in plasma concentrations of CORT in turkeys injected intramuscularly with porcine ACTH after 1, 2, and 4 h (Davis and Siopes, 1985, 1987). Moreover, in some cases, plasma concentrations of CORT were decreased after ACTH challenge (Davis and Siopes, 1985, 1987). However, an increase was consistently observed 6 h after intramuscular (i.m.) ACTH challenge (Davis and Siopes, 1985, 1987). On the contrary, in one study, plasma concentrations of CORT were elevated 30 min after i.v. challenge with 1 and 5 IU ACTH (Davis and Siopes, 1987). Moreover, surprisingly, plasma concentrations of CORT were depressed after i.v. challenge with 10 IU ACTH (Davis and Siopes, 1987). The lack of consistency might be attributable to differences with the turkey HPA axis and/or to species specificity of the response to exogenous porcine ACTH, that is, structural differences between porcine ACTH used in the studies and endogenous turkey ACTH. Turkey ACTH has been purified and sequenced (see Figure 1) (Chang et al., 1980; Yamashiro et al., 1984). However, the published structure differs markedly from the sequence of turkey ACTH deduced from cDNA and from the sequences of amino acids in other birds, irrespective of the closely related species in the order Galliformes and other species in superorders Galloanserae (containing both galliforms and ducks of the order Anseriformes) or Neaves or even in the infraclass Palaeognathae (Figure 1). The present studies reexamine the effects of ACTH on plasma concentrations of CORT using the synthesized turkey ACTH to conform with the
deduced sequence. In addition, a fragment of human ACTH was used for comparison based on the previous report of its ability to stimulate release of CORT from turkey adrenal cells (Carsia and McIlroy, 1998; Carsia and Weber, 2000).

Circulating concentrations of CORT have been widely used in chickens as a physiological index of stress (Carsia, 2016; Scanes, 2016), with plasma concentrations of CORT being increased when birds experience either prolonged or acute stresses. For instance, plasma concentrations of CORT progressively increased with duration of manual restraint (placing the bird on its side) in adult laying hens (Korte et al., 1997). Similarly, there were progressively higher increases in plasma concentrations of CORT when broiler chickens were placed in mock shackles in an inverted position (Kannan and Mench, 1997; Kannan et al., 1997; Bedanova et al., 2007).

There is limited information on the effects of stress and/or physiological manipulation on plasma concentrations of CORT in turkeys (Carsia, 1998; Scanes, 2016). However, most studies have been conducted at least 15 yr ago. Based on the limited information, plasma concentrations of CORT progressively increased with duration of manual restraint (placing the bird on its side) in adult laying hens (Korte et al., 1997). Similarly, there were progressively higher increases in plasma concentrations of CORT when broiler chickens were placed in mock shackles in an inverted position (Kannan and Mench, 1997; Kannan et al., 1997; Bedanova et al., 2007).

Figure 1. Comparison of the structures of turkey adrenocorticotropic hormone (ACTH) as deduced from cDNA with those of other poultry, human, and porcine, turkey, and ostrich ACTH as determined from peptide sequencing. Black background, white letters: identical sequence with turkey ACTH as deduced from the cDNA sequence. White background, black letters: amino acid residue different from turkey ACTH as deduced from the cDNA sequence. Italic letters: different amino acid residue in turkey ACTH as determined by peptide sequencing compared with the structure deduced from cDNA. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine. Polypeptide structures deduced from cDNA sequences: turkey XM_021391833.1 (identical to turkey variant 1 Genebank XM_010708068.2 and variant 2 XM_019613648.1); chicken NM_001031098.1; quail (Japanese) AB620013.1; guinea fowl XM_021391833.1; duck XM_013103743.2; pigeon XM_021295822.1; human NM_001035256.2, and pig X00135.1. Structures by peptide sequencing: turkey – Chang et al., 1980; Yamashiro et al., 1984; ostrich – Naudé et al., 2006.

Moreover, the stress of trapping is accompanied by increases in plasma concentrations of CORT in wild turkeys (Whatley et al., 1977). Other factors influencing plasma concentrations of CORT include ghrelin (concentration increase shown in the study by Shahryar and Lotfi, 2017), parachlorophenylalanine (an inhibitor of serotonin synthesis), α-methyl tyrosine (an inhibitor of dopamine and norepinephrine synthesis) (concentration increase shown in the study by Martin et al., 1982), and refeeding after starvation (concentration decrease shown in the study by Vizcarra et al., 2018). There is evidence for a diurnal pattern of CORT release. In turkeys on a photoperiod of 12 h of light and 12 h of darkness (12L:12D), there are 2 peaks in plasma concentrations of CORT, one at the beginning of the photophase (the period of daylight) and one at the beginning of the scotophase (the period of night) (Martin et al., 1982). In other studies, there were diurnal shifts in plasma concentrations of CORT in turkeys with peaks in the night and midmorning (as observed in ovariectomized turkeys in the study by Proudman and Opel, 1989 and observed in incubating turkeys in the study by Proudman, 1991). The wide variation in plasma concentrations of CORT in individual incubating hens (Proudman and Opel, 1989; Proudman, 1991) and young turkeys (Bartz et al., 2018) is consistent with episodic CORT secretion. The present study examines the effects of the following putative stressors, namely, simulated shackling, handling, herding, and endotoxin,
on plasma concentrations of CORT in market-weight male turkeys.

**MATERIALS AND METHODS**

**Animals**

Basal plasma concentrations of CORT were determined in market-weight male turkeys raised in 8 open-sided houses in commercial farms in North Carolina, Missouri, and Kansas. This was a part of other studies on plasma concentrations of CORT in young turkeys. Effects of putative stressors and ACTH were determined in market-weight male birds at the Butterball research farm (La Grange, Wayne County, NC). The turkeys were raised on litter under normal industry conditions in accordance with Butterball Animal Welfare Standards. All people who work with the turkeys are trained, at least on a quarterly basis, on the correct care and handling of turkeys.

**Blood Sampling**

All blood samples were taken by board-certified veterinarians by venipuncture into heparinized syringes. Blood samples to examine basal plasma concentrations of CORT were taken from either the jugular vein or brachial vein. Studies examining effects of ACTH or putative stressors (handling, herding, fasting, shackling, or endotoxin challenge) used blood samples taken from the brachial vein. After centrifugation and separation, plasma samples were frozen on dry ice from transportation and stored at −20°C until assay.

**Effect of Stressors or ACTH Challenge**

A series of studies were conducted in a pen in the research farm. These examined effects of a series of stressors on plasma concentrations of CORT: the stressors being handling (for 4 min), herding (after either 4 or 15 min), inversion and being held in shackles (for 4 min), fasting for 24 h (with water available), and either i.v. ACTH challenge (sampled after 30 min) or i.m. endotoxin administration (sampled after 30 min) together with their respective vehicle excipients (saline).

**Herding**

Herding was performed by 4–5 people walking behind groups of 4 to 6 birds to encourage the birds to move into the next pen about 5 m away. Care was taken not to touch the birds during this process. Turkeys that escaped the herding process were herded with the next group.

**Shackling**

A line of 9 shackles was set up to perform the mock shackling study. The shackles used in the study were similar to the ones used in processing plants. Unlike those in a processing plant, the shackles were not movable. Moreover, the light intensity was ambient as opposed to the dim lighting in processing plants. The shackles were suspended at a height of 2 m above the floor level by ropes hanging from the roof supports (3.5 m above the floor level), with each shackle 0.7 m apart. Turkeys were carried (~6 m) to the mock shackles, inverted, placed into the shackles, and suspended for 4 min, at which time blood samples were taken from the brachial vein.

**ACTH Challenge**

Synthetic turkey ACTH (H2N-SYSMEHFRWGKPV GRKRRPIKVYPNGVDEESAESYPVEF-OH) was obtained from New England Peptide, Gardner, MA. The synthetic polypeptide used in the present study was identical to the predicted structure of turkey ACTH from the transcripts of turkey propiomelanocortin (NCBI Reference Sequence: XM_021391833.1) together with both variants X1 (NCBI Reference Sequence: XM_010708068.2) and X2 (NCBI Reference Sequence: XM_019613648.1). The predicted sequence is also very similar to the predicted structures of other poultry species (Figure 1) and other birds (Scanes, unpublished observations). It differs by 8 amino acid residues from that predicted in earlier studies, in which turkey ACTH was sequenced by peptide sequencing (Chang et al., 1980; Yamashiro et al., 1984) (see Figure 1). Vehicle saline or turkey ACTH were injected intravenously (1 mL; 2 mg kg−1; 0.2 IU kg−1; 0.43 mmol kg−1). Human ACTH fragment 1-24 (Sigma-Aldrich, Milwaukee, WI, USA) was injected intravenously (1 mL; 17.2 µg per bird; 1.7 IU; 0.86 µg kg−1; 0.29 µmol kg−1). Blood samples were taken 30 min after the challenge.

**Endotoxin Challenge**

Lipopolysaccharides from *Salmonella enterica* serotype Typhimurium (Sigma-Aldrich, Milwaukee, WI) were used in the study. The endotoxin was suspended in saline by ~20 inversions of the mixture. The suspension was injected intramuscularly into the breast muscle at a concentration of 8 mg per bird (1 mL; 0.4 mg kg−1); similar doses were being used in turkeys (Ball et al., 1962; Emory et al., 1991), chickens (Cheng et al., 2004; Wideman et al., 2004; Baert et al., 2005), and ducks (1, 10, and 100 µg kg−1; Gray and Maloney, 1998). The vehicle, saline, was similarly injected via the i.m. route. Blood samples were taken 30 min after the challenge.

**Physiological Index of Stress: Plasma Concentrations of CORT**

Plasma concentrations of CORT were determined using an ELISA kit (ADI-901–097, Enzo Life Sciences, Farmingdale, NY), the assay being previously used for poultry species (Huth and Archer, 2015; Archer, 2018).
The interassay and intra-assay coefficient of variance was less than 5%.

**Statistics**

Statistical analysis was conducted on both plasma concentrations of CORT and log-transformed plasma concentrations of CORT, the latter following the approach of Proudman and Opel (1989) for plasma concentrations of CORT in turkeys. Data were analyzed using one-way ANOVA and Tukey’s range test. The paired t test was used for repeated sampling, with a maximum of 2 samples taken from the same bird. Differences were considered significant (P < 0.05) when analyzed as both plasma concentrations of CORT and log-transformed plasma concentrations of CORT.

**RESULTS**

**Basal Plasma Concentrations of CORT**

The mean plasma concentration of CORT across houses (n = 8) was 9.01 ± 1.07 ng mL⁻¹. The mean plasma concentration of CORT across birds (n = 139) was 8.86 ± 0.61 ng mL⁻¹, with the difference between the averages between houses and individual birds being explicable by differences in the number of samples taken per house. It is generally assumed that plasma concentrations of CORT in poultry follow a Gaussian distribution. This was examined with the frequency distribution of plasma concentrations of CORT, which is shown in Figure 2. The majority (81.9%) of plasma concentrations of CORT followed a Gaussian or normal distribution and were lower than 15 ng mL⁻¹. On the contrary, 18.1% of plasma concentrations of CORT in individual male turkeys were higher than 15 ng mL⁻¹, 8.7% of plasma concentrations of CORT were higher than 20 ng mL⁻¹, and 5.1% of plasma concentrations of CORT were higher than 25 ng mL⁻¹.

**ACTH Challenge**

Plasma concentrations of CORT were elevated (P < 0.05) 30 min after turkey ACTH challenge (Table 1). Plasma concentrations of CORT were increased by 67.9% compared with those of the saline vehicle–injected birds and by 3.2-fold compared with those of the untreated controls. Plasma concentrations of CORT were also elevated in the saline vehicle–injected and handled birds compared with the untreated controls (Table 1).

**Effect of Putative Stressors**

Effects of some potential stressors on plasma concentrations of CORT are summarized in Tables 1 and 2. Plasma concentrations of CORT were increased (P < 0.05) after handling and i.v. administration of saline (Table 1). However, there were no effects of handling alone on plasma concentrations of CORT (Table 2).

**DISCUSSION**

The circulating concentrations of CORT in market-weight turkeys determined in the present study were similar to those reported by Kiezen et al. (2015) and Vizcarra et al. (2018), but both markedly lower than those reported by El Halawani et al. (1973) or Carsia and McIlroy (1998) and higher than those reported by Bartz et al. (2018). The present data (Figure 2) are consistent with episodic CORT secretion in young turkeys. The possibility of episodic CORT secretion in laying hens has been previously suggested (Etches, 1979). Moreover, there were wide variations in plasma concentrations of CORT in individual cannulated ovariectomized female turkeys with a coefficient of variation of about 100% (Proudman and Opel, 1989). Similarly, high variances of plasma concentrations of CORT were observed in studies conducted on young turkeys (Bartz et al., 2018). Not only these reports and the present data (Figure 2) are consistent with episodic secretion of CORT but also reevaluation of data on plasma concentrations of CORT in cannulated individual ovariectomized turkeys (Proudman and Opel, 1989) provides
direct support for pulsatile release of CORT in turkeys. Episodic or ultradian release of glucocorticoids has been established in mammals (Wallace et al., 1991, analyzed cortisol release in children; Ladewig and Smidt, 1989, analyzed cortisol release in cattle; Fulkerson, 1978, analyzed cortisol release in sheep; Ingram et al., 1999, analyzed cortisol release in deer; Spiga et al., 2011, analyzed CORT release in rats) together with fish (Nichols and Weisbart, 1984, analyzed cortisol release in salmon).

Turkeys responded to turkey ACTH with increases in plasma concentrations of CORT after 30 min, the response being similar to that in chickens (Radke et al., 1985; Webb and Mashaly, 1985). Plasma concentrations of CORT tended to be increased by a fragment of human ACTH, hACTH₁₋₂₄ (Table 1), this being consistent with elevated synthesis and release of CORT from turkey adrenal cortical cells in response to hACTH₁₋₂₄ (Kocsis and Carsia, 1989). Previous in vivo studies also provide support for ACTH influencing CORT release in turkeys but requiring multiple hours to achieve this. For instance, plasma concentrations of CORT were elevated 6 h after i.m. administration of porcine ACTH, but with ACTH reducing plasma concentrations of CORT after 2 and 3 h (Davis and Siopes, 1985). On the contrary, in chickens, plasma concentrations of CORT were consistently elevated 30 min after the ACTH challenge (Davis et al., 1980; Decuyper et al., 1989; Minozzi et al., 2008; Ralph et al., 2015).

Some putative stressors did not influence plasma concentrations of CORT in male turkeys. For instance, plasma concentrations of CORT were not affected by handling alone (Table 2) but were increased by handling and i.v. saline injection (Table 1). In chickens, plasma concentrations of CORT were reported to be elevated by handling (as shown in the study by Kannan et al., 1997 on broiler chickens and Beuving and Vonder, 1978 on laying hens) or repeated blood sampling (Radke et al., 1985). There was also no effect of endotoxin challenge on plasma concentrations of CORT in turkeys. Similarly, there were no overt clinical signs after the administration of endotoxin to young turkeys in an attempt to induce shock (Ball et al., 1962). The lack of an effect of endotoxin challenge on plasma concentrations of CORT in turkeys is in contrast to the reports of increased plasma concentrations of CORT in endotoxin-challenged chickens (Scanes et al., 1980; Johnson et al., 1993; Shini et al., 2008). The lack of an effect of endotoxin in turkeys in the present study may reflect the doses used, species specificity, and/or a relative refractoriness of turkeys to endotoxin (Emory et al., 1991).

Plasma concentrations of CORT tended to be increased by shackling compared with pretreatment in male turkeys and to be higher ($P < 0.05$) than in birds that have been handled (Table 2). The plasma concentrations of CORT in turkeys subjected to mock shackling were very similar to those in turkeys shackled in a commercial processing plant (Scanes et al., 2019). The magnitude of tendency for increased plasma concentrations of CORT is markedly lower than the increases in plasma concentrations of CORT observed in broiler chickens after shackling (Kannan and Mench, 1997; Kannan et al., 1997; Bedanova et al., 2007) or after handling and being inverted multiple times (Kannan and Mench, 1997; Kannan et al., 1997). This is again consistent with turkeys being relatively refractory to this specific stressor. Alternatively following the conceptual model of Grandin and Shivley (2015), turkeys may be exhibiting less fear and/or aversion in response to shackling, therefore perceiving it as less stressful. It is suggested that this may be due to sufficient cervical flexion to enable the head to move from an inverted to vertical or upright position.

The stressor herding evoked marked increases in plasma concentrations of CORT (Tables 1 and 2). It is suggested that this was related to, at least, 3 factors: (1) a fear response to the humans herding the turkeys, (2) the novelty of being herded, and (3) disruption of the social structure of the turkeys. It is suggested that herding may be perceived by turkeys as equivalent to extreme handling in cattle (Grandin and Shivley, 2015). Plasma concentrations of the glucocorticoid cortisol were elevated in cattle with handling and during transportation (as reviewed in the study by Grandin, 1997). It is also to be noted that the increase in plasma concentrations of CORT was not affected by handling alone (Table 2) but were increased by handling and i.v. saline injection (Table 1).

Table 1. Effect of ACTH on plasma concentrations of corticosterone, mean ± (n) SEM, in young male turkeys.

| Procedure               | Plasma concentration of corticosterone, ng mL⁻¹ |
|-------------------------|-----------------------------------------------|
|                         | Before procedure | After procedure |
| ACTH challenge (i.v.)   |                 |                 |
| Saline vehicle          | 17.1 ± (10) 3.05 * |                 |
| Turkey ACTH (0.43 μmol kg⁻¹) | 28.7 ± (10) 3.14 * |                 |
| Human ACTH (0.29 μmol kg⁻¹) | 19.4 ± (10) 2.45 * |                 |

*Different superscript letters in a column indicate difference, $P < 0.05$.

Table 2. Effect of putative stressors on plasma concentrations of corticosterone, mean ± (n) SEM, in young male turkeys.

| Procedure           | Plasma concentration of corticosterone, ng mL⁻¹ |
|---------------------|-----------------------------------------------|
|                     | Before procedure | After procedure |
| Herding             | 11.2 ± (10) 2.12 | 17.9 ± (10) 2.75 * |
| Handling            | 8.5 ± (10) 1.73  | 8.6 ± (10) 1.69 b |
| Shackling           | 9.6 ± (2) 2.67   | 13.8 ± (2) 2.40 b |

*Different superscript letters in a column indicate difference, $P < 0.05$.
concentrations of CORT after herding was of a similar magnitude to that observed in broiler chickens after catching (Nijdam et al., 2005). Interestingly, the plasma concentrations of CORT in turkeys after herding were higher than those after 4 min of shackling. It is argued that further research is needed to develop husbandry techniques that are less stressful for the movement of turkeys.

ACKNOWLEDGMENTS

The helpful comments of Temple Grandin (Colorado State University) and Jesse Grimes (North Carolina State University) on the manuscript are gratefully acknowledged.

REFERENCES

Archer, G. S. 2018. Color temperature of light-emitting diode lighting matters for optimum growth and welfare of broiler chickens. Animal 12:1015–1021.

Baert, K., L. Duchateau, S. De Baover, M. Cherlet, and P. De Baeker. 2005. Antipyretic effect of oral sodium salicylate after an intravenous E. coli LPS injection in broiler chickens. Br. Poult. Sci. 46:137–143.

Ball, R. A., J. H. Shautter, R. E. Burger, and B. S. Pomeroy. 1962. Effects of endotoxin on turkey poult's. Proc. Soc. Exper. Biol. Med. 110:753–756.

Bartz, B. M., D. R. McIntyre, and J. L. Grimes. 2018. Effects of management related practices on turkey hen performance supplemented with either Original XPCTM or AviCareTM. Front. Vet. Sci. 5:1–8.

Bedanova, I., E. Voslarova, P. Chloupek, V. Pistekova, P. Suchy, J. Blahova, R. Dobsikova, and V. Vecerek. 2007. Stress in broilers resulting from shackling. Poult. Sci. 86:1065–1069.

Beuving, G., and G. M. Vonder. 1978. Effect of stressing factors on corticosterone levels in the plasma of laying hens. Gen. Comp. Endocrinol. 35:153–159.

Carsi, R. V. 2016. Adrenal. Pages 577–611 in C. G. Scanes (Ed.), Sturkie’s Avian Physiology, Academic Press, New York.

Carsi, R. V., and P. J. McClory. 1998. Dietary protein restriction stress in the domestic turkey (Meleagris gallopavo) induces hypofunction and remodeling of adrenal steroidogenic tissue. Gen. Comp. Endocrinol. 109:140–153.

Carsia, R. V., and H. Weber. 2000. Remodeling of turkey adrenal steroidogenic tissue induced by dietary protein restriction: the potential role of cell death. Gen. Comp. Endocrinol. 118:471–479.

Chang, W. C., D. Chung, and C. H. Li. 1980. Isolation and characterization of beta-lipotropin and adrenocorticotropin from turkey pituitary glands. Int. J. Pept. Protein Res. 17:270–276.

Cheng, H. W., R. Freire, and E. A. Pajor. 2004. Endotoxin stress responses in chickens from different genetic lines. 1. Sickness, behavioral, and physical responses. Poult. Sci. 83:707–715.

Davis, G. S., and T. D. Siopes. 1985. Adrenal cortical response of toms poult's. Poult. Sci. 64:2189–2194.

Davis, G. S., and T. D. Siopes. 1987. Plasma corticosterone response of turkeys to adrenocorticotropic hormone: age, dose, and route of administration effects. Poult. Sci. 66:1527–1532.

Davison, T. F., C. G. Scanes, S. Harvey, and I. H. Flack. 1980. The effect of an injection of corticosteroid on plasma concentrations of corticosteroid, growth hormone and prolactin in two strains of domestic fowl. Br. Poult. Sci. 21:287–293.

Decuypere, E., V. M. Darras, K. Vermijlen, and E. R. Kihm. 1989. Developmental changes in the corticosterone response to corticotrophin and in the adrenal corticosterone content of rapid and slow growing strains of chickens (Gallus domesticus). Br. Poult. Sci. 30:699–709.

El Halawani, M. E., P. E. Waibel, J. R. Apple, and A. L. Good. 1973. Effects of temperature stress on catecholamines and corticosterone of male turkeys. Am. J. Physiol. 224:384–388.

Emory, D. A., K. V. Nagarava, V. Sivanandan, B. W. Lee, C. L. Zhang, and J. A. Newman. 1991. Endotoxin lipopolysaccharide from Escherichia coli and its effects on the phagocytic function of systemic and pulmonary macrophages in turkeys. Avian Dis. 35:901–909.

Erasmus, M. 2017. Welfare in Turkey production. Pages 263–292 in J. Mench (Ed.), Advances in Poultry Welfare, 1st ed. Woodhead Publishing, Duxford, United Kingdom.

Etches, R. J. 1979. Plasma concentrations of progesterone and corticosterone during the oestrus cycle in the hen (Gallus domesticus). Poult. Sci. 58:211–216.

Fullerton, W. J. 1978. Synchronous episodic release of cortisol in the sheep. J. Endocrinol. 79:131–132.

Grandin, T. 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75:240–257.

Grandin, T., and C. Shively. 2015. How farm animals react and perceive stressful situations such as handling, restraint, and transport. Animals (Basel) 5:1233–1251.

Gray, D. A., and S. K. Maloney. 1998. Antiinflammatory and anxiolytic effects of environmental enrichment on plasma corticosterone concentrations in feedlot fed animals. J. Physiol. 514:605–610.

Huth, J. C., and G. S. Archer. 2015. Comparison of two LED light bulbs to a dimmable CFL and their effects on broiler chicken growth, stress and fear. Poult. Sci. 94:2027–2036.

Ingram, J. R., J. N. Crockford, and L. R. Matthews. 1999. Ultradian, circadian and seasonal rhythms in cortisol secretion and adrenal responsiveness to ACTH and yarning in unrestrained red deer (Cervus elaphus) stags. J. Endocrinol. 162:289–300.

Johnson, R. W., S. F. Curtis, R. Dantzer, J. M. Bahr, and K. W. Kelley. 1993. Sickness behavior in birds caused by peripheral or central injection of endotoxin. Physiol. Behav. 53:343–348.

Kannan, G., and J. A. Mench. 1997. Prior handling does not significantly reduce the stress response to pre-slaughter handling in broiler chickens. Appl. Anim. Behav. Sci. 51:87–99.

Kannan, G., J. L. Heath, C. J. Wabeck, and J. A. Mench. 1997. Shackling of broilers: effects on stress responses and breast meat quality. Br. Poult. Sci. 38:323–332.

Kiezen, J. B. Kaminska, J. Jankowski, and L. Dusza. 2015. Concentrations of the adrenocorticotropic hormone, corticosterone and sex steroid hormones and the expression of the androgen receptor in the pituitary and adrenal glands of male turkeys (Meleagris gallopavo) during growth and development. Gen. Comp. Endocrinol. 217:218–220.

Koecsia, J. F., and R. V. Carsia. 1989. Steroidogenetic properties of isolated turkey adrenocortical cells. Domest. Anim. Endocrinol. 6:121–131.

Korte, S. M., G. Beuving, W. Ruesing, and H. J. Blokhuis. 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. Physiol. Behav. 62:437–441.

Ladewig, J., and D. Smidt. 1989. Behavioral, episodic secretion of cortisol, and adrenocortical reactivity in bulls subjected to tethers. Horm. Behav. 23:344–360.

Martin, J. T., M. El Halawani, and R. E. Phillips. 1982. Diurnal variation in hypothalamic monamines and plasma corticosterone in the turkey after inhibition of tyrosine hydroxylase or tryptophan hydroxylase. Neuroendocrinology 34:191–196.

Minozzi, G., D. Guénée, M. Couty, D. Gourichon, F. Minvielle, and M. H. Pinaud-van der Laan. 2008. Circulating corticosterone re- action to restraint and adrenocorticotropic hormone administration in white leghorns selected for immune response traits. Poult. Sci. 87:2225–2230.

Nagarajan, G., A. Jurkevich, S. W. Kang, and W. J. Kuenzel. 2017. Anatomical and functional implications of corticotrophin-releasing hormone neuropeptides in a septal nucleus of the avian brain: an emphasis on ghial-neuronal interaction via V1a receptors in vitro. J. Neuroendocrinol. 29:1249–1259.

Naudé, R. W., O. Oelofsen, A. Takahashi, M. Amano, and H. Kawachi. 2006. Molecular cloning and characterization of preproopipecolactin (prePOMC) cDNA from the ostrich (Struthio camelus). Gen. Comp. Endocrinol. 146:1051–1057.

Nichols, D. J., and M. Weisbart. 1984. Plasma cortisol concentrations in Atlantic salmon, Salmo salar: episodic variations, diurnal change, and short-term response to adrenocorticotropic hormone. Gen. Comp. Endocrinol. 56:169–176.
Nijdam, E., E. Delezie, E. Larnbooij, M. J. A. Nabuurs, E. Decuyper, and J. A. Stegeman. 2005. Comparison of bruises and mortality, stress parameters, and meat quality in manually and mechanically caught broilers. Poult. Sci. 84:467–474.

Proudman, J. A. 1991. Daily rhythm of prolactin and corticosterone in unrestrained, incubating turkey hens. Dom. Anim. Endocrinol. 8:65–70.

Proudman, J. A., and H. Opel. 1989. Daily changes in plasma prolactin, corticosterone, and luteinizing hormone in unrestrained ovariectomized turkeys. Poult. Sci. 68:177–184.

Radke, W. J., C. M. Albsasi, A. Rees, and S. Harvey. 1985. Stress and ACTH stimulate aldosterone secretion in the fowl (Gallus domesticus). Comp. Biochem. Physiol. A. 82:285–288.

Ralph, C. R., P. H. Hemsworth, B. J. Leury, and A. J. Tilbrook. 2015. Relationship between plasma and tissue corticosterone in laying hens (Gallus gallus domesticus): implications for stress physiology and animal welfare. Domest. Anim. Endocrinol. 50:72–82.

Scanes, C. G. 2016. Biology of stress in poultry with emphasis on glucocorticoids and the heterophil to lymphocyte ratio. Poult. Sci. 95:2208–2215.

Scanes, C. G., G. F. Merrill, R. Ford, P. Mauser, and C. Horowitz. 1980. Effects of stress (hypoglycaemia, endotoxin, and ether) on the peripheral circulating concentration of corticosterone in the domestic fowl (Gallus domesticus). Comp. Biochem. Physiol. 66C:183–186.

Shahryar, A. H., and A. Lotfi. 2017. Effect of ghrelin administration on serum corticosterone, T3, T4 and some biochemical indices in the turkey (Meleagris gallopavo). Int. J. Pept. Res. Ther. 23:541–547.

Shini, S., P. Kaiser, A. Shini, and W. L. Bryden. 2008. Biological response of chickens (Gallus gallus domesticus) induced by corticosterone and a bacterial endotoxin. Comp. Biochem. Physiol. 149B:324–333.

Spiga, F., E. J. Waite, Y. Liu, Y. M. Kershaw, G. Aguilera, and S. L. Lightman. 2011. ACTH-dependent ultradian rhythm of corticosterone secretion. Endocrinology 152:1448–1457.

Vizcarra, F. R., M. Verghez, and J. A. Vizcarra. 2018. Effect of short- and long-term feed restriction on ghrelin concentrations in turkeys. Poult. Sci. 97:2183–2188.

Wallace, W. H., E. C. Crowne, S. M. Shalet, C. Moore, S. Gibson, M. D. Littley, and A. White. 1991. Episodic ACTH and cortisol secretion in normal children. Clin. Endocrinol. (Oxf.) 34:215–221.

Webb, M. L., and M. M. Mashaly. 1985. Maturation of the diurnal rhythm of corticosterone in female domestic fowl. Poult. Sci. 64:744–750.

Whatley, H. E., M. E. Lisano, and J. E. Kennamer. 1977. Plasma corticosterone level as an indicator of stress in the eastern wild turkey. J. Wildl. Manag. 41:189–193.

Wideman, R. F., M. E. Chapman, W. Wang, and G. F. Erf. 2004. Immune modulation of the pulmonary hypertensive response to bacterial lipopolysaccharide (endotoxin) in broilers. Poult. Sci. 83:624–637.

Yamashiro, D., C. H. Li. 1984. Adrenocorticotropic. 57. Synthesis and biological activity of ostrich and turkey hormones. Int. J. Pept. Protein Res. 23:42–46.