PHYSIOLOGY AND REPRODUCTION

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

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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, herding, the administration of *Escherichia coli* endotoxin, 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

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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 Neoaves 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

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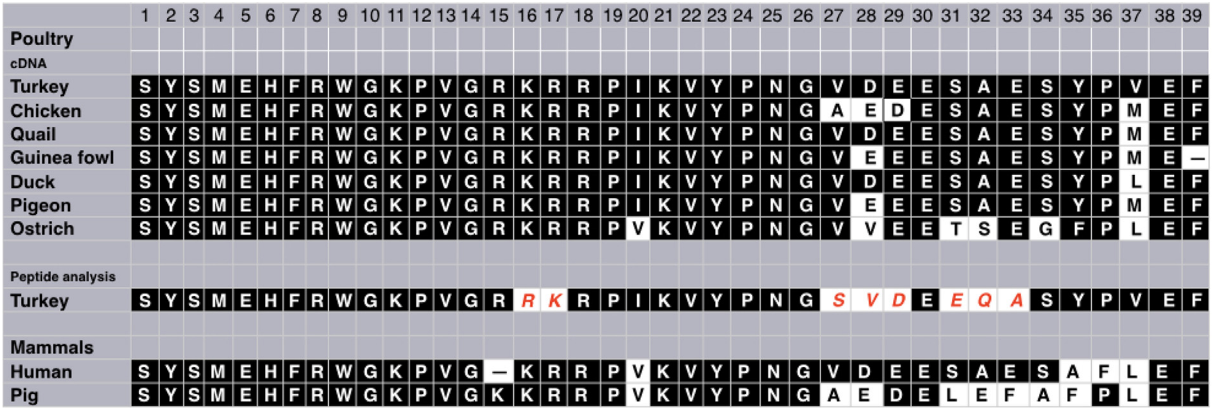

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Poultry																																							
cDNA																																							
Turkey	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	I	K	V	Y	P	N	G	V	D	E	E	S	A	E	S	Y	P	V	E	F
Chicken	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	I	K	V	Y	P	N	G	A	E	D	E	S	A	E	S	Y	P	M	E	F
Quail	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	I	K	V	Y	P	N	G	V	D	E	E	S	A	E	S	Y	P	M	E	F
Guinea fowl	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	I	K	V	Y	P	N	G	V	E	E	E	S	A	E	S	Y	P	M	E	–
Duck	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	I	K	V	Y	P	N	G	V	D	E	E	S	A	E	S	Y	P	L	E	F
Pigeon	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	I	K	V	Y	P	N	G	V	E	E	E	S	A	E	S	Y	P	M	E	F
Ostrich	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	K	R	R	P	V	K	V	Y	P	N	G	V	V	E	E	T	S	E	G	F	P	L	E	F
Peptide analysis																																							
Turkey	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	R	*R*	*K*	R	P	I	K	V	Y	P	N	G	*S*	*V*	*D*	E	*E*	*Q*	*A*	S	Y	P	V	E	F
Mammals																																							
Human	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	–	K	R	R	P	V	K	V	Y	P	N	G	V	D	E	E	S	A	E	S	A	F	L	E	F
Pig	S	Y	S	M	E	H	F	R	W	G	K	P	V	G	K	K	R	R	P	V	K	V	Y	P	N	G	A	E	D	E	L	E	F	A	F	P	L	E	F

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_021298522.1; 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.

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 (Erasmus, 2017). Moreover, most studies have been conducted at least 15 yr ago. Based on the limited information, plasma concentrations of CORT in turkeys are increased by stressors. Although turkey poults fed with a protein-deficient feed have elevated plasma concentrations of CORT, there was no change in the circulating concentrations of ACTH (Carsia and McIlroy, 1998), this apparent discrepancy being attributable to adrenal remodeling (Carsia and McIlroy, 1998; Carsia and Weber, 2000). Similarly, plasma concentrations of CORT are elevated in growing turkeys subjected to feed restriction (Bartz et al., 2018; Vizcarra et al., 2018). In addition, nocturnal plasma concentrations of CORT were increased by either high or low environmental temperatures in turkeys (El Halawani et al., 1973). 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 addition to the crepuscular peaks, there were higher plasma concentrations of CORT during the day than during the night (Martin et al., 1982). On the contrary, plasma concentrations of CORT were reported to be depressed during the night on a photoperiod of 14 h of light and 10 h of darkness (14L:10D) (El Halawani et al., 1973). 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 (H_2N-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 proopiomelanocortin (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). Plasma concentrations of CORT were not influenced by endotoxin challenge (Table 1). Shackling for 4 min tended ($P < 0.2$) to increase plasma concentrations of CORT (Table 2). It should be noted that no wing flapping was observed in any of the shackled turkeys. Plasma concentrations of CORT were higher ($P < 0.05$) in turkeys subjected to herding than in those remaining in the pen (Table 1), these birds being the pretreatment controls for the shackling and handing studies (Table 2). Plasma concentrations of CORT were reduced ($P < 0.01$) between the herded birds and the same birds later and after 24 h of fasting (10.0 ± (10) 3.19 ng mL^{-1}). In a second study, herding turkeys from one pen to another was followed by a trend for an increase ($P < 0.1$) in plasma concentrations of CORT compared with pretreatment and an increase ($P < 0.05$) compared with handling (Table 2).

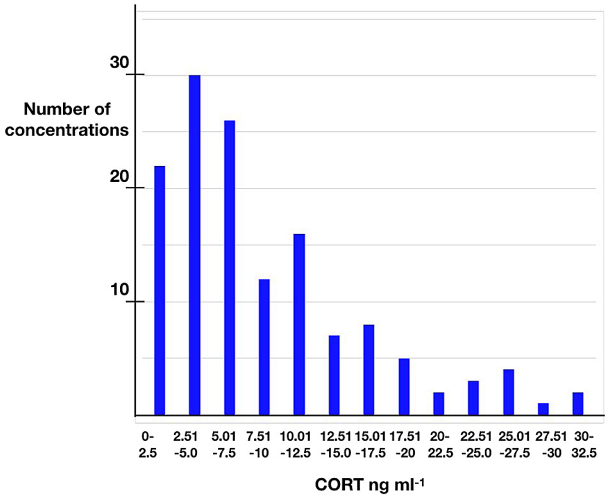

Figure 2. Frequency distribution of plasma concentrations of corticosterone (CORT) in market-weight male turkeys.

DISCUSSION

The circulating concentrations of CORT in market-weight turkeys determined in the present study were similar to those reported by Kiezun 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

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

Treatment	Plasma concentration of corticosterone, ng mL^{-1}
ACTH challenge (i.v.)	
Saline vehicle	17.1 ± (10) 3.05^{c}
Turkey ACTH (0.43 μmol kg^{-1})	28.7 ± (10) 3.14^{e}
Human ACTH (0.29 μmol kg^{-1})	19.4 ± (10) 2.45^{c}
Effect of putative stressors	
Control[1]	9.0 ± (19) 1.54^{a}
Herding[2]	21.4 ± (10) 3.04^{d}
Saline vehicle, i.m.[3]	10.0 ± (10) $1.76^{a,b}$
Endotoxin, i.m.[3]	11.4 ± (10) 1.74^{b}

[a,b,c,d,e] Different superscript letters indicate difference, $P < 0.05$.

Abbreviations: ACTH, adrenocorticotropic hormone; i.m., intramuscular; i.v., intravenous.

[1] Untreated and sampled in pens.

[2] Herding (sampled 4 min after manually moving birds ∼30 m from one pen to a new pen).

[3] Sampled after 30 min.

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, hACTH$_{1\text{-}24}$ (Table 1), this being consistent with elevated synthesis and release of CORT from turkey adrenal cortical cells in response to hACTH$_{1\text{-}24}$ (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 (Davison et al., 1980; Decuypere 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

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[3]	11.2 ± (10) 2.12	17.9 ± (10) 2.75^{c}[1]
Handling[4]	8.5 ± (10) 1.73	8.6 ± (10) 1.69^{a}
Shackling[2]	9.6 ± (9) 2.67	13.0 ± (9) 2.02^{b}

[a,b,c] Different superscript letters in a column indicate difference, $P < 0.05$.

[1] Difference $P < 0.1$ compared with pretreatment.

[2] Sampled before and 4 min after shackling.

[3] Sampled before and 15 min after manually moving birds ∼30 m from one pen to another.

[4] Sampled before and 4 min after handling.

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.

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