Simulated transport of well- and poor-feathered brown-strain end-of-cycle hens and the impact on stress physiology, behavior, and meat quality

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ABSTRACT Transportation of poultry is stressful, especially for end-of-cycle hens (**EOCH**) experiencing metabolic stress. The aim of this study was to evaluate the effects of simulated transport on well- and poor-feathered brown-strain EOCH. The study (5 × 3 × 2 factorial arrangement) consisted of 5 temperature and relative humidity (**RH**) combinations applied directly at crate level (−10°C uncontrolled RH [−10], +21°C 30%RH [21/30], +21°C 80%RH [21/80], +30°C 30%RH [30/30], or +30°C 80%RH [30/80]), 3 durations (4, 8, or 12 h), and 2 feather covers (well [**WF**] or poor [**PF**]). Hens (n = 540) from 3 commercial farms were housed for a 3- to 5-d adaptation period, then feed was withdrawn before treatment exposure (crate density 54.5 kg/m^2). Data collected included chamber conditions, feather condition score, behavior, blood physiology, core body temperature, mortality, and meat quality. Data were analyzed (randomized complete block design) using ANOVA; significance declared at $P \leq 0.05$. Time spent performing thermoregulatory behaviors increased for hot (30/30 and 30/80) and cold (−10) treatments. Mortality only occurred in hens exposed to −10 and increased with longer duration. Cold exposure impacted meat quality, resulting in higher thigh pH and lower L* (lightness) and b* (yellowness). Prolonged exposure duration resulted in dehydration, indicated by blood physiology (hematocrit and hemoglobin) and live shrink. PF hens struggled with thermoregulation in −10, while WF hens struggled in 30/30 and 30/80. These results demonstrate that EOCH exposed at crate level to hot (+30) conditions experience thermal stress, while hens exposed to cold (−10) are unable to cope, compromising welfare and meat quality.

Key words: thermal stress, feather cover, core body temperature, thermoregulatory behavior, spent hen

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INTRODUCTION

The transportation of livestock is a necessary practice in modern agriculture; however, it is important to assess the possible stressors to reduce the negative impact of transportation on animal welfare and productivity parameters. Common factors involved in transport stress are feed and water withdrawal, catching and handling, crating density, thermal stress, trip duration or distance, and many others (Mitchell and Kettlewell, 2009); yet there is little research concerning the transport of end-of-cycle hens (**EOCH**). The current Canadian Codes of Practice (NFACC, 2001) do not list separate requirements for the transport of EOCH, and as a result, they are included with broiler chickens. Because of this, there are multiple factors that may be overlooked, as EOCH pose unique issues compared with broiler chickens. They are often metabolically challenged, with low energy, protein, and calcium stores, and are therefore more prone to fatigue and bone breakage (Gregory and Wilkins, 1989; Gregory and Devine, 1999; Whitehead and Fleming, 2000; Richards et al., 2012). In addition, EOCH often have poor feather cover (Knowles and Broom, 1990; Richards et al., 2012), which may affect the bird's ability to thermoregulate (Leeson and Morrison, 1978). Another challenge associated with hen transport is the increased distance or duration, as there are fewer slaughter plants equipped to process EOCH (Knowles, 1994; Newberry et al., 1999; Weeks et al., 2012). Despite these challenges, EOCH continue to be used in the meat industry, and it is important to ensure that these hens can be transported safely and humanely.

The literature regarding the transportation of EOCH has primarily focused on dead-on-arrival (**DOA**) numbers,

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as this is considered both an economic loss and a welfare concern (Petracci et al., 2006; Weeks et al., 2012). It has been suggested that DOA values increase in the summer (Petracci et al., 2006), likely as a result of thermal stress. A study conducted in the Czech Republic demonstrated increased mortality throughout the winter months (December and January), with the highest incidence of mortality occurring at −3.1°C to −6.0°C (Vecerkova et al., 2019). It is important to note that the study did not attain temperatures below −6.0°C, which are frequently reached in North America. Therefore, there is still a lack of data concerning the transport of EOCH in winter conditions frequently experienced in North America. Weeks et al. (2012) found that hens were more susceptible to cold temperatures if they were light, poorly feathered, or transported to longer distances. In a companion article, the authors noted that hens were experiencing cold stress even at temperatures below 10°C (Richards et al., 2012). Although these field studies provide helpful data regarding the risks of transportation commercially, they did not assess the physiological stress or behavioral response of hens during transportation. As a result, data concerning the health and welfare of EOCH in transport are lacking. It is also important to note that the majority of the EOCH transport research studies have been completed in climates that are much warmer than Western Canada, and as a result, there are limited data for the transport of hens in cold conditions. Furthermore, poor feather condition may exacerbate the effects of cold conditions during transport.

In contrast to layers, the effects of transportation on broiler chickens and turkeys are well documented as they are more commonly used for meat production. One of the predominant stressors evaluated in both broilers and turkeys is thermal stress, either in the form of hyperthermia or hypothermia (Knezacek et al., 2010; Caffrey et al., 2017; Henrikson et al., 2018). The initial response to stress is to alter behavior to cope with the stressor at hand. Broilers and turkeys exposed to cold temperatures will use behavioral adaptations such as shivering, piloerection, and huddling (Strawford et al., 2011; Henrikson et al., 2018), whereas broilers and turkeys exposed to hot temperatures use panting to help dissipate heat (Mitchell and Kettlewell, 1998; Wichman et al., 2012). When behavior alone is unsuccessful at maintaining coping ability, stress physiological changes may occur, such as change in core body temperature (**CBT**) and change in blood or muscle physiology and, as a result, changes in meat quality. Broilers transported in cold weather conditions (below 0°C) demonstrated a decrease in CBT (Dadgar et al., 2012a). The authors also observed a decrease in blood glucose after only 3 h of exposure and a decrease in both thigh and breast muscle glycogen stores (Dadgar et al., 2012a). In addition, it is well documented that exposure to cold temperatures increases the incidence of dark, firm, dry meat (Petracci et al., 2004; Dadgar et al., 2011, 2012a,b). As a result of behavioral adaptations to heat stress, particularly panting, the partial pressure of CO_2 decreases, and changes in the acid-base balance occur (El Hadi and Sykes, 1982; Mitchell and Kettlewell, 1998). In addition to acid-base changes, Mitchell and Kettlewell (1998) also observed an increase in the heterophil-to-lymphocyte (**H/L**) ratio as a result of heat stress. Heat stress during transport also impacts muscle physiology and has been associated with pale, soft, exudative meat (Mitchell and Kettlewell, 1998; Dadgar et al., 2010; Jiang et al., 2016). While it is likely that laying hens will respond similarly to broilers, EOCH may experience greater difficulty coping with thermal stress due to greater tendency for metabolic imbalances in these birds.

The objectives of this study were to evaluate the effects of simulated transportation conditions relevant to North America (temperature, relative humidity, and duration) on both well-feathered and poor-feathered brown-strain EOCH to provide comprehensive data on both hen physiology and welfare. Temperatures were chosen to be indicative of hot (30°C), moderate or thermo-neutral (21°C), and cold (−10°C). Hen behavior, blood physiology, mortality, CBT, and muscle tissue characteristics (meat quality) will be evaluated to complete this assessment.

MATERIALS AND METHODS

The procedures for this experiment adhered to the guidelines set out by the Canadian Council on Animal Care (CCAC, 1993, 2009) and were approved by the University of Saskatchewan's Animal Research Ethics Board (AUP # 20160066).

Experimental Design

This study consisted of a 5 × 3 × 2 factorial arrangement, with 5 temperature (T)/relative humidity (**RH**) treatment groups (−10°C uncontrolled RH [−10], +21°C 30%RH [21/30], +21°C 80%RH [21/80], +30°C 30%RH [30/30], or +30°C 80%RH [30/80]), 3 transport durations (4, 8, or 12 h), and 2 feather condition scores (F; well feathered [**WF**] and poor feathered [**PF**]). Brown EOCH were obtained from 3 separate commercial farms (Table 1), with farm of origin used as block. Each flock was exposed to the same treatment combinations as indicated previously.

Birds and Housing

Hens (n = 540; 180/replicate) were feather scored on farm to obtain 50% WF and 50% PF hens. This allowed the use of equal number of WF and PF birds for each T/RH grouping. Four areas of the body were evaluated: neck, back, breast, and wings. Hens scoring 3 (less than 50% of the plumage is missing) or 4 (full intact plumage) were classified as well-feathered, and hens scoring 1 (no feather cover) or 2 (greater than 50% of the plumage is missing) were classified as poorly feathered (adapted from the study by Davami et al., 1987, and Sarica et al., 2008). The hens were then crated and transported to the University of Saskatchewan in a climate-controlled van. Hens were housed in 2 floor

Table 1. Brown-strain end-of-cycle hen flock information.

Farm	Strain	Age (wk)	Average BW (kg)
1	Lohmann Brown	68	1.82
2	Lohmann Brown Lite	66	1.90
3	Bovan Brown	72	1.81

pens (3.9 m × 3.0 m) with wheat straw bedding for an acclimatization period (3–5 d; as 2 T/RH treatments were tested each day) to allow the birds to settle in. Feed (obtained from farm of origin) and water were provided ad libitum. Feed was provided in aluminum tube feeders (38 cm pan diameter), and water was provided using bell drinkers (36 cm diameter). Lighting was provided via incandescent bulbs, and the lighting program from the farm of origin was maintained for each flock.

Prior to Simulated Transport

Six hours before treatment exposure, hens were placed in 1 of 4 feed withdrawal pens (18 hens/F resulting in 6 hens/d; 1.2 m × 1.3 m pen) with wheat straw bedding and an aluminum drinker pail (30 cm diameter). Hens (6 WF and 6 PF) were then randomly assigned to one of the T/RH and D combinations. Hen preparation involved taking initial body weights (n = 6/replicate), then hens were orally administered a miniature data logger (iButton Thermochron DS1922 L; Maxim Integrated, San Jose, CA), which travelled to the crop/gizzard and recorded CBT every minute. Baseline readings were obtained after hen preparation, by allowing hens to rest in the holding crates for 15 min. Hens received 2 wing bands for identifications, and blood samples were also taken via the brachial vein into a dipotassium EDTA tube (n = 5/replicate). Blood smears were prepared (n = 5/replicate) for analysis of the H/L ratio at a later date. Blood slides were stained using PROTOCOLTM Hema 3 (Fisher Scientific, Ottawa, Canada) and were stored in a slide box until read. Slides were read under 100X oil magnification (microscope B-290 TB; Optika, Bergamo, Italy), and the H/L ratio was determined by counting the number of heterophils and lymphocytes in the field of view until a total of 100 cells was reached. Blood samples were also analyzed immediately after collection (n = 3/replicate) for pH, partial pressure of carbon dioxide (pCO_2; mmHg), partial pressure of oxygen (pO_2; mmHg), base excess in the extracellular fluid compartment (BE; mmol/L), bicarbonate (HCO_3-; mmol/L), total carbon dioxide (tCO_2; mmol/L), oxygen saturation (sO_2; %), sodium (mmol/L), glucose (mmol/L), hematocrit (% packed cell volume), and hemoglobin (mmol/L) using a CG8+ cartridge in an i-STAT1 handheld analyzer (Abbott Point of Care Inc, Princeton, NJ). Hens were then transported 750 m in a van to the environmental chambers (College of Engineering, University of Saskatchewan, Saskatoon, Canada) and placed into one half of the crate (0.56 m × 0.39 m) at a target crate density of 54.5 kg/m^2 resulting in 6 WF hens on one side and 6 PF hens on the other side of each crate.

Table 2. Behavioral ethogram has been adapted from the study by Rault et al. (2016) and Henrikson et al. (2018).

Behavior	Definition
Motionless	No apparent movement—bird may be standing or in a crouched position, bird may be in a collected posture with its head retracted. Eyes may be open or closed.
Active	Bird is moving feet and/or wings, and is changing position/location in the crate.
Rustle	Bird is shifting position without changing location in the crate.
Survey	Quick head movements in an alert bird, suggesting visual surveillance of the environment.
Burrow	Movement in a downward motion in attempt to get underneath another bird.
Preen	Manipulation of the bird's own feathers with the beak.
Pant	Increase in the breathing of the bird characterized by an open beak, rapid breathing, or increased thoracic movements.
Shiver	The wings or body of the hen quiver or move from side to side in a rapid motion coupled with fluffed feathers.
Piloerection	Feathers are erect or being ruffled in an organized manner.
Object peck	Bird is using the beak in short quick forward motions to make contact with the crate or crate lid.
Bird peck	Bird is using the beak in short quick forward motions to make contact with another hen. May or may not include the removal of feathers.
Sham nibble	Movement of the mouth that simulates eating.
Gulp	Head of the bird is pointed vertically upward, beak is opened, and the bird takes a large gasp of air.
Head shake	Quick movement consisting of small displacement of the head in any direction or rotation of the of the head around its horizontal or vertical axis.
Stretch	Extending the leg or wing away from the body.
Head scratch	Bird raised its leg over its wing and repeatedly rubs its head with its talons.
No observation	Hens cannot be seen or behavior cannot be characterized.

Low frequency (<1%) nonthermoregulatory behaviors have been combined for analysis including: object pecking, bird pecking, sham nibbling, head shaking, stretching, head scratching, and no observation.

During Simulated Transport

Chamber conditions were monitored in real time using thermocouples attached to a multimeter (Omega HH509; Omega Engineering Inc., Laval, Canada) and RH sensors (HM1500LF; Measurement Specialties, Inc., Toulouse, France). Chamber set points were adjusted, if needed, to achieve the targeted environmental conditions. A miniature data logger (iButton Hygrochron DS1923-#F5; Maxim Integrated, San Jose, CA) was placed in each crate and recorded the T/RH at bird level every minute. Infrared video cameras (Panasonic WV-CF224FX; Panasonic Corporation of North America, Newark, NJ) were used to monitor behavior over the entire duration of exposure. Behavior was evaluated at 5-min intervals using the scan sampling technique. Behaviors observed included motionless, active, rustling, surveying, burrowing, preening, panting, shivering, piloerection, object pecking, bird pecking, sham nibbling, gulping, head shaking, stretching, and head scratching and are defined in Table 2.

Postsimulated Transport

After 4, 8, or 12 h, the designated crate was removed from the chamber, and the hens were weighed to obtain live shrink (%). A second blood sample was collected and analyzed, and delta (Δ) values were calculated for

Table 3. Average crate and chamber temperature (T; °C) and relative humidity (RH; %) conditions achieved for brown-strain end-of-cycle hens exposed to 5 T/RH combinations (−10°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a duration of 4, 8, or 12 h.

	T/RH treatment									
	−10		21/30		21/80		30/30		30/80	
Crate	T	RH	T	RH	T	RH	T	RH	T	RH
4 h	−4.3	66.5	23.7	41.2	26.3	68.1	33.7	31.9	34.1	69.6
8 h	−4.4	64.5	24.3	40.2	25.8	68.4	33.6	30.7	34.3	67.1
12 h	−3.3	61.3	24.8	39.2	26.8	67.9	34.0	32.8	35.2	68.3
Chamber	−9.5	70.8	20.8	42.3	21.9	81.6	31.5	32.8	31.7	80.3

each blood parameter listed previously (Δ = final-initial measure). Hens were then slaughtered using a small-scale slaughter line, where hens were shackled, electrically stunned, and exsanguinated. Each hen was scalded, plucked, and eviscerated, and the miniature data logger was retrieved from either the crop or the gizzard. Meat quality analyses were performed on 5 randomly selected hens/replicate. The initial meat pH was taken using the upper portion of the right breast (pectoralis major) and the right thigh (iliotibialis muscle), using a portable handheld pH meter (H1 9025; Hanna Instruments, Woonsocket, RI). The carcasses were placed in a chill tank for 1 h and then transferred to ice and stored in a +4°C cooler for 5 h. The carcasses were then removed from ice; the left breast was removed, sealed in a Ziploc bag, and stored at −30°C for further analysis. The right breast and right thigh were removed, weighed, and placed on a Styrofoam tray with a Dri-loc pad and covered with cling wrap. The trays were then placed back in the +4°C cooler for an additional 24 h. After 24 h, the breast and thigh muscles were weighed to calculate the drip loss (%), and a final pH reading was taken by cutting a small incision next to the previous cut. The trays were allowed to sit for 30 min to allow for oxygen exposure to the cut portion followed by 2 color readings taken using a color meter (Minolta Chroma Meter CR-400; Konica Minolta Sensing Americas Inc, Ramsey, NJ). The readings were taken on the incision of both the breast and thigh at 90° angles from each other. The output from the meter was converted (SpectraMagicTM NX Software; Konica Minolta Sensing Americas, Inc., Ramsey, NJ) using illuminant source C and 2° to obtain lightness (L*), redness (a*), and yellowness (b*), and the 2 readings were averaged to obtain the final values.

The left breast was analyzed for thaw loss (%) and cook loss (%) 4–6 wk after slaughter. The breast muscle was removed from the freezer and allowed to thaw for 24 h in a 4°C cooler. The breast was then removed from the bag, blotted with white paper towel, and weighed to calculate thaw loss. Breast muscles were then transferred to a Ziploc bag and placed in a water bath at 80°C until the internal temperature reached 75°C using a thermocouple and a multimeter (Omega HH509; Omega Engineering Inc., Laval, Canada), then left in the water bath for an additional 5 min. The breast was then removed from the water bath and allowed to cool to a minimum of 50°C, then it was removed from the bag, blotted with white paper towel, and weighed to obtain the cook loss.

Table 4. Significant interactions for the behavior (% of time) of well- (WF) and poor-feathered (PF) brown-strain end-of-cycle hens exposed to 5 temperature (T)/relative humidity (RH) combinations (−10°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30% RH, and 30°C 80%RH) for a duration (D) of 4, 8, or 12 h.

	T/RH x feather cover interaction—panting ($P = 0.03$)				
Feather cover	−10	21/30	21/80	30/30	30/80
WF	0.11^{d}	0^{d}	0.07^{d}	34.99^{b}	48.32^{a}
PF	0^{d}	0^{d}	0^{d}	20.66^{c}	36.96^{b}

	T/RH x D x feather cover interaction—piloerection (P = 0.01)									
	−10		21/30		21/80		30/30		30/80	
Duration	WF	PF	WF	PF	WF	PF	WF	PF	WF	PF
4 h	0^{c}	0.25^{a}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}
8 h	$0.18^{a,b}$	$0.09^{b,c}$	0^{c}	0.06^{c}	0^{c}	0.06^{c}	0^{c}	0^{c}	0^{c}	0^{c}
12 h	0^{c}	0^{c}	0^{c}	0.04^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0^{c}

Means within a parameter with different superscript letters are significantly different ($P \leq 0.05$).

Statistical Analyses

The data were analyzed as a randomized complete block design, with farm of origin as block, and data analysis was completed using SAS 9.4 (SAS Institute; Cary, NC). The data were verified for normality using PROC UNIVARIATE, and all mortality and behavior data were log transformed (log+1) before analyses. PROC MEANS was used to obtain treatment means and the SEM. ANOVA were conducted with 5 T/RH combinations × 3 D × 2 F in a factorial arrangement using PROC MIXED. The experimental unit for each variable was a half-crate (6 hens/half-crate; 3 experimental units per treatment combination). Error was estimated using DDFM Kenwardroger, and means separation was conducted using Tukey's test. Differences were considered significant when $P \leq 0.05$.

RESULTS

Chamber Environment

Actual environment conditions achieved (average crate temperature for each T/RH and D combination and actual chamber conditions) are shown in Table 3.

Table 5. Behavior (% of time) of brown-strain end-of-cycle hens with 2 feather covers (F; well- [WF] and poor-feathered [PF]) exposed to 5 temperature (T)/relative humidity (RH) combinations (−10°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a duration (D) of 4, 8, or 12 h.

	T/RH					D			F		
Behavior	−10	21/30	21/80	30/30	30/80	4 h	8 h	12 h	WF	PF	SEM[1]
Motionless	93.38^{a}	94.73^{a}	92.33^{a}	63.54^{b}	46.74^{c}	77.02	79.14	75.40	74.98	79.57	2.456
Active	0.13^{b}	0.11^{b}	0.25^{b}	0.36^{a,b}	0.75^{a}	0.50^{a}	0.18^{b}	0.29^{a,b}	0.23	0.43	0.060
Rustle	2.12^{b,c}	1.38^{c}	2.12^{b,c}	2.84^{a,b}	3.91^{a}	2.92^{a}	2.11^{b}	2.44^{a,b}	2.08^{b}	2.92^{a}	0.189
Burrow	0.15	0.06	0.01	0.07	0.14	0.09	0.09	0.07	0.08	0.09	0.022
Preen	0.13^{b,c}	0.56^{a}	0.32^{a,b}	0.15^{b,c}	0.04^{c}	0.15	0.29	0.32	0.20	0.29	0.037
Gulp	0^{b}	0^{b}	0^{b}	0.07^{a,b}	0.10^{a}	0.02	0.04	0.05	0.04	0.03	0.012
Pant	0.06	0	0.03	27.82	42.64	13.57	14.80	16.67	17.47	12.35	2.248
Survey	2.00^{b,c}	2.20^{c}	4.13^{a}	4.19^{a}	3.80^{a,b}	3.82^{a}	2.47^{b}	3.74^{a}	3.40	3.28	0.230
Shiver	0.41^{a}	0.02^{b}	0.01^{b}	0^{b}	0^{b}	0.12	0.05	0.03	0.04	0.10	0.032
Piloerection	0.11	0.02	0.01	0	0	0.02	0.04	0.01	0.01	0.03	0.008
Other[2]	2.19	0.96	0.70	1.14	2.19	2.12	1.02	0.97	1.59	1.20	0.275

Means within a main effect with different superscript letters are significantly different ($P \leq 0.05$).
[1]Pooled standard error of the mean.
[2]Other combined low-frequency (<1%) nonthermoregulatory behaviors—object peck, bird peck, sham nibble, head shake, stretch, scratch, no observation.

The crate temperature typically increased compared with chamber set points, as birds within a crate were in close proximity to one another.

Feather Condition Score

As WF and PF birds were divided equally among treatments before exposure, mean feather scores did not differ for T/RH (2.9, 2.7, 2.7, 2.8, and 2.7 for treatments −10, 20/30, 21/80, 30/30, and 30/80, respectively) or D (2.7, 2.7, and 2.7 for 4 h, 8 h, and 12 h treatments, respectively). Feather scores were different for WF and PF, as expected, with an average of 3.5 for WF and 2.0 for PF ($P < 0.01$).

Behavior

The significant interactions for behavior are shown in Table 4, and the main effects are shown in Table 5. There was a T/RHxDxF interaction for hens demonstrating piloerection, with PF hens in −10 for 4 h displaying this behavior most ($P = 0.01$). There was an interaction for T/RHxF for time spent panting, with WF hens exposed to 30/80 panting most, followed by 30/30 WF hens and

Table 6. Blood physiology of brown-strain end-of-cycle hens with 2 feather covers (F; well- [WF] and poor-feathered [PF]) exposed to 5 temperature (T)/relative humidity (RH) combinations (−10°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a duration (D) of 4, 8, or 12 h.

	T/RH					D			F		
Parameter[1]	−10	21/30	21/80	30/30	30/80	4 h	8 h	12 h	WF	PF	SEM[2]
ipH	7.05^{a,b}	7.01^{b}	7.06^{a}	7.03^{a,b}	7.01^{b}	7.03	7.03	7.04	7.04	7.03	0.005
ΔpH	−0.01^{b}	0.06^{a,b}	0.02^{b}	0.06^{a,b}	0.08^{a}	0.03	0.05	0.06	0.05	0.04	0.007
ipCO_2 (mmHg)	72.1^{a,b}	75.6^{a}	67.6^{b}	75.9^{a}	76.9^{a}	74.0	73.4	73.9	73.8	73.7	0.96
ΔpCO_2 (mmHg)	−2.9^{a}	−11.8^{a,b}	−9.5^{a}	−18.8^{b,c}	−19.6^{c}	−10.0^{a}	−13.4^{a,b}	−17.0^{b}	−13.8	−12.8	1.15
ipO_2 (mmHg)	62.7^{b}	67.1^{a}	67.8^{a}	63.8^{b}	63.1^{b}	65.2	64.6	65.5	64.2	66.0	0.70
ΔpO_2 (mmHg)	18.6^{a}	2.4^{b}	3.8^{b}	5.3^{b}	6.6^{b}	6.0	7.9	5.2	7.2	5.5	0.99
itCO_2 (mmol/L)	22.1	21.4	21.2	22.0	21.6	21.5	21.4	22.0	21.8	21.4	0.19
ΔtCO_2 (mmol/L)	−1.4	0.9	−2.0	−3.0	−2.5	−1.5	−1.9	−2.7	−2.1	−1.9	0.30
isO_2 (%)	78.4^{b}	79.2^{a,b}	81.9^{a}	78.0^{b}	76.0^{b}	78.8	78.4	79.0	78.4	79.0	0.46
ΔsO_2 (%)	9.1^{a}	4.4^{b,c}	3.4^{c}	6.2^{a,b,c}	8.1^{a,b}	5.1	6.7	6.3	6.4	5.6	0.57
iHCO_{3-} (mmol/L)	19.9	19.2	19.3	19.8	19.3	19.4	19.3	19.8	19.7	19.2	0.18
ΔHCO_{3-} (mmol/L)	−1.2	−0.6	−1.7	−2.5	−2.0	−1.2	−1.6	−2.2	−1.7	−1.5	0.28
iBE (mmol/L)	−10.5	−11.9	−11.0	−11.1	−11.8	−11.4	−11.6	−10.9	−11.0	−11.6	0.21
ΔBE (mmol/L)	−1.4	0.4	−1.3	−1.5	−0.8	−0.7	−0.7	−1.3	−1.0	−0.8	0.35
iSodium (mmol/L)	143.8	144.2	144.7	144.6	144.4	144.3	144.3	144.6	144.4	144.4	0.25
ΔSodium (mmol/L)	−0.2^{c}	1.8^{b,c}	3.5^{a,b}	5.0^{a}	4.7^{a}	1.1^{c}	3.1^{b}	5.8^{a}	3.2	3.2	0.38
iHematocrit (%)	21.8	21.7	21.0	21.6	21.4	21.3	21.5	21.6	21.3	21.6	0.17
ΔHematocrit (%)	−1.3	0.1	0.2	0.6	0.6	−0.6^{b}	0.1^{a,b}	0.9^{a}	0.1	0.2	0.19
iHemoglobin (mmol/L)	4.6	4.6	4.4	4.6	4.5	4.5	4.5	4.6	4.5	4.6	0.04
ΔHemoglobin (mmol/L)	−0.3	0	0	0.1	0.1	−0.1^{b}	0^{a,b}	0.2^{a}	0	0	0.04
iGlucose (mmol/L)	13.4^{a,b}	13.3^{a,b}	12.8^{b}	13.4^{a}	13.0^{a,b}	13.2	13.3	13.0	13.0	13.3	0.08
ΔGlucose (mmol/L)	−2.9^{c}	−1.3^{b}	−0.6^{a,b}	−0.7^{b}	0.1^{a}	−1.0	−1.1	−0.6	−0.7^{a}	−1.1^{b}	0.14
iH/L	0.92^{a,b}	0.81^{b}	1.07^{a}	0.91^{a,b}	0.84^{b}	0.88	0.94	0.92	0.95	0.87	0.029
ΔH/L	1.00^{a}	0.36^{b}	0.44^{a,b}	0.48^{a,b}	0.76^{a,b}	0.68	0.59	0.43	0.68	0.46	0.055

Means within a main effect with different superscript letters are significantly different ($P \leq 0.05$).
[1]Delta values (Δ) = final–initial (i). Partial pressure of carbon dioxide (pCO_2) and oxygen (pO_2), total carbon dioxide (tCO_2), oxygen saturation (sO_2), bicarbonate (HCO_{3-}), base excess in the extracellular fluid compartment (BE), heterophil/lymphocyte ratio (H/L).
[2]Pooled standard error of the mean.

Table 7. Delta core body temperature (ΔCBT) and percent mortality (%) of brown-strain end-of-cycle hens with 2 feather covers (F; well- [WF] and poor-feathered [PF]) exposed to 5 temperature (T)/relative humidity (RH) combinations (−10°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a duration (D) of 4, 8, or 12 h.

	T/RH					D			F		
Main effect	−10	21/30	21/80	30/30	30/80	4 h	8 h	12 h	WF	PF	SEM[1]
ΔCBT[2]	-2.5^{b}	1.6^{a}	0^{b}	1.8^{a}	1.6^{a}	-0.1^{b}	1.0^{a}	1.46^{a}	0.6	0.9	0.25
Mortality (%)	18.9	0	0	0	0	0.6	3.4	5.7	2.6	4.0	1.36

	T/RH × D interaction—mortality (P = 0.01)				
Duration	−10	21/30	21/80	30/30	30/80
4 h	$3.3^{b,c}$	0^{c}	0^{c}	0^{c}	0^{c}
8 h	$20.0^{a,b}$	0^{c}	0^{c}	0^{c}	0^{c}
12 h	33.3^{a}	0^{c}	0^{c}	0^{c}	0^{c}

Means within a parameter with different superscript letters are significantly different ($P \leq 0.05$).
[1]Pooled standard error of the mean.
[2]ΔCBT = average CBT in last h of exposure–average 15-min baseline CBT; values are derived from all live birds at end of specific duration period.

30/80 PF hens, then by 30/30 PF hens, with both WF and PF hens in −10, 21/30, and 21/80 panting less ($P = 0.03$). Hens spent more time motionless in −10, 21/30, and 21/80, followed by 30/30, and the least amount of time motionless in 30/80 ($P < 0.01$). Hens spent the most time active in 30/80 compared with −10, 21/30, and 21/80, with 30/30 not differing from all other treatments ($P < 0.01$). Hens spent the most time rustling in 30/80 compared with −10, 21/30, and 21/80 ($P < 0.01$). Hens preened the most in 21/30 compared with −10, 30/30, and 30/80 ($P < 0.01$). Gulping only occurred under hot conditions with hens in 30/80 gulping most ($P = 0.02$). Hens spent more time surveying the environment in 21/80, 30/30, and 30/80 compared with 21/30 ($P < 0.01$). Hens spent the most time shivering in −10 compared with all other treatments ($P = 0.01$). Duration effects were observed for active, rustling, and survey behaviors. Hens were most active and spent more time rustling in 4 h compared with 8 h, with 12 h not differing from either ($P = 0.02$ and $P = 0.04$, respectively). Hens spent more time surveying the environment in 4 h and 12 h than in 8 h exposure ($P < 0.01$). There was an effect of feather cover on rustling behavior, with PF hens spending more time rustling than WF hens ($P = 0.02$).

Blood Physiology

Brown EOCH blood physiology parameters are shown in Table 6. There were no significant interactions between T/RH, D, or F for the variables shown. Before chamber exposure, EOCH demonstrated effects of T/RH for the following initial blood physiology parameters: pH, pCO_2, pO_2, sO_2, Glu, and H/L. The initial pH was higher for hens in 21/80 than for hens in 21/30 and 30/80 ($P < 0.01$). The initial pCO_2 was lowest for hens in 21/80 compared with that for hens in 21/30, 30/30, and 30/80, with hens in −10 not differing from other treatments ($P = 0.01$). The initial pO_2 was higher for 21/30 hens and 21/80 hens than that for hens exposed to −10, 30/30, and 30/80 ($P = 0.02$). The initial sO_2 was higher for 21/80 than that for hens exposed to −10, 30/30, and 30/80, with hens exposed to 21/30 not differing from other treatments ($P < 0.01$). The initial Glu was higher in hens subjected to 30/30 than in hens subjected to 21/80, with all other treatments not differing from either ($P = 0.03$). The initial H/L was higher for hens exposed to 21/80 than that for hens exposed to 21/30 and 30/80, with hens exposed to −10 and 30/30 not differing from either ($P = 0.01$).

There were also significant differences for T/RH effects after treatment for the following Δ parameters: pH, pCO_2, pO_2, sO_2, sodium, glucose, and H/L. The ΔpH was larger for birds exposed to 30/80 than that of birds exposed to 21/80 and −10 (The latter was negative.), with 21/30 and 30/30 birds not differing ($P = 0.01$). The ΔpCO_2 had the largest negative value in 30/80 hens compared with −10, 21/30, and 21/80, with hens in −10 and 21/80 exhibiting the smallest negative value but not differing from hens in 21/30 ($P < 0.01$). The ΔpO_2 was largest for hens in −10 compared with all other treatments ($P < 0.01$). The ΔsO_2 was larger for hens exposed to −10 (30/30 and 30/80 did not differ) and smallest for hens exposed to 21/80 (21/30 and 30/30 did not differ) ($P < 0.01$). The ΔSodium demonstrated the largest value for hens in 30/30 and 30/80, with hens in 21/80 not differing, and a negative value for hens in −10, with hens in 21/30 not differing ($P < 0.01$). The ΔGlu has the largest negative value for hens exposed to −10 compared with all other treatments and had a larger negative value in hens exposed to 21/30 and 30/30 compared with 30/80 ($P < 0.01$). The ΔH/L was largest for −10 hens and lowest for 21/30 hens, with all other treatments not differing from either ($P = 0.01$).

Duration effects were observed for ΔpCO_2, Δsodium, Δhematocrit, and Δhemoglobin. The ΔpCO_2 demonstrated a greater negative value for hens in 12 h than for hens in 4 h exposure ($P < 0.01$). The hen's Δsodium had larger positive values with increasing duration ($P < 0.01$). Both Δhematocrit ($P = 0.02$) and Δhemoglobin ($P = 0.02$) were negative for hens in the 4 h exposure and positive for hens in the 12 h exposure, with hens in the 8 h being intermediate but not differing from either.

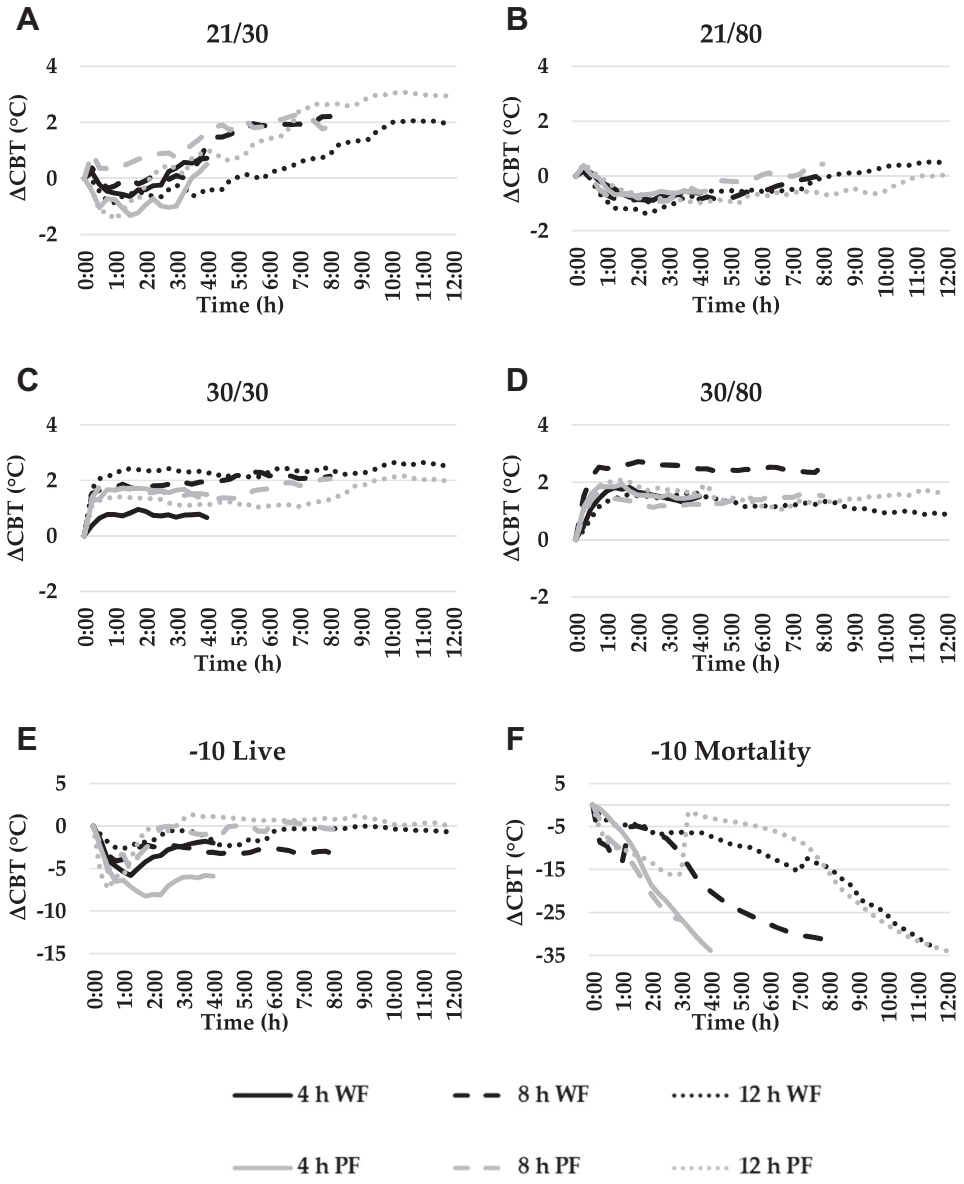

Figure 1. Delta core body temperature (ΔCBT; °C) over time of hens exposed to (A) +21°C/30% relative humidity (RH), (B) +21°C/80%RH, (C) +30°C/30%RH, (D) +30°C/80%RH (a-d N = 108), (E) −10°C uncontrolled RH (birds that lived through exposure; N = 73), (F) −10°C uncontrolled RH–data includes any mortality including those from crates removed prior to duration end for humane end-point reasons (N = 17).

Feather cover effects were only observed for ΔGlu, with PF hens having a larger negative value than WF hens ($P < 0.01$).

Mortality

Mortality only occurred in the −10 treatments, with percent mortality being highest in the 12 h treatment; 8 h was intermediate and not different from either 4 or 12 h, and the 4 h treatment was lowest; however, it was not different from all other T/RH treatments that had no mortality ($P = 0.01$; Table 7).

Core Body Temperature

The average delta core body temperature (**ΔCBT**) for each 15-min period for EOCH are shown in Figure 1 and were not analyzed statistically. For hens in the 21/30, the average ΔCBT changed relatively little in the 4 h treatment, but it increased over the 8 and 12 h periods. The hens in the 21/80 treatment all followed a similar pattern and did not have a large change in CBT. The ΔCBT of hens in the 30/30 treatment increased initially and then plateaued, with the 12 h WF hens having the largest increase. The ΔCBT of hens in the 30/80 treatment also increased similarly to the 30/30 hens, with the 8 h WF hens having the highest overall ΔCBT. The hens in the −10 treatment were separated into live and dead birds as the mean ΔCBT was greatly impacted when crates were removed from treatment before the set duration being reached because of mortality. The hens in the −10 treatment that lived all showed an initial decrease in CBT, with most hens showing an ability to thermoregulate and increase the CBT within

Table 8. Breast and thigh meat quality of brown-strain end-of-cycle hens with 2 feather covers (F; well- [WF] and poor-feathered [PF]) exposed to 5 temperature (T)/relative humidity (RH) combinations (−10°C uncontrolled RH, 21°C 30% RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a duration (D) of 4, 8, or 12 h.

	T/RH					D			F		
Parameter	−10	21/30	21/80	30/30	30/80	4 h	8 h	12 h	WF	PF	SEM[1]
Initial BW	1.84	1.84	1.81	1.85	1.86	1.85	1.84	1.84	1.85	1.83	0.008
Live shrink (%)	2.53^{a,b}	2.18^{b}	1.81^{b}	3.36^{a}	3.07^{a}	1.33^{c}	2.56^{b}	4.05^{a}	2.50	2.70	0.169
Breast meat quality											
Drip loss (%)	0.72	0.32	0.27	0.35	0.21	0.44	0.30	0.28	0.36	0.33	0.045
Thaw loss (%)	1.95	2.44	1.97	1.87	1.92	2.39^{a}	1.94^{a,b}	1.73^{b}	2.07	2.00	0.097
Cook loss (%)	18.98	20.65	20.57	19.10	20.11	20.26	19.58	20.01	19.91	20.01	0.322
L*	47.66	47.52	47.67	48.51	47.42	48.85^{a}	47.20^{b}	47.15^{b}	47.60	47.93	0.221
a*	6.45	6.58	6.91	6.89	6.60	6.36^{b}	6.67^{a,b}	7.12^{a}	6.64	6.78	0.117
b*	0.14	0.02	0.48	0.46	−0.03	0.46	0.12	0.05	0.20	0.24	0.100
Initial pH	6.53	6.50	6.46	6.41	6.49	6.46	6.44	6.52	6.46	6.49	0.019
Final pH	6.05^{a}	5.86^{b}	5.87^{b}	5.83^{b}	5.79^{b}	5.86	5.88	5.85	5.85	5.88	0.015
Thigh meat quality											
Drip loss (%)	0.93	0.27	0.32	0.32	0.27	0.52	0.28	0.33	0.44	0.31	0.074
L*	43.56	50.28	50.41	49.63	50.00	50.10	48.97	48.51	48.79	49.68	0.375
a*	6.30^{a,b}	6.14^{b}	6.11^{b}	6.88^{a}	6.66^{a,b}	6.20^{b}	6.28^{b}	6.85^{a}	6.33	6.53	0.132
b*	−5.31^{b}	−2.09^{a}	−1.78^{a}	−2.25^{a}	−1.80^{a}	−2.55	−2.39	−2.31	−2.59	−2.24	0.183
Initial pH	6.83^{a}	6.54^{b}	6.60^{a,b}	6.45^{b}	6.53^{b}	6.55	6.55	6.60	6.58	6.55	0.024
Final pH	6.78^{a}	6.08^{b}	6.14^{b}	6.08^{b}	6.10^{b}	6.22	6.20	6.14	6.21	6.16	0.030

Means within a main effect with different superscript letters are significantly different ($P \leq 0.05$).
[1]Pooled standard error of the mean.

an hour. The hens in the −10 treatment that died typically decreased quite rapidly, with some of the hens increasing CBT for a short period of time before decreasing again. ΔCBT (Table 7) for the live hens only, for the last hour of exposure, was lower at −10 and 21/80 ($P < 0.01$) and increased with 8 and 12 h durations ($P = 0.01$).

Live Shrink and Meat Quality

Live shrink and meat quality are shown in Table 8 with significant interactions shown in Table 9. Initial body weight before treatment exposure was not significantly different. There were no interactions for live shrink (%); however, both T/RH and D had an effect on live shrink. Live shrink (%) was highest for hens in the 30/30 and 30/80 treatments and lowest for hens in the 21/30 and 21/80 treatments, with hens in the −10 treatment not differing from either ($P < 0.01$). Live shrink was also affected by D with longer D increasing live shrink loss (1.33, 2.56, and 4.05% for 4, 8, and 12 h, respectively; $P < 0.01$).

Breast meat quality demonstrated one significant interaction, with initial pH after slaughter being higher in hens exposed to 21/30 for 12 h than that in 21/30 4 h hens and 30/80 8 h hens ($P = 0.03$). Hens in all other T/RH and D treatments were not different. Breast drip loss, cook loss, and b* demonstrated no significant differences. Final breast pH was significantly different for T/RH, with −10 being higher than all other treatments ($P < 0.01$). Thaw loss, L*, and a* demonstrated D effects. Thaw loss was highest in 4 h compared with 12 h, with 8 h not differing from either ($P < 0.01$). L* was higher for hens in the 4 h than that for hens in the 8 or 12 h ($P < 0.01$). Breast a* was lower in hens in the 4 h than that in hens in the 12 h, but hens in the 8 h treatment were not different from either ($P < 0.01$).

Thigh drip loss was not significantly affected by treatment. Thigh meat quality had one significant interaction with L* having a T/RH and D interaction. Thigh L* was higher for 30/80 4 h than those for 30/30 12 h, and all D in the −10 treatment ($P = 0.04$). Thigh L* was also lower for hens in the −10 8 h and 12 h than that for hens in all D in 21/30, 21/80, 30/30, and 30/80 but was not different compared to hens in the −10 4 h treatment. Thigh a*, b*, initial pH, and final pH were affected by T/RH. Thigh a* was higher for 30/30 than for 21/30 and 21/80, but hens in −10 and 30/80 were intermediate ($P < 0.01$). Thigh b* was lower for −10 than that for all other T/RH treatments ($P < 0.01$). Thigh initial pH was highest for −10 compared with 21/30, 30/30, and 30/80, with 21/80 not differing ($P < 0.01$). Thigh final pH was highest for −10 compared with all other T/RH treatments ($P < 0.01$). Finally, thigh a* also had a duration effect, with hens in the 12 h treatment having a higher

Table 9. Significant interactions for meat quality of brown-strain end-of-cycle hens exposed to 5 temperature (T)/relative humidity (RH) combinations (−10°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a duration of 4, 8, or 12 h.

	T/RH treatment				
Duration	−10	21/30	21/80	30/30	30/80
Breast initial pH (P = 0.03)					
4 h	6.57^{a,b}	6.34^{b}	6.42^{a,b}	6.41^{a,b}	6.56^{a,b}
8 h	6.49^{a,b}	6.49^{a,b}	6.49^{a,b}	6.41^{a,b}	6.34^{b}
12 h	6.51^{a,b}	6.67^{a}	6.47^{a,b}	6.41^{a,b}	6.55^{a,b}
Thigh L* (P = 0.04)					
4 h	45.32^{c,d}	51.18^{a,b}	50.73^{a,b}	51.00^{a,b}	51.75^{a}
8 h	42.51^{d}	51.50^{a,b}	49.77^{a,b}	49.69^{a,b}	49.28^{a,b}
12 h	42.27^{d}	48.25^{a,b,c}	50.75^{a,b}	48.18^{b,c}	48.98^{a,b}

Means within a parameter with different superscript letters are significantly different ($P \leq 0.05$).

a* value than that of birds in the 4 h and 8 h treatments ($P < 0.01$).

DISCUSSION

Most transportation studies have focused on broiler and turkey transport; however, with the increasing transport of EOCH to slaughter, it is important to note there are major differences in these birds. EOCH may experience difficulty coping with thermoregulation during transport because of their lack of energy and protein stores and potential poor feather cover (Gregory and Devine, 1999; Richards et al., 2012).

The chamber conditions achieved were comparable to the targeted temperature and humidity. Interestingly the temperature and humidity achieved within the crate differed from those of the chamber. Hens exposed to the cold treatment (−10°C) only experienced between −3.3°C and −4.4°C. The differences in external and crate temperatures have been observed in commercial settings, with many studies noting the temperature gradient in trailers as a result of lack of air flow and heat production from the birds (Knezacek et al., 2010). In addition, the hens exposed to 21°C and 30°C typically experienced conditions 2°C–5°C warmer than the chamber conditions, and hens exposed to 80% relative humidity experienced 10–13% lower humidity within the crate rather than chamber conditions.

EOCH have the ability to cope with stressors in 2 ways, the first being behavioral responses and the second being physiological responses. To the authors' knowledge, the literature concerning hen behavior in various thermal conditions during transport has not previously been reported. In response to cold exposure, hens coped behaviorally by spending more time shivering compared to hens in all other treatments. Similarly, when broilers were exposed to cold temperatures, huddling behavior increased (Strawford et al., 2011). Turkeys exposed to cold temperatures (−18°C) spent more time huddling, shivering, and feathers erected (Henrikson et al., 2018). As EOCH spent more time shivering, they likely required mobilization of energy stores, as shown by the decrease in blood glucose, to help cope with thermal stress. With cold exposure, the use of glucose and break down of glycogen is needed to maintain CBT, allowing the hen to maintain homeostasis; however, this can lead to meat quality defects (Dadgar et al., 2012a,b). As seen in previous broiler studies, cold exposure led to dark, firm, dry meat classified by high meat pH (>6.1) and low L* (lightness; <46.0) (Barbut et al., 2005; Dadgar et al., 2012b). Once hens deplete their available energy stores, they will not be able to maintain homeostasis resulting in a decrease in CBT. With prolonged cold exposure and energy depletion, CBT decreases past a point of recovery, resulting in death. This was not well observed in the average CBT data, as hens that were removed from trial early due to high mortality levels were not included in that analysis. Mortality in this study was not only prevalent in the cold treatment but also increased with exposure duration. Although many studies have not considered the effects of transport environment on CBT, research has suggested that EOCH do not cope well with cold exposure, especially with longer transport durations, resulting in a high percentage of DOA (Weeks et al., 2012). The hens that survived cold exposure exhibited other signs of thermal stress that were noted in the blood physiology parameters (increase in H/L ratio, pO_2, and sO_2 and decreased blood glucose), suggesting that they experienced negative welfare during simulated transport.

Hens exposed to hot conditions (30/30 and 30/80) spent the least amount of time motionless, which is likely a result of performing other behaviors such as panting, active, rustling, and surveying. EOCH responded behaviorally to thermal stress by panting to dissipate heat, which functions as a mechanism of evaporative cooling to cope with heat stress in poultry (Yahav et al., 2000; Mack et al., 2013; Vermette et al., 2017). It has also been suggested that increases in RH result in panting being less efficient at dissipating heat because of the decreased efficiency of evaporative cooling (Warriss et al., 2005). Hens exposed to hot temperatures also surveyed their environment more, which may be in an effort to increase surface area by extending the neck. EOCH also demonstrated a gulping behavior when exposed to heat; however, this behavior has not been characterized, and therefore, its purpose is uncertain. While hens spend a lot of their time performing thermoregulatory behavior, the ΔCBT over time increased slightly for the hot treatments. The increase in ΔCBT was not substantial, and there was no mortality observed in relation to heat exposure suggesting that hens coped successfully with heat stress. Contrary to the results in this study, heat stress has been shown to increase DOA numbers (Petracci et al., 2006). Although EOCH were able to cope successfully, there are some physiological indicators that suggest hens experienced stress. Live shrink was highest for both hot treatments compared with the neutral treatments, which is likely a result of moisture loss due to evaporative cooling and dehydration and likely corresponds with the increase in percentage of time spent panting. This loss of moisture also relates to the higher Δsodium, indicating hens were exhibiting physiological signs of dehydration. Heat stress had minor impacts on meat quality. Typically, high levels of stress before slaughter has been associated with pale, soft, exudative meat characterized by a low pH and a high L* indicative of lightness (Petracci et al., 2004) which was not seen in this study.

Increasing duration of exposure or trip journey had minor effects on EOCH behavior; however, there were differences in blood physiology and meat quality which may correspond with increasing levels of dehydration. Dehydration may be reflected by the increase in live shrink, Δsodium, Δhematocrit, and Δhemoglobin with increasing duration of exposure. In addition, the T/RH by duration interaction for mortality demonstrates that increasing exposure duration in cold conditions (−10°C) may result in higher levels of DOA, which has been shown in both broilers and layers (Warriss et al., 1992; Weeks et al., 2012).

Feather cover had very little effect on the behavior and physiology of brown EOCH. It is important to note that regardless of feather cover, hens did not cope well under cold stress. The laying hen Codes of Practice recommends gradually lowering the barn temperature to acclimatize birds to weather conditions; however, it is important to note that this was not done in the present study (NFACC, 2017). Gradually acclimatizing the birds to cooler temperatures may help them cope with cold exposure; however, there is little research regarding these practices.

Overall brown-strain EOCH are susceptible to transport stress regardless of feather cover, particularly when exposed to cold conditions. Simulated transportation at −10°C for durations of up to 12 h resulted in significant levels of mortality; however, it is important to note that although the chamber was set at −10°C, conditions in the crate were not identical. For this reason, it is important that future research evaluates the thermal microclimate within the transport crate under commercial conditions which may result in temperature gradients within a truck. This research also suggests that brown-strain EOCH are less susceptible to heat stress than cold stress, which suggests that transportation during the summer months may be less stressful for the hen. Further research regarding the crating density, crate design, and trailer microclimate for hens in transport may be helpful for establishing proper regulations to optimize the welfare of EOCH.

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DISCLOSURES

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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