The influence on behavior and physiology of white-feathered end-of-cycle hens during simulated transport

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ABSTRACT Transportation is a stressful procedure that can alter end-of-cycle hen (**EOCH**) behavior and physiology. This study (5 × 3 × 2 factorial arrangement) aimed to assess the effects of temperature (**T**)/relative humidity (**RH**) (−10°C uncontrolled RH (−10), +21°C 30%RH (21/30), +21°C 80%RH (21/80), +30°C 30%RH (30/30), +30°C 80%RH (30/80)), duration (4, 8, 12 h), and feather cover [well (**WF**) and poorly-feathered (**PF**)] on white-feathered EOCH (65−70 wk) behavior and physiology. EOCH (n = 630) from 3 commercial farms were housed for adaptation (3−5 d), fasted (6 h), crated (53 kg/m^2), and placed in a climate-controlled chamber. Data collected included chamber and crate conditions, feather condition score, mortality, core body temperature (**CBT**), behavior, and delta (Δ) blood physiology. Analyses were conducted via ANOVA in a randomized complete block design (farm of origin) with significance declared at $P \leq 0.05$. PF EOCH had higher mortality than WF hens during cold exposure (−10). EOCH ΔCBT demonstrated a greater (positive) change at 12 h for all T/RH compared to 4 h at 21/30, 21/80, and −10 (negative). Cold exposure (−10) resulted in a higher percentage of time spent shivering and motionless, while heat exposure resulted in a higher percentage of time spent panting for WF EOCH exposed to 30/30 and WF and PF hens exposed to 30/80. Hen Δglucose had a greater (negative) change at 4 and 12 h for −10 compared to 4 h at 21/30, and all durations for 21/80, 30/30, and 30/80. PF hens exposed to −10 had a greater (positive) change in Δsodium, Δhemoglobin, and Δhematocrit compared to WF birds (negative). The development of metabolic alkalosis was supported by the increase in Δblood pH over time and the increase in Δpartial pressure of carbon dioxide, Δbicarbonate, and Δbase excess extracellular fluid during cold exposure (−10). These results indicated that EOCH exposed to heat endured thermal stress while PF hens exposed to cold were unable to cope with cold stress.

Key words: thermal stress, feather cover, thermoregulatory behavior, spent hens, welfare

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INTRODUCTION

Transportation is an integral component of poultry production; however, it can result in welfare concerns due to factors such as feed and water withdrawal, loading procedures, social disruption, sensory changes, lairage, and many other variables (Freeman, 1984; Dadgar et al., 2010). There has been extensive research on the impacts of transportation of meat birds, but there are limited studies investigating the impact on end-of-cycle hens (**EOCH**). Therefore, the Canadian Code of Practice (NFACC, 2001) and the Canadian Food Inspection Agency (CFIA, 2020) have not outlined separate requirements (particularly for feed and water withdrawal) between the 2 species, EOCH and broilers, despite significant metabolic differences. Age, metabolic exhaustion, body condition, feather cover (**FC**), and limited customized slaughter plant equipment resulting in longer transport durations are just a few of the unique challenges associated with the transportation of EOCH (Gregory and Wilkins, 1989; Knowles and Broom, 1990; Knowles, 1994; Gregory and Devine, 1999; Newberry et al., 1999; Richards et al., 2012; Weeks et al., 2012).

In Canada, passive ventilation trailers equipped with side curtains to combat ambient weather conditions are the primary method of commercial poultry transport (Knezacek et al., 2010). Passively ventilated trailers do not facilitate environmental control and are vulnerable to poor airflow, potentially causing heat and moisture buildup, in turn creating a thermal gradient (Knezacek et al., 2010). Crate location on the trailer as well as the bird's location in the crate can result in inescapable thermal stress. Extended transport duration and poor bird

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condition, such as poor FC, low body mass, or feather wetness, may exacerbate the effects of thermal stress. To cope with transport stress, poultry use mitigation strategies including initial changes in behavior followed by alterations to physiology if necessary (Broom, 1986, 1990).

Since thermal stress often accompanies transport, birds in transit may demonstrate thermoregulatory behaviors such as panting during heat stress (Mitchell and Kettlewell, 1998) and pteroerection, shivering, or huddling behavior during cold stress (Strawford et al., 2011; Henrikson et al., 2018; Beaulac et al., 2020). Heat stress research with broilers and turkeys have demonstrated increased mortality, core body temperature (**CBT**), and changes to blood physiology parameters (Ait-Boulahsen et al., 1989; Toyomizu et al., 2005; Warriss et al., 2005; Menten et al., 2006; González et al., 2007; Vosmerova et al., 2010; Vermette et al., 2017). Cold stress studies in broilers and turkeys have reported some mortality and decreased CBT (Dadgar et al., 2010; 2011; Knezacek et al., 2010; Strawford et al., 2011; Vecerek et al., 2016; Henrikson et al., 2018). Unfortunately, there have been limited analyses conducted on blood physiology parameters for birds during cold exposure (Hester et al., 1996; Henrikson et al., 2018). Beaulac et al. (2020) found that hens exposed to cold temperatures responded with an increase in the H/L ratio, partial pressure of oxygen ($\mathbf{pO_2}$), soluble oxygen ($\mathbf{sO_2}$) as well as a decrease in blood glucose. The hens exposed to the hot treatments had fewer changes to blood physiology, but indicated dehydration via increased blood sodium concentrations (Beaulac et al., 2020).

The transportation literature for EOCH focuses primarily on dead-on-arrival (**DOA**) numbers, which can be utilized as an indicator of both welfare and economic loss. Studies have demonstrated increased DOA numbers in both summer and winter months, suggesting that temperature greatly influences bird welfare (Petracci et al., 2006; Vecerkova et al., 2019). This study evaluated the influence on well-feathered (**WF**) and poorly-feathered (**PF**) EOCH exposed for pre-determined durations (**D**) to temperature **(T)**/relative humidity (**RH**) combinations on behavior and physiology. In addition to the findings reported in this study, data on muscle characteristics have been previously reported (Frerichs et al., 2021).

MATERIALS AND METHODS

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

Experimental Design

This study was designed as a 5 × 3 × 2 factorial arrangement, with 5 temperature/relative humidity (T/RH) combinations (-10°C, uncontrolled RH (**-10**), 21°C 30%RH (**21/30**), 21°C 80%RH (**21/80**), 30°C 30%RH (**30/30**), 30°C 80%RH (**30/80**)), 3 exposure durations (D; 4, 8, or 12h), and 2 feather covers (FC; WF and PF). Since there is limited work evaluating the response of EOCH to transport conditions, these temperatures were selected to obtain a temperature close to the thermoneutral range (21°C), a temperature above (30°C) and below (−10°C) the thermoneutral range. The two RH values were selected to be representative of a high vs. low humidity environment. In addition, RH was not controlled in the cold treatment as cold air does not have the same water holding capacity as warm air, making humidity control extremely difficult. Due to the trial reaching a humane endpoint in the second replicate for PF hens exposed to −10 (all durations), there were only 2 replicates completed for that specific group. The third replicate was replaced with WF hens to ensure crate density was maintained.

Birds and Housing

White-feathered EOCH (Lohmann LSL-Lite; 65−70 wk; n = 630) were sourced from 3 independent commercial farms housed in conventional layer cages (sourced within 120-km radius of Saskatoon, Saskatchewan, Canada). Each farm of origin was treated as an individual block to minimize flock differences. The EOCH were feather scored on farm to obtain 105 WF and 105 PF hens/replicate (210/replicate). Hens were scored by one observer on 4 parts of the body (neck, back, breast, and wings) using a 4-point system. Score 1 (no feather cover) and score 2 (greater than 50% of the plumage is missing) were grouped together for PF and score 3 (less than 50% of the plumage is missing) and score 4 (full intact plumage) were grouped together for WF (adapted from Davami et al., 1987; Sarica et al., 2008). Hens were then crated and transported in an enclosed van to the research facility. The birds were provided an acclimatization period of 3 to 5 d (2 T/RH simulated transport treatments were conducted per day) in 2 floor pens (3.9 × 3.0 m) with wheat straw litter. Ad libitum feed (obtained from farm of origin) and water were provided via aluminum tube feeders (38 cm diameter) and bell drinkers (36 cm diameter). Housing T was kept between 15°C and 18°C and RH was 40 to 60%. Lighting program was consistent with the farm of origin.

Prior to Simulated Transport

EOCH were moved to 1 of 4 feed withdrawal pens (21 hens/FC resulting in 7 hens/D; 1.2 × 1.3 m pen) 6 h prior to simulated transport. Each pen had wheat straw litter and access to an aluminum waterer (30 cm diameter). Hens (7 WF and 7 PF) were randomly allocated to one T/RH and D combination. All EOCH were wing banded and a subsample of birds (n = 5/replicate) were orally administered a miniature data logger (iButton Thermochron DS1922L, Maxim Integrated, San Jose,

CA) which moved to the crop/gizzard and recorded CBT every minute. Baseline CBT readings were obtained in the final 5 min in temporary transport crates after hen preparation. Blood samples were taken (n = 5/replicate) via brachial vein into an ethylenediamine dipotassium tetraacetic acid (**EDTA**) anticoagulation tube. The blood samples were used for blood physiology analysis (n = 3/replicate) and heterophil to lymphocyte (**H/L**) ratio analyses (n = 5/replicate). Blood physiology parameters were evaluated via CG8+ cartridge in an iSTAT handheld analyzer (Abbott Point of Care Inc., Princeton, NJ). Parameters evaluated included: blood pH, glucose (mmol/L), sodium (mmol/L), partial pressure of carbon dioxide (**pCO_2**; mm Hg), total carbon dioxide (**tCO_2**; mmol/L), partial pressure oxygen (**pO_2**; mm Hg), oxygen saturation (**sO_2**; %), bicarbonate concentration (**HCO_3^-**; mmol/L), base excess in the extracellular fluid compartment (**BE**; mmol/L), hemoglobin (mmol/L), and hematocrit (% packed cell volume (**PCV**)). Blood smears were prepared manually using a two-slide wedge method, dried, and later stained using PROTOCOL Hema 3 (Fisher Scientific; Ottawa, ON, Canada) and stored for analysis. During analysis slides were read at 100× oil magnification (microscope B-290TB; Optika; Bergamo, Italy) and the number of heterophils and lymphocytes were counted until a total of 100 was reached. After baseline readings were collected, EOCH were transported (750 m) in an enclosed van to the climate-controlled chambers (College of Engineering, University of Saskatchewan, Saskatoon, Canada).

Simulated Transport

Hens were transferred to the experimental crates (0.56 × 0.39 m; density 53 kg/m^2); each crate was divided in half to hold 7 WF hens on one side and 7 PF hens on the other. The chambers and each crate were equipped with a T/RH data logger (iButton Hygrochron DS1923-#F5, Maxim Integrated; San Jose, CA) at bird level, which recorded T/RH every minute. Chamber conditions were monitored in real time via thermocouple and a multimeter (Omega HH509, Omega Engineering; Laval, Canada) and RH sensors (HM1500LF, Measurement Specialities, Inc.; Toulouse, France). Infrared video cameras (Panasonic WV-CF224FX; Panasonic Corporation of North America, Neward, NJ) were used to record bird behavior during simulated transport. Instantaneous scan sampling at 5-min intervals was used to evaluate EOCH behaviors. The observer conducting the scan samples was blind to T/RH treatment, however, duration and feather cover were unable to be blinded. The behavioral ethogram used is outlined in Table 1.

Post-simulated Transport

Each crate of EOCH was removed after the designated D (4, 8, or 12 h). A second blood sample was collected, analyzed, and delta (Δ) values were calculated for each blood parameter listed above (Δ = final-initial). Hens were slaughtered using a small-scale slaughter line (shackled, stunned, and exsanguinated with an electric stunning knife [VS200, Midwest Processing Systems; Minneapolis, MN]). The data loggers were retrieved from the crop or gizzard of the hen. The ΔCBT was calculated (mean baseline CBT - mean CBT during last h of exposure; Henrikson et al., 2018) for each 15-min interval and overall (4, 8, or 12 h).

Table 1. Behavioural ethogram adapted from Webster and Hurnik, 1990; Hurnik et al., 1995; Webster, 2000; EFSA, 2011; Rault et al., 2016; Henrikson et al., 2018.

Behaviour	Definition
Motionless	Hen is stationary sitting, crouching, or standing with both feet and potentially body in contact with the floor. Hen has no apparent movement and may be in a collected posture (while either standing or sitting) with head and neck retracted and eyes open or closed. Beak may potentially be oriented towards the floor.
Active	Locomotive movement in an attempt to move feet, wings, or location.
Object peck	Beak used in short, quick forward motion to make contact with objects (sensors, wall, or floor of the crate). This is often performed in a repetitive, stereotyped manner.
Aggressive peck	Beak used forcefully in a short and quick forward motion, making contact with another hen with intent to injure.
Burrowing	Downward motion to get underneath another bird.
Preen	Manipulation of feather cover along the bird's body with the beak.
Gulp	Opening the mouth wide and shutting it in one quick exaggerated motion.
Head shake	Body of hen is immobile except for quick, short, sharp movement consisting of small displacement of the head in any direction or rotation of the head around its vertical or horizontal axis.
Panting	The hen's beak is open while breathing and respiration rate is abnormally rapid. Distinct 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.
Pteroerection	Erection of feathers or fluffing.
Survey	Quick head movements (alert bird), suggesting visual surveillance of the environment.
Rustling	Bird shifts position in the crate without change in location.
Stretch	A muscular activity, characterized by brief, forceful extension of limbs.
Head movement	Body of hen is immobile except small displacement of head in any direction.
Wing shake	Quick movement of the wing.
Tail movement	Tail moves vertically, horizontally, fans in or out.
Twitch	A brief contraction of skeletal muscle.
No observation	Hens cannot be seen and behavior cannot be characterized. Potentially deceased hens placed in this category.

Low incidence behaviors have been combined for analysis including: head movement, wing shake, tail movement, stretch, twitch, scratch, object peck, aggressive peck, and no observations.

Statistical Analyses

The data collected were analyzed as a randomized complete block design, with farm of origin as block using SAS 9.4 (SAS 9.4, Cary, NC). Each crate section (half crate) was considered the experimental unit. Prior to

analyses, data were checked for normality (PROC UNIVARIATE) and mortality, blood physiology, and behavior data were log transformed. PROC MEANS was used to obtain treatment means and standard error of the means (**SEM**) followed by an ANOVA (PROC MIXED) with 5 T/RH combinations × 3 D × 2 FC in a factorial arrangement. Tukey's test was used for means separation and differences were declared significant at $P \leq 0.05$.

RESULTS

Chamber and Crate Conditions

The simulated transport conditions hens were exposed to (average crate T for each T/RH and D combination and average chamber T/RH combination) is reported in Frerichs et al. (2021). The attained chamber conditions closely aligned with the target T/RH combinations reaching −8.9, 20.9, 21.8, 30.7, and 30.0°C and 70.3, 48.1, 81.9, 39.0, and 80.9%RH for the treatments −10, 21/30, 21/80, 30/30, and 30/80, respectively. Inside the crate, at bird level, T was typically higher, and RH was generally lower than T/RH conditions observed inside the chamber.

Mortality

There was a significant interaction between T/RH combination and FC for percent mortality (Table 2). Higher mortality was observed in hens exposed to −10 PF compared to all other treatment combinations ($P < 0.01$), while no effect was observed for D.

Core Body Temperature

An interaction was observed between T/RH combinations and D for ΔCBT of EOCH (Table 2). Hens ΔCBT had a greater positive change from baseline in hens exposed for 12 h to all T/RH combinations compared to those exposed for 4 h to 21/30 and 21/80, followed by 4 h at −10 which had a negative change from baseline. No effect of FC was reported on ΔCBT from baseline (Table 2).

The graphs in Figures 1A−1F outline the change in CBT for all EOCH during each 15-min interval when exposed to the T/RH combinations. Hens exposed to the hot (30/30 and 30/80) and neutral (21/30 and 21/80) T/RH combinations demonstrated a slight CBT increase; however, D and FC played a limited role. Birds exposed to the cold T/RH (−10) were split into 2 categories: live hens (Figure 1E), which included hens that survived exposure for the entire D, and mortality (Figure 1F), which included hens that died during exposure, 55% of which were PF hens from the 12 h D.

Behavior

Behavior data are outlined in Table 3. There were 2 two-way interactions for T/RH and FC. Hens exposed to 30/30 and 30/80 that were WF spent the least amount of time (%) motionless compared with WF hens exposed to −10, 21/30, and 21/80 and PF hens exposed to 21/30, 21/80, and 30/30, with PF −10 and 30/80 being intermediate ($P = 0.03$). The opposite effect is seen on percentage of time spent panting, with WF hens exposed to 30/30 and 30/80 and PF hens exposed to 30/80 spending more time panting compared with WF and PF hens exposed to −10, 21/30, and 21/80, with PF hens exposed to 30/30 being intermediate ($P = 0.03$).

Temperature/RH main effects were observed for percentage of time spent performing the following behaviors: active, rustle, head shake, shiver, preen, gulp, pteroerection, and other (low incidence). Hens spent more time performing active behaviors in 30/30 and 30/80 compared with 21/30, 21/80, and −10 combinations ($P < 0.01$). Similarly, the birds spent more time rustling in 30/30 and 30/80 compared with the 21/30 and 21/80 combinations ($P < 0.01$). EOCH spent a greater percentage of time performing head-shaking behavior when exposed to −10 compared with 21/30, 21/80, and 30/30 ($P = 0.01$). Hens shivered and performed pteroerection more when exposed to −10 compared with all other T/RH combinations ($P < 0.01$ for both). EOCH spent more time preening in 30/80 and 30/30 combinations compared with 21/30 and −10 ($P < 0.01$). Hens spent

Table 2. Mortality (%) and delta core body temperature (ΔCBT; °C) of white-feathered end-of-cycle hens with 2 feather covers (FC; well [WF] and poorly-feathered [PF]) exposed to 5 temperature (T) and RH combinations (−10°C uncontrolled RH (−10), 21°C 30%RH (21/30), 21°C 80%RH (21/80), 30°C 30%RH (30/30), and 30°C 80%RH (30/80)) for a duration (D) of 4, 8, and 12 h.

	T/RH combinations						D				FC			
Parameter	−10	21/30	21/80	30/30	30/80	*P*-value	4 h	8 h	12 h	*P*-value	WF	PF	*P*-value	SEM
Mortality	15.2[a]	0.8[b]	0[b]	0[b]	0[b]	<0.01	1.5	2.0	4.9	0.24	1.0[b]	4.8[a]	0.03	1.30
ΔCBT[1]	−1.31	0.61	0.20	0.51	1.14	0.23	−1.01[c]	0.47[b]	1.71[a]	<0.01	0.19	0.54	0.53	0.247
T/RH × FC interaction − mortality ($P < 0.01$)														
	-10			21/30			21/80			30/30			30/80	
WF	3.2[b]			1.6[b]			0[b]			0[b]			0[b]	
PF	33.3[a]			0[b]			0[b]			0[b]			0[b]	
T/RH × D interaction − ΔCBT[1] ($P = 0.02$)														
	−10			21/30			21/80			30/30			30/80	
4 h	−4.97[c]			−0.88[b]			−1.06[b]			0.29[ab]			0.91[ab]	
8 h	1.12[ab]			0.71[ab]			0.11[ab]			−0.10[ab]			0.86[ab]	
12 h	2.34[a]			1.99[a]			1.55[a]			1.33[a]			1.65[a]	

[a,b,c]Means within a main effect or an interaction with different superscripts are significantly different ($P \leq 0.05$).

[1]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.

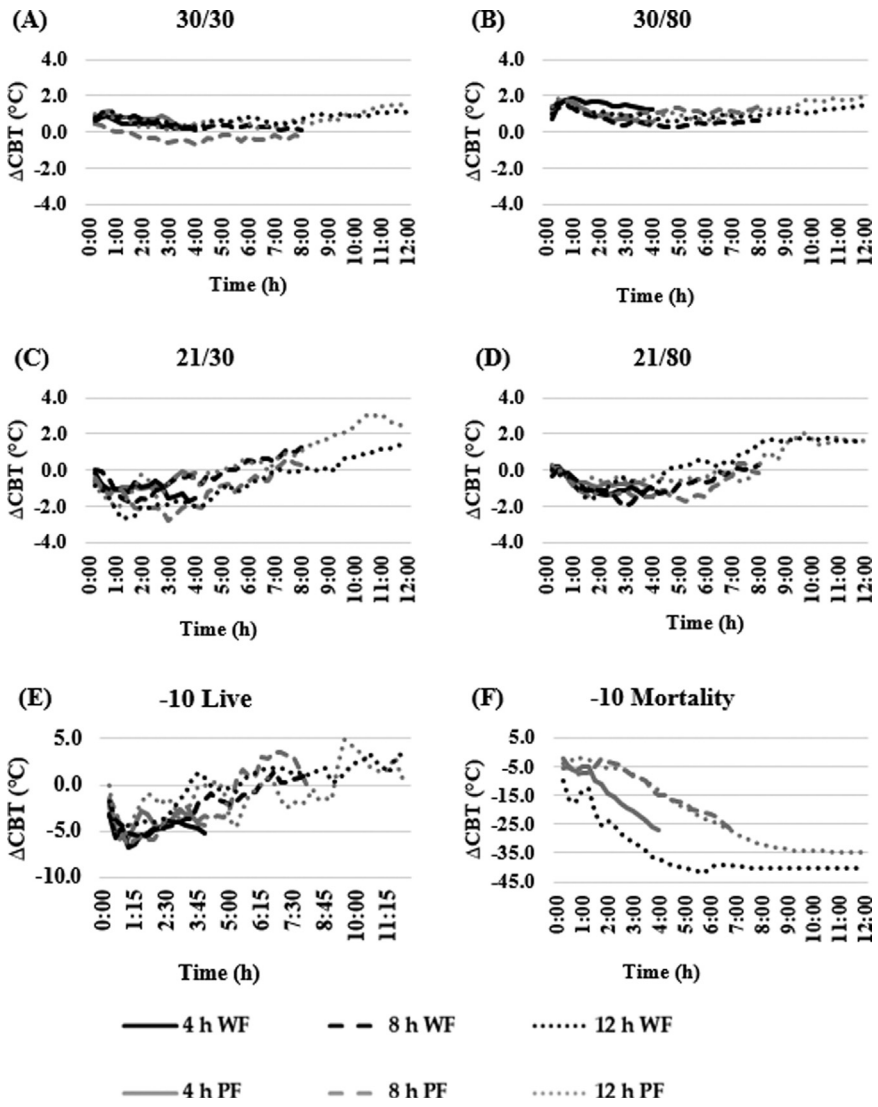

Figure 1. Delta core body temperature (ΔCBT;°C) over time of white-feathered end-of-cycle hens exposed to (A) +30°C/30%RH, (B) +30°C/80%RH (C) +21°C/30%RH (D) +21°C/80%RH, (E) −10°C uncontrolled RH (birds that lived through exposure; N = 108), (F) −10°C uncontrolled RH (data includes any mortality including those from crates removed prior to duration end for humane end-point reasons; N = 11).

Table 3. Behavior parameters (% of time) for temperature (T) and RH combinations (−10°C uncontrolled RH (−10), 21°C 30%RH (21/30), 21°C 80%RH (21/80), 30°C 30%RH (30/30), and 30°C 80%RH (30/80), duration of exposure (D; 4, 8, and 12 h), and feather cover (FC; well [WF] and poorly-feathered [PF]) of white-feathered end-of-cycle hens.

	T/RH combinations						D				FC			
Behavior	−10	21/30	21/80	30/30	30/80	*P*-value	4 h	8 h	12 h	*P*-value	WF	PF	*P*-value	SEM
Motionless	83.8^{ab}	90.9^{a}	90.7^{a}	75.8^{bc}	70.2^{c}	<0.01	81.6	82.5	82.3	0.84	79.5^{b}	85.0^{a}	0.01	1.44
Active	0.3^{b}	0.4^{b}	0.4^{b}	1.0^{a}	1.3^{a}	<0.01	0.8	0.5	0.8	0.24	0.8	0.7	0.23	0.08
Rustle	1.9^{ab}	0.8^{b}	1.0^{b}	2.2^{a}	1.9^{a}	<0.01	1.8^{a}	1.4^{ab}	1.3^{b}	0.02	1.6	1.5	0.43	0.11
Survey	3.1	2.8	3.0	3.3	2.5	0.70	3.8^{a}	1.8^{b}	3.1^{ab}	<0.01	3.0	2.8	0.74	0.21
Head shake	2.1^{a}	0.9^{b}	1.0^{b}	1.1^{b}	1.3^{ab}	0.01	1.3	1.0	1.3	0.22	1.2	1.2	0.35	0.09
Pant	0.1^{b}	1.2^{b}	0.2^{b}	11.2^{a}	17.7^{a}	<0.01	6.5	6.4	6.9	0.97	8.7^{a}	4.3^{b}	<0.01	1.19
Shiver	2.5^{a}	0^{b}	0^{b}	<0.1^{b}	0^{b}	<0.01	0.7	0.1	0.2	0.08	0.4	0.3	0.59	0.15
Burrow	<0.1	0	0	0	<0.1	0.11	<0.1	<0.1	0	0.28	<0.1	<0.1	0.53	0.01
Preen	0^{c}	0.4^{bc}	0.6^{ab}	0.9^{a}	1.0^{a}	<0.01	0.6	0.6	0.8	0.24	0.6	0.7	0.24	0.06
Gulp	0^{b}	0.1^{ab}	0.2^{a}	0.1^{ab}	0.1^{ab}	0.02	0.1	0.2	<0.1	0.06	0.2^{a}	0.1^{b}	0.03	0.02
Pteroerection	0.3^{a}	<0.1^{b}	<0.1^{b}	<0.1^{b}	<0.1^{b}	<0.01	0.1	0.1	0.1	0.58	<0.1	0.1	0.13	0.02
Other[1]	6.1^{a}	2.6^{b}	2.8^{b}	4.4^{ab}	4.0^{ab}	<0.01	2.8^{b}	5.4^{a}	3.2^{ab}	<0.01	4.2	3.3	0.51	0.40
T/RH × FC interaction – Motionless ($P = 0.03$)														
	−10			21/30			21/80			30/30			30/80	
WF	85.4^{a}			91.4^{a}			90.1^{a}			67.8^{bc}			64.0^{c}	
PF	80.9^{ab}			90.3^{a}			91.3^{a}			83.8^{a}			76.4^{abc}	
T/RH × FC interaction – Pant ($P = 0.03$)														
	−10			21/30			21/80			30/30			30/80	
WF	<0.1^{c}			1.2^{c}			0.2^{c}			16.3^{ab}			23.8^{a}	
PF	0.1^{c}			1.3^{c}			0.2^{c}			6.1^{bc}			11.6^{b}	

^{a,b,c}Means within a main effect or an interaction with different superscripts are significantly different ($P \leq 0.05$).

[1]Other defined as low incidence behaviors such as: head movement, wing shake, tail movement, stretch, twitch, scratch, object peck, aggressive peck, and no observations.

Table 4. Delta (Δ; final-initial) blood physiology parameters of temperature (T) and RH combinations (−10°C uncontrolled RH (−10), 21°C 30%RH (21/30), 21°C 80%RH (21/80), 30°C 30%RH (30/30), and 30°C 80%RH (30/80)), duration of exposure (D; 4, 8, and 12 h), and feather cover (FC; well [WF] and poorly-feathered [PF]) for white-feathered end-of-cycle hens.

	T/RH combinations						D				FC			
Parameter[1]	-10	21/30	21/80	30/30	30/80	*P*-value	4h	8h	12h	*P*-value	WF	PF	*P*-value	SEM
ΔpH	0	0.03	0.02	0.01	0.02	0.35	0[b]	0.02[ab]	0.04[a]	0.04	0.02	0.01	0.14	0.006
ΔpCO_2	−6.3	−8.1	−5.3	−7.8	−3.5	0.48	−3.2[a]	−5.5[ab]	−10.1[b]	0.02	−7.9[b]	−4.3[a]	<0.01	1.09
ΔpO_2	9.9[a]	−2.2[ab]	−4.5[ab]	1.0[ab]	−10.6[b]	0.03	−4.1	−1.2	−1.2	0.39	−2.0	−2.5	0.93	1.42
ΔBE	−2.0	−0.8	−0.5	−1.6	0.1	0.12	−1.4	−0.6	−0.7	0.35	−1.0	−0.8	0.24	0.28
ΔHCO_3^-	−1.9	−1.3	−0.8	−1.8	−0.2	0.11	−1.3	−0.9	−1.3	0.60	−1.3[b]	−0.9[a]	0.05	0.24
ΔtCO_2	−2.0	−1.6	−0.9	−2.0	−0.3	0.14	−1.3	−1.1	−1.6	0.65	−1.6[b]	−1.0[a]	0.02	0.25
ΔsO_2	2.7[a]	0.2[ab]	−0.9[ab]	1.5[a]	−2.9[b]	0.05	−1.8	0.1	1.5	0.10	0	−0.2	0.77	0.54
Δsodium	0.3[c]	1.9[bc]	2.6[bc]	3.9[ab]	4.7[a]	<0.01	2.0[b]	2.9[ab]	3.8[a]	0.04	2.8	3.0	0.31	0.31
Δglucose	−3.0[b]	−1.6[a]	−1.2[a]	−1.1[a]	−1.2[a]	<0.01	−1.3	−1.5	−1.7	0.06	−1.4	−1.6	0.07	0.12
Δhematocrit	−0.2	−0.1	0.1	0	0.8	0.68	−0.2	−0.2	0.8	0.09	−0.1	0.4	0.09	0.23
Δhemoglobin	0	0	0	0	0.2	0.62	0	−0.1	0.2	0.06	0	0.1	0.06	0.05
ΔH/L ratio	0.88[a]	0.04[b]	0.08[b]	0.18[b]	0.04[b]	<0.01	0.23	0.14	0.19	0.89	0.21	0.16	0.27	0.06

[a,b,c]Means within a main effect with different superscripts are significantly different ($P \leq 0.05$).

[1]Partial pressure of carbon dioxide (pCO_2; mm Hg), partial pressure of oxygen (pO_2; mm Hg), base excess in extracellular fluid compartment (BE; mmol/L), bicarbonate (HCO_3^-; mmol/L), total carbon dioxide (tCO_2; mmol/L), sodium (mmol/L), glucose (mmol/L), hemoglobin (mmol/L), oxygen saturation (sO_2; %), hematocrit (%PVC), heterophil to lymphocyte ratio (H/L ratio).

more time gulping in the 21/80 treatment compared with −10 ($P = 0.02$). Hens performed low incidence behaviors more frequently in −10 treatment compared with 21/30 and 21/80 ($P < 0.01$). There was no effect of T/RH on survey or burrow behavior.

Duration main effects were observed for percentage of time spent performing the following behaviors: rustle, survey, and other (low incidence). Hens exposed for 4 h spent more time rustling than hens exposed for 12 h, with 8 h being intermediate ($P = 0.02$). EOCH spent more time surveying for 4 h compared to the 8 h exposure, with 12 h being intermediate ($P < 0.01$). Low incidence behaviors were performed most frequently in the 8 h duration compared with the 4 h duration ($P < 0.01$). There was no impact of duration on motionless, active, head shake, pant, shiver, burrow, preen, gulp, or pteroerection behaviors.

Feather cover main effects were observed for percentage of time spent performing gulping behavior. Hens that were WF spent more time gulping than PF hens ($P = 0.03$). There was no effect of feather cover on active, rustle, survey, head shake, shiver, burrow, preen, pteroerection, or other (low incidence) behaviors.

Blood Physiology

T/RH effects were observed for ΔpO_2 and ΔsO_2 (Table 4). Hens were found to have a positive ΔpO_2 (increase) from baseline when exposed to −10 compared with a negative ΔpO_2 (decrease) from baseline in hens exposed to 30/80 ($P = 0.03$). The birds demonstrated a greater positive ΔsO_2 from baseline when exposed to −10 and 30/30 compared with a negative ΔsO_2 when exposed to 30/80 ($P = 0.05$). No effect of T/RH conditions were observed on Δ blood pH. An effect of duration was observed for Δblood pH (Table 4). Hens exposed for 12 h to simulated transport conditions had a larger positive Δblood pH (increase) from baseline compared to hens exposed for 4 h. No effect of D was observed on ΔpCO_2, ΔpO_2, ΔBE, ΔHCO_3^-, ΔtCO_2, ΔsO_2, Δsodium, Δhematocrit, or Δhemoglobin values. There was no effect observed for FC of EOCH on Δblood pH, ΔpO_2, ΔsO_2, or Δglucose.

Two-way interactions between T/RH and FC were observed for ΔpCO_2, ΔBE, ΔHCO_3-, ΔtCO_2, Δsodium, Δhematocrit, and Δhemoglobin (Table 5). Hens demonstrated a positive ΔpCO_2 (increase) from baseline in the −10 PF treatment and a negative ΔpCO_2 (decrease) in the −10 WF treatment ($P = 0.03$). A large positive ΔBE

Table 5. Blood physiology parameter interactions (2-way) for white-strain end-of-cycle hens (2 feather covers [FC]: well [WF] and poor-feathered [PF]) under simulated transport conditions: temperature (T) and RH (−10°C uncontrolled RH (−10), 21°C 30%RH (21/30), 21°C 80%RH (21/80), 30°C 30%RH (30/30), and 30°C 80%RH (30/80)) and exposure duration (D) of 4, 8, or 12 h.

	−10	21/30	21/80	30/30	30/80
T/RH × FC interaction – ΔpCO_2 (mm Hg; $P = 0.03$)					
WF	−11.0[b]	−8.4[ab]	-5.8[ab]	−8.7[ab]	−6.3[ab]
PF	2.1[a]	−7.9[ab]	-4.8[ab]	−6.9[ab]	−0.7[ab]
T/RH × FC interaction – ΔBE (mmol/L; $P = 0.01$)					
WF	−3.4[c]	0[ab]	0[ab]	−1.7[bc]	−0.4[ab]
PF	0.4[ab]	−1.7[b]	−1.0[ab]	−1.5[b]	0.5[a]
T/RH × FC interaction – ΔHCO_3^-(mmol/L; $P < 0.01$)					
WF	−3.3[b]	−0.7[ab]	−0.5[a]	−2.0[ab]	−0.7[ab]
PF	0.5[a]	−1.9[ab]	−1.1[ab]	−1.7[ab]	0.3[a]
T/RH × FC interaction – ΔtCO_2 (mmol/L; $P < 0.01$)					
WF	−3.6[b]	−1.0[ab]	−0.7[ab]	−2.2[ab]	−1.0[ab]
PF	0.8[a]	−2.2[ab]	−1.2[ab]	−1.8[ab]	0.3[a]
T/RH × FC interaction – Δsodium (mmol/L; $P = 0.02$)					
WF	−1.1[b]	2.2[a]	3.2[a]	4.0[a]	4.9[a]
PF	2.8[a]	1.7[ab]	2.0[a]	3.8[a]	4.5[a]
T/RH × FC interaction – Δhematocrit (% PCV; $P = 0.01$)					
WF	−1.5[d]	−0.4[bcd]	1.0[abc]	−0.2[bcd]	0.3[abcd]
PF	1.9[a]	0.1[abcd]	−0.7[cd]	0.1[abcd]	1.3[ab]
T/RH × FC interaction – Δhemoglobin (mmol/L; $P < 0.01$)					
WF	−0.3[b]	−0.1[ab]	0.2[ab]	0[ab]	0[ab]
PF	0.4[a]	0[ab]	−0.2[ab]	0[ab]	0.3[ab]
T/RH × D interaction – Δglucose (mmol/L; $P = 0.05$)					
4 h	−3.2[bc]	−0.7[a]	−0.9[a]	−1.0[a]	−1.1[a]
8 h	−1.9[abc]	−1.8[ab]	−1.5[a]	−1.4[a]	−1.1[a]
12 h	−3.8[c]	−2.2[abc]	−1.3[a]	−0.9[a]	−1.3[a]

Δ = final-initial; partial pressure of carbon dioxide (pCO_2); base excess in extracellular fluid compartment (BE), bicarbonate (HCO_3^-), total carbon dioxide (tCO_2).

[a,b,c,d]Means with different superscripts within a parameter are significantly different ($P \leq 0.05$).

Table 6. Delta (Δ; final-initial) heterophil to lymphocyte (H/L) ratio for white-feathered end-of-cycle hens: Three-way interaction between temperature and RH combinations (−10°C uncontrolled RH (−10), 21°C 30%RH (21/30), 21°C 80%RH (21/80), 30°C 30%RH (30/30), and 30°C 80%RH (30/80)), duration of exposure (4, 8, and 12 h), and feather cover (well [WF] and poor-feathered [PF]).

	−10		21/30		21/80		30/30		30/80	
	WF	PF	WF	PF	WF	PF	WF	PF	WF	PF
4 h	0.83[b]	1.07[ab]	0.23[b]	−0.11[b]	0.08[b]	0.07[b]	0.25[b]	0.03[b]	−0.25[b]	0.38[b]
8 h	0.07[b]	2.70[a]	0.07[b]	0.08[b]	0.15[b]	−0.10[b]	0.20[b]	−0.02[b]	−0.04[b]	−0.03[b]
12 h	0.62[b]	0.99[ab]	0.14[b]	−0.16[b]	0.19[b]	0.08[b]	0.63[b]	−0.03[b]	0.13[b]	0.03[b]

[a,b]Means with different superscripts are significantly different ($P \leq 0.05$).

(increase) from baseline was observed for PF hens exposed to 30/80 compared with a negative ΔBE (decrease) in the PF hens exposed to 21/30 and 30/30, followed by a larger negative ΔBE for WF hens exposed to −10 ($P = 0.01$). A large negative ΔHCO_3^- (decrease) was observed for WF hens exposed to −10, compared with mild ΔHCO_3^- (positive or negative) from baseline for PF hens exposed to −10 and 30/80 and WF hens exposed to 21/80 ($P < 0.01$). The ΔtCO_2 from baseline was positive (increase) for PF hens exposed to −10 and 30/80 and negative (decrease) for WF hens exposed to −10 ($P < 0.01$). Hens of both FC had a greater positive Δsodium (increase) from baseline for 30/80, 30/30, 21/80 combinations, WF birds in the 21/30 combination, and PF EOCH in the −10 combination compared to the negative Δsodium for WF hens exposed to −10 (decrease; $P = 0.02$). EOCH demonstrated a greater positive Δhematocrit (increase) from baseline for PF hens exposed to −10 compared with the negative Δhematocrit (decrease) for PF hens exposed to 21/80 and WF hens exposed to −10, 21/30, and 30/30 ($P = 0.01$). PF hens experienced a greater positive Δhemoglobin (increase) from baseline when exposed to −10 compared to the negative Δhemoglobin (decrease) for WF hens exposed to −10 ($P < 0.01$).

A 2-way interaction between T/RH and D was observed for Δglucose (Table 5). Hens were reported to have a small negative Δglucose (decrease) from baseline when exposed to 21/30 for 4 h, as well as 21/80, 30/30, and 30/80 combinations for all D compared with a large negative Δglucose (decrease) when exposed to −10 for 4 or 12 h ($P = 0.05$).

A 3-way interaction for H/L ratio was observed between T/RH, D, and FC. EOCH experienced a greater positive (increase) ΔH/L ratio from baseline in the −10 PF 8 h treatment compared to all other T/RH, D, and FC combinations except for the 4 and 12 h −10 PF hens ($P = 0.02$; Table 6).

DISCUSSION

Thermal stress may be experienced by EOCH within a transport trailer as a result of the microclimate, which can be created by lack of adequate air circulation or external ambient conditions (Knezacek et al., 2010). The effects of thermal stress have been well documented for broilers; however, EOCH tend to respond differently due to differences in feather cover and poor body reserves (Gregory and Devine, 1999; Richards et al., 2012).

Hens responded to transport stressors, as expected by altering their behavior and physiology in an attempt to maintain body homeostasis. As seen in this study, increased time spent performing pteroerection and shivering are well known behavioral responses to the cold. Henrikson et al. (2018) observed shivering and pteroerection in turkey toms (2.2 and 56.5% of time) and hens (5.9 and 27.7% of time) when exposed to −18°C for 8 h and concluded that both responses assisted with generating or capturing heat. Head-shaking behavior was also increased with cold exposure; however, little is known about head-shaking behavior in cold stressed poultry. The increased prevalence suggests that it may be a response to the environment and could be related to the high incidence of mortality associated with cold exposure in this study, especially in PF hens. Euthanasia studies have demonstrated head-shaking behavior prior to loss of consciousness in attempt to regain alertness (Raj and Gregory, 1994). Burrowing, another common poultry response to the cold, has been observed in other studies (Strawford et al., 2011; Henrikson et al., 2018); however, no effect was observed in the present study for this behavior.

One of the primary physiological coping mechanisms for cold stress is energy mobilization, which can be measured by a decrease in blood glucose (Dadgar et al., 2012a,b). Hens in the current study demonstrated lower blood glucose when exposed to cold, compared with hot T or neutral T regardless of RH especially with short and longer exposure times. Beaulac et al. (2020) also found that hens exposed to cold temperatures (−10) had a larger decrease in blood glucose. Similarly, Dadgar et al. (2011) reported higher concentrations of blood glucose in broilers exposed to neutral environments (20°C) compared to cold environments (ranging from −18°C to −4°C). This has been seen in other broiler studies as well, with blood glucose decreasing with increasing transportation distance (Vosmerova et al., 2010). The current study also noted differences in pO_2 and sO_2 when comparing the cold treatment (−10) and hot and low humidity treatment (30/30; for sO_2 only) to the hot and high humidity treatment (30/80); these effects were also noted in the study with brown-feathered hens (Beaulac et al., 2020). Sauer et al. (2019) reported that pO_2 were positively correlated to blood pH values, which suggests that it may be tied to respiratory alkalosis. Meanwhile little is known about sO_2 in poultry. The current study also noted a rise in blood pH, supporting the development of metabolic alkalosis. This is further demonstrated by the increase in ΔpCO_2,

Δ HCO_3^-, and ΔBE during cold exposure (−10) for PF hens.

Well-feathered hens exposed to hot temperatures (30/30 and 30/80) spent less time motionless compared with other treatments, which was also observed in brown-feathered EOCH exposed to hot temperatures, regardless of feather cover (Beaulac et al., 2020). Similarly, to the Beaulac et al. (2020) study, the hens in this study also responded to heat exposure with behavioral responses such as increased time spent panting, active, and rustling. Panting behavior functions as an evaporative cooling mechanism and has been demonstrated in both broilers and turkeys as a thermoregulatory response to the heat stress (Toyomizu et al., 2005; Menten et al., 2006; Vermette et al., 2017). Increased active and rustling behavior in response to heat exposure may be a result of hens trying to move away from conspecifics to assist in dissipating heat. Increased preening during heat exposure was also observed in this study, which may suggest that EOCH were trying to pull feathers away from their skin to facilitate cooling. Sherry (1981) observed that red junglefowl exposed to cold T had a reduced incidence of preening behavior, which was suggested to slow heat loss. Physiological blood parameter changes can also be related to heat exposure. This heat exposure, coupled with feed and water withdrawal, and transport duration, can lead to dehydration. Blood sodium, hematocrit, and hemoglobin concentrations increased during simulated transport. Beaulac et al. (2020) found that the blood sodium concentration increased with heat exposure, while hematocrit and hemoglobin remained unaffected by exposure T. Studies on other poultry species have found that hot T and fasting can result in increases in blood sodium concentrations (Ait-Boulahsen et al., 1989; González et al., 2007).

To maintain homeostasis during thermal stress, poultry will alter their CBT by either shifting heat from the internal core to the periphery when exposed to hot conditions (Wolfenson et al., 1981; Giloh et al., 2012) or by initiating thermogenesis when exposed to the cold (Block, 1994; Schwartzkopf-Genswein et al., 2012). The hens in this study that were unable to maintain body temperature were primarily those exposed to cold, which is likely due to reduced energy stores from the demands of egg production. This study also demonstrated that FC significantly influences the hen's ability to cope with environmental stressors. In addition to behavior and blood physiology, one key indicator of distress during transport is mortality or DOA. The majority of EOCH in this study were able to utilize behavioral and physiological mechanisms to cope, however, hens exposed to the cold demonstrated difficulty coping. Some birds survived the entire D by mobilizing energy stores to maintain homeostasis. Others, particularly the PF hens in the 12-h D were unable to cope resulting in a decline in CBT leading to hypothermia and ultimately death from insufficient energy reserves. The PF birds exposed to the cold (−10) combination had the highest mortality, with only two replicates completed for humane reasons, compared to all other treatments, however mortality was also observed in the WF hens exposed to the cold. This has also been observed in other studies where higher mortality was reported for poultry exposed to T between −6.0 and −3.1°C, indicating that birds struggle to cope with cold (Vecerek et al., 2016; Vecerkova et al, 2019). Weeks et al. (2012) noted a higher percentage of DOA with cold exposure, especially with longer transport durations and identified low body weights, flock mortality, and poor feather cover as factors increasing the risk of DOAs. Contrary to this study, brown EOCH demonstrate difficulty coping with cold exposure (−10°C) as indicated by high mortality, regardless of FC (Beaulac et al., 2020). The inability to cope with cold stress was further seen with PF EOCH experiencing larger increases in the H/L ratio when exposed to cold for an intermediate time period (8 h). Hester et al. (1996) reported that layers exposed to the cold (average T of 0°C, RH between 50 and 74%) for 72 h had increased H/L ratios compared to hens in a neutral environment (average T of 21°C, RH between 35 and 44%). Conversely, brown-feathered hens did not demonstrate an effect of FC or D, but had increased H/L ratios when exposed to cold (−10) compared with neutral temperatures (21/30) (Beaulac et al., 2020).

This study also reinforced that the length of time EOCH are exposed to transport conditions can exacerbate the impact on hen welfare. Rustling and surveying were observed more frequently in the initial 4 h of exposure. However, these behaviors ceased with time suggesting either the conservation of energy for thermoregulation, acceptance of the hen's circumstances, or a reflection of the feedback of behavior and physiological mechanisms allowing the hens to cope and maintain homeostasis, potentially decreasing the level of stress. Similar results were seen for brown-feathered hens (Beaulac et al., 2020), while Henrikson et al. (2018) reported that surveying behavior was not expressed in turkeys exposed to T between −18°C and 20°C with a RH of 30% or 80% for 8 h. The remaining low incidence behaviors became statistically significant when pooled. However, individually these behaviors were observed at a low frequency suggesting they may not have biological relevance. In addition, blood pH slightly increased and pCO_2 decreased with increased D likely because of birds experiencing respiratory distress for an increased D. Further, blood sodium concentrations were highest with 12-h D likely due to increasing dehydration over time. These effects were also demonstrated in brown-feathered hens exposed to the same conditions (Beaulac et al., 2020).

Overall, this research demonstrated changes to CBT, EOCH behavior, and blood physiology parameters, particularly for T/RH and FC. During heat stress white-feathered EOCH had a rise in CBT, increased observation of heat related behaviors, and indicators of dehydration from increased respiration. Cold stress in EOCH demonstrated a decline in CBT, increased observation of cold related behaviors, decreased glucose from increased energy consumption, development of

metabolic alkalosis, higher stress levels, and higher mortality. Lastly, white-feathered EOCH demonstrated the importance of good FC to cope with exposure to the cold. More research is needed concerning crating density and trailer microclimates to ensure stress during transport is minimized for EOCH.

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DISCLOSURES

The authors have no conflicts of interest to dislcose.

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