The effects of simulated transport conditions on the muscle tissue characteristics of white-strain layer pullets

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ABSTRACT The objective of this study was to evaluate the effects of temperature (\mathbf{T}) /relative humidity (**RH**) combinations and exposure duration (**D**) on the muscle tissue characteristics of layer pullets during simulated transport. While layer pullets are not processed for meat, muscle physiology can be used as an indicator to assess welfare. Pullets (n = 240) were randomly assigned to 1 of 5 T/RH combinations (-15°C uncontrolled RH [-15], 21°C 30%RH [21/30], 21°C 80% RH [21/80], 30°C 30%RH [30/30], and 30°C 80%RH [30/ (30) and 2 D (4 or 8 h) in a 5 x 2 factorial arrangement (3 replications). Birds were weighed before exposure, crated (density 45.5 kg/m²) and exposed to the conditions above. After exposure, birds were weighed (live shrink calculated) and slaughtered using a small-scale facility. Postslaughter, carcasses were eviscerated, and an initial pH was obtained from the right breast and thigh. Final breast and thigh pH and color values (lightness $[L^*]$, yellowness $[b^*]$, and redness $[a^*]$) were obtained 30 h postslaughter. Left breast muscles were frozen and analyzed for thaw and cook loss 4 wk postslaughter. Data were analyzed as a randomized complete block design via ANOVA (Proc Mixed; SAS 9.4), with farm of origin as block. Differences were considered significant when P ≤ 0.05 . Live shrink (kg) was higher for pullets exposed to 30/30 and 30/80 compared with those exposed to 21/80(P = 0.04) and for pullets exposed for 8 h compared with 4 h (P < 0.01). Breast muscle thaw loss (%) was higher in pullets exposed for 4 h compared with 8 h (P = 0.01). Breast and thigh muscle a^{*} were higher for pullets exposed to 30/30 compared with 21/30 (P = 0.02). Thigh muscle b^{*} was lower for pullets exposed to -15 compared with 21/80 (P = 0.05). Breast b* was higher for pullets exposed for 8 h compared with 4 h (P = 0.04). The results from this study demonstrates that increasing exposure D had minor effects on pullet muscle characteristics. In addition, layer pullets coped well with thermal stressors associated with simulated transport.

Key words: live shrink, thermal stress, muscle pH, muscle color, muscle physiology

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INTRODUCTION

Though transportation is an essential component of the egg production industry, it can present many stressors for pullets that will be coming into lay. One of the most significant stressors for poultry during transportation may be the microclimate environment, which is a combination of temperature (**T**) and relative humidity (**RH**), within the trailer, as it can vary immensely within a load (Knezacek et al., 2010). High **T** and high RH microclimates can be concerning, as it can be challenging for poultry to thermoregulate under these conditions because of both nonevaporative and

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evaporative heat loss mechanisms becoming less effective (Freeman, 1984; Lin et al., 2005). However, cold conditions can also present challenges in relation to thermoregulation (Knezacek et al., 2010). Weight loss during transport, commonly referred to as live shrink, is a common occurrence and is typically related to moisture loss as a result of respiration and fecal output. However, live shrink may be exacerbated by thermal stress, as thermoregulation can be energy demanding. Bianchi et al. (2005) noted that broilers transported for over 5 h compared with those transported for less than 3.5 h had a higher live shrink; however, transportation conditions were not mentioned in this study. Nonetheless, this demonstrates that as a result of a longer duration (**D**) without feed and water and potentially the need to thermoregulate against poor conditions for a longer D, an increase in live shrink can be expected (Bianchi et al., 2005). Evaluating bird welfare during transport can be difficult; therefore, it is important to

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use several parameters such as physiological and behavioral changes to evaluate how the bird copes with a stressor. The primary areas of concern when evaluating the physiological response to a stressor is the blood chemistry; however, changes in blood parameters can also impact the muscle tissue characteristics postmortem. Although layer pullets are not used for subsequent meat consumption, live shrink and muscle tissue characteristics, including muscle pH, color, and water-holding capacity, have been identified as indicators of either heat or cold stress during transportation in broilers (Dadgar et al., 2010, 2012a, 2012b). Previous research has shown that heat stress preslaughter may result in characteristics associated with pale, soft, and exudative (**PSE**) muscle tissue postslaughter, which is characterized by low pH, lighter muscle tissue color (higher L^*), and a low water-binding capacity (Barbut et al., 2005). Whereas exposure to cold stress preslaughter may result in changes associated with dark, firm, and dry (**DFD**) muscle tissue postslaughter, which is characterized by high pH, a darker muscle tissue color (lower L^{*}), and high water-binding capacity (Barbut et al., 2005; Dadgar et al., 2012a).

Previous research has observed an impact of exposure to either heat stress or cold stress preslaughter on the muscle pH, water-holding capacity, and color. However, an increased journey D may also present a concern for the well-being of birds during transportation as layer pullets are often transported over longer distances compared with broiler chickens. Exposure to poor environmental conditions for a longer period of time may exacerbate the stress response and the resultant changes in muscle tissue characteristics postslaughter (Petracci et al., 2001; Zhang et al., 2009). Little research has been conducted studying the effects of increased exposure D on muscle tissue characteristics. Therefore, it is not well known as to how the muscle pH, waterholding capacity, and color would change as a result of an increased transportation D.

Egg production pullets are transported to a laying facility at approximately 17 wk of age (NFACC, 2017). Despite many studies being conducted on the transportation of broilers, very little is known regarding the transportation of pullets and pullet stress responses during transportation. Additionally, little is known regarding the effect of increased transportation D on the stress responses and the welfare of pullets. The objective of this study was to evaluate the effects of exposure to hot and neutral T, paired with a high or low RH, as well as to cold T during simulated transportation for a short or long D on pullet live shrink and muscle tissue characteristics, which are used as a measure of stress.

MATERIALS AND METHODS

The experimental protocol for this research was approved by the University of Saskatchewan Animal Care Committee and was performed under the guidelines of the Canadian Council on Animal Care (1993, 2009). This study was part of a larger study which aimed to examine the effects of simulated transportation conditions on the health and well-being of whitefeathered egg production pullets. While this study's focus is primarily on changes that occur in muscle tissue physiology, the data pertaining to core body and extremity temperature, blood physiology, and behavior has been previously reported (Lalonde et al., 2020).

Experimental Design

The impacts of exposing white-feathered egg production pullets to simulated transport conditions were evaluated using 5 T and RH combinations $(-15^{\circ}\text{C} \text{ uncontrolled RH } [-15], 21^{\circ}\text{C} 30\%$ RH $[21/30], 21^{\circ}\text{C} 80\%$ RH $[21/80], 30^{\circ}\text{C} 30\%$ RH [30/30], and $30^{\circ}\text{C} 80\%$ RH [30/80]) and 2 exposure durations (D; 4 or 8 h). The study was arranged as a 5 x 2 factorial arrangement and completed in 3 replicates.

Birds and Housing

A total of 240 commercially raised white-feathered egg production pullets (18–19 wk of age) that were obtained from 3 separate flocks within a 250-km radius of Saskatoon, Saskatchewan. One flock was derived from a floor-rearing system, and 2 from cage-rearing systems. Only birds were selected with full feather cover so as not to have an impact on thermoregulation during the study. Once at the study site, birds were housed in a floor pen (3.9 m \times 3.0 m) with straw litter. Feed and water were provided ab libitum via 2 bell drinkers (38 cm diameter) and 3 aluminum tube feeders (36 cm diameter). The lighting program used was obtained from the farm of origin for each flock. The birds were given a 3 to 5 d acclimatization period before being exposed to the simulated transportation conditions.

Before Simulated Transport

Before simulated transport, 8 pullets were randomly assigned to 1 of the T/RH and D combinations. Pullets were not feed restricted before simulated transportation. An initial individual body weight was recorded for each pullet, and each bird was wing banded for identification purposes. Once all birds were weighed and banded, they were then crated, and remained in lairage for a 15-min period. After the lairage period, both crates containing the pullets were transported to the environmental simulation chambers.

Simulated Transport

The environmental chambers were set to the T/RH conditions listed above and were monitored in real time via a multimeter and thermocouples (Omega HH509, Omega Engineering Inc., Laval, Quebec, Canada) and RH sensors (HM1500LF; Measurement Specialties, Inc., Toulouse, France). The chamber T and RH conditions were also recorded every minute via data logger (iButton Hygrochron DS1923-#F5, Maxim

Integrated; San Jose, CA). Pullets were then placed in transport crates inside the environmental chamber at a stocking density of 45.5 kg/m² (8 pullets/crate). Each crate was equipped with a miniature data logger that recorded the T and RH inside the crate once every minute (iButton Hygrochron DS1923-#F5, Maxim Integrated; San Jose, CA). The pullets then remained in the chamber for the assigned duration (4 or 8 h) before being removed. The T/RH data for the chamber and crate conditions have been reported in Lalonde et al. (2020).

Postsimulated Transport

At the end of the respective exposure D, the respective crate was removed from the chamber. Final individual body weights were taken for each pullet, and live shrink was calculated (live shrink (%) = ((final body weightinitial body weight)/initial body weight) \times 100). The pullets were then shackled, stunned, and exsanguinated using an electric knife (VS200, Midwest Processing Systems; Minneapolis, MN). Additionally, 5 pullets per T/RH and D combination per replicate were then scalded in a hot water bath (65°C–68°C), plucked (Featherman Pro Plucker; Granbury, TX), and manually eviscerated.

Following evisceration, an initial pH reading was taken using a portable pH meter (H1 9025, Hanna Instruments; Woonsocket, RI) for both the right pectoralis and right iliotibial muscles by making a small vertical incision in the muscle. Afterward, the whole carcasses were chilled in an ice water bath for 1 h and then transferred to ice for 5 h. Six h postslaughter, the right pectoralis and iliotibial muscles were removed from the carcass, weighed, placed on a drip-tray with a dri-lock pad, covered with plastic wrap, and placed in a cooler $(4^{\circ}C)$ for 24 h. The left pectoralis muscle was also removed and sealed in a Ziploc bag and stored at -30° C until further analyzed. After 24 h (30 h postslaughter) of refrigeration, a second pH reading was taken (final pH) by making a second small incision adjacent to the previous one. Additionally, the right breast and thigh were reweighed (drip loss (%) = ((final weight-initial))weight)/initial weight) $\times 100$), and the muscles were set aside to allow oxygen exposure to the incision for 30 min. Two color readings, at 90° angles from each other, were then taken using a Minolta Chroma Meter (CR-400; Konica Minolta Sensing Americas Inc.; Ramsey, NJ). The data were downloaded, and the output was converted via SpectraMagic NX Software (Konica Minolta Sensing Americas Inc.) using illuminant source C and 2° to obtain values for lightness (L^*) , redness (a^*) , and yellowness (b^*) , and an average of the 2 readings was taken. Muscle color readings were only obtained for 2 replications (5 birds per replicate).

Thaw loss and cook loss were analyzed 4 wk postslaughter. Breast muscle samples were removed from the freezer and placed in a cooler (4°C) to thaw for 24 h. After refrigeration, samples were then removed from the bag, blotted with paper towel, weighed, and thaw loss was calculated (thaw loss (%) = ((final weight-initial weight)/initial weight) x 100). The breast samples were then placed in a new labeled Ziploc bag and placed in a hot water bath at 80°C until the internal temperature reached 75°C (measured via thermocouple and multimeter [Omega HH509, Omega Engineering Inc.; Laval, Canada]) and then left for an additional 5 min. The samples were then removed and allowed to cool to 50°C, blotted with a paper towel, re-weighed, and cook loss was calculated (cook loss (%) = ((final weight-initial weight)/initial weight) × 100).

Statistical Analyses

The experiment was analyzed as a randomized complete block design with the farm of origin as block (SAS 9.4; Cary, NC). The experimental unit for each parameter was the crate. Data were checked for normality using PROC UNIVARIATE; if the data did not fit a normal distribution curve, then log transformations were applied (log+1). Treatment means and standard error of the means were calculated via PROC MEANS. An ANOVA (PROC MIXED) was conducted with the 5 T/RH combinations and 2 D in a factorial arrangement. Means separation was done via Tukey's test. Significant differences were considered at $P \leq 0.05$, and trends were noted when $0.05 < P \leq 0.10$.

RESULTS

Chamber Environment

The environmental conditions achieved for each treatment are outlined in Lalonde et al. (2020). The mean chamber conditions were close to target for each treatment with a T of -14.7, 22.1, 21.9, 31.3, and 31.5° C and an RH of 69.7, 38.4, 81.4, 29.4, and 78.0% for treatments -15, 21/30, 21/80, 30/30, and 30/80, respectively. The conditions achieved within the crate varied slightly from chamber conditions.

Live Shrink

There were no interactions for T/RH and D on pullet live shrink. Live shrink (kg) was impacted by T/RH combinations with pullets exposed to the 30/30 and 30/80 conditions demonstrating an increased live shrink, compared with pullets exposed to the 21/80 treatment, with pullets exposed to -15 and 21/30 being intermediate (P = 0.04; Table 1). However, when live shrink was expressed as percentage of body weight, there was only a tendency for T/RH combination to impact pullet live shrink (P = 0.07). Transport D also impacted live shrink, with pullets exposed for 8 h demonstrating higher live shrink values (kg; P < 0.01 and %; P = 0.01) compared with pullets exposed for a 4 h D (Table 1).

Drip, Thaw, and Cook Loss

There were no interaction effects for T/RH and D on the drip, thaw, or cook loss values. The right breast

muscle was evaluated for drip loss (%), whereas there was no effect of T/RH on drip loss (%) pullets exposed to 21/30 and 21/80 had a heavier initial breast weights, compared with those exposed to 30/30 (Table 2). The left breast muscle was evaluated for thaw and cook loss, and results are shown in Table 2. There was a tendency for the initial breast muscle weight to be higher in pullets exposed to 21/30 (P = 0.09) and a tendency for higher that loss (%) values associated with pullets exposed to 21/80 (P = 0.08). There were no effects of T/RH on breast muscle cook loss (%). There was no impact of transport D on drip loss (%). Transport D resulted in higher that $\log (\%)$ in pullets exposed for 4 h compared with pullets exposed for the 8 h D (P = 0.01), as well as a tendency for increased cook loss (%) in pullets exposed for 4 h compared with 8 h (P = 0.06).

Breast and Thigh Muscle pH

There were no interaction effects between T/RH and D on pullet breast or thigh muscle pH (Tables 2 and 3). Additionally, there was no impact of T/RH or D individually on breast muscle pH (Table 2) nor was there an impact of T/RH or D individually on thigh muscle pH (Table 3).

Muscle Color

There were no interactions for T/RH and D on pullet breast and thigh muscle color (Tables 2 and 3). The T/RH treatments did not impact breast muscle lightness (L^*) or yellowness (b^*) ; however, redness (a^*) was higher in pullets exposed to 30/30 compared with 21/30, with -15, 21/80 and 30/80 being intermediate (P = 0.02; Table 2). Transport D had no effect on breast muscle lightness (L^*) or redness (a^*) ; however, yellowness (b^*) was higher in pullets transported for 4 h compared with those transported for 8 h (P = 0.04; Table 2). Thigh muscle lightness (L^*) was not affected by the T/RH treatments. Thigh muscle redness (a^{*}) followed the same pattern as with the breast, with a^{*} being higher in pullets exposed to 30/30 compared with 21/30(P = 0.02; Table 3). Thigh muscle vellowness (b^{*}), was lower in pullets exposed to -15 compared with 21/80, with all other T/RH treatments being intermediate (P = 0.05; Table 3). Transport D had no impact on thigh muscle color.

DISCUSSION

Implementing thermoregulatory and physiological mechanisms during exposure to either hot or cold T can be energy costly (Holm and Fletcher, 1997). Live shrink can be a useful measure to indicate the amount of energy mobilized and used during transportation (Holm and Fletcher, 1997; NFACC, 2001). Large losses in live weight during transportation can, therefore, indicate that birds are expending large amounts of energy, which may be a welfare concern. The data from the current study may indicate that compared with the pullets in the 21/80 treatment, pullets in both of the hot T (30°C) treatments mobilized and expended more energy to respond to the heat stress. Furthermore, these pullets likely lost more moisture to the environment through other thermoregulatory behaviors, such as panting which has been reported in Lalonde et al. (2020). These results agree with previous research by Holm and Fletcher (1997) and Petracci et al. (2001) who found a 6.29% and a 5.67% decrease in the live weight, respectively, of broilers exposed to T above 29°C for 12 h. In both of the previously mentioned studies, the broilers were feed restricted for 12 h, as opposed to the pullets in the current study who were not feed restricted. In addition to feed restriction, a higher loss in live weight in the mentioned studies, as opposed to the current study, could also be a result of species differences between broilers and layer pullets. However, both the current study and previous research indicate that thermoregulation to control the effects of heat stress can be energy-demanding as shown by higher losses in live weight, which may be a concern for the well-being of birds during transport. The increase in live shrink with increasing transport D seen in this study is in accordance with previous research and is to be expected as birds are away from feed and water and thermoregulating for a longer period of time. Bianchi et al. (2005) found a higher live shrink (2.09%) in broilers transported for over 5 h, compared with those transported for less than 5 h; however, transportation conditions and feed restriction before transport were not mentioned.

Changes in muscle pH associated with either PSE or DFD muscle tissue can be related to exposure to hot or cold T before slaughter, respectively (Zhang et al., 2009; Dadgar et al., 2010, 2012a). Muscle tissue that can be classified as PSE typically has an ultimate pH (24 h postslaughter) of approximately 5.7 or lower,

Table 1. Live shrink of white-feathered layer pullets during simulated transport with 5 temperature (T)/relative humidity (RH) combinations (-15° C uncontrolled RH, 21° C 30%RH, 21° C 80%RH, 30° C 30%RH, and 30° C 80%RH) for a 4 or 8 h duration (D).

Parameter	T/RH combinations					D					
	-15	21/30	$rac{21/}{80}$	$rac{30}{30}$	$rac{30/}{80}$	<i>P</i> -value	4 h	8 h	<i>P</i> -value	Interaction P -value	SEM^1
Initial BW (kg) Live Shrink (kg) Live Shrink (%)	${\begin{array}{c} 1.29 \\ 0.03^{\rm a,b} \\ 2.44 \end{array}}$	${\begin{array}{c} 1.29 \\ 0.02^{\rm a,b} \\ 2.02 \end{array}}$	$1.29 \\ 0.02^{b} \\ 1.44$	$1.26 \\ 0.04^{\rm a} \\ 3.28$	$1.29 \\ 0.05^{a} \\ 3.47$	$0.34 \\ 0.04 \\ 0.07$	$1.28 \\ 0.03^{ m b} \\ 1.87^{ m b}$	${\begin{array}{c} 1.29 \\ 0.04^{\rm a} \\ 3.20^{\rm a} \end{array}}$	$0.19 < 0.01 \\ 0.01$	$0.18 \\ 0.90 \\ 0.91$	$0.011 \\ 0.004 \\ 0.295$

 $^{\rm abc} {\rm Differences}$ considered significant when P < 0.05.

¹Pooled standard error of mean.

Table 2. Breast muscle characteristics of white-feathered layer pullets during simulated transport with 5 temperature (T)/relative humidity (RH) combinations (-15° C uncontrolled RH, 21° C 30%RH, 21° C 80%RH, 30° C 30%RH, and 30° C 80%RH) for a 4 or 8 h duration (D).

	T/RH combinations					<i>P</i> -	D		<i>P</i> -	Interaction	
Parameter	-15	21/30	21/80	30/30	30/80	value	4 h	8 h	value	<i>P</i> -value	SEM^1
Right breast											
Initial weight (g)	$76.43^{\mathrm{a,b}}$	80.47^{a}	81.83^{a}	71.85^{b}	$78.18^{\mathrm{a,b}}$	0.01	78.34	77.16	0.47	0.53	1.643
Drip loss (%)	0.73	0.77	1.00	0.98	0.64	0.47	0.84	0.81	0.93	0.42	0.154
Initial pH	6.64	6.51	6.58	6.59	6.63	0.61	6.59	6.59	0.97	0.32	0.029
Final pH	5.70	5.83	5.68	5.70	5.74	0.34	5.71	5.75	0.45	0.96	0.028
Lightness $(L^*)^2$	50.06	48.95	49.31	49.22	49.73	0.79	49.96	48.95	0.13	0.65	0.287
Redness $(a^*)^2$	$5.31^{\mathrm{a,b}}$	4.94^{b}	$5.44^{a,b}$	6.09^{a}	$5.97^{ m a,b}$	0.02	5.47	5.64	0.41	0.28	0.134
Yellowness $(b^*)^2$	1.25	0.79	0.92	0.96	1.05	0.65	1.22^{a}	0.76^{b}	0.04	0.33	0.150
Left breast											
Initial weight (g)	74.76	80.60	73.77	73.37	75.53	0.09	76.37	74.84	0.38	0.80	3.497
Thaw loss (%)	4.07	3.42	4.23	3.14	3.35	0.08	4.04^{a}	3.25^{b}	0.01	0.69	0.196
$\operatorname{Cook} \operatorname{loss}(\%)$	24.63	23.21	24.61	23.47	23.25	0.94	24.56	23.11	0.06	0.25	0.775

^{abc}Differences considered significant when P < 0.05.

¹Pooled standard error of mean.

²Color readings obtained for 2 replications only.

whereas muscle tissue classified as DFD has an ultimate pH of approximately 6.1 or higher; however, this can vary depending on the muscle type (Dadgar et al., 2010, 2011). In the current study, there was no effect of either the T/RH or D on breast or thigh muscle pH. However, the pH of the breast muscle ranged from 5.68 to 5.83, which may be classified as PSE, whereas the pH of the thigh ranged from 5.95 to 6.28, which is suggestive of DFD muscle tissue. Interesting to note in the current study, regardless of the T/RH exposure conditions or exposure D, the final pH for both the breast and thigh muscles decreased compared with the initial pH reading. The results of the present study differ from that found by Dadgar et al. (2012a) who observed a higher breast muscle pH of broilers (5–6 wk of age) exposed to cold T (below -9° C) for 3 h, as opposed to broilers in the control group (20°C exposure for 3 h). However, the mentioned study used core samples to determine breast muscle pH, which may be more accurate compared to using a pH probe. With regards to heat exposure, Holm and Fletcher (1997) observed a lower pH in the breast muscle of broilers exposed to 29°C for 12 h preslaughter compared with those exposed to temperature below 18°C for the same D. Dadgar et al. (2010) subjected broilers to 25°C with 46% RH for a 3 h D

and observed a significant decrease in the ultimate pH post-slaughter. An initial pH was not indicated in the previously mentioned studies, though the ultimate pH was found to be consistent with that of the breast muscle in the current study. In the current study, there was no impact of treatment exposure on muscle pH, this may be a result of birds having to use energy stores in order to cope to stressors during transport, regardless of T/RH exposure; therefore, only small amounts of glycogen would be left in the muscle for post-mortem glycolysis (Lyon and Buhr, 1999). However, no significant changes in muscle pH resulting from the T/RH conditions could also indicate that pullets were able to implement mechanisms that were effective in maintaining a homeostatic body T, resulting in no changes in muscle tissue characteristics.

According to previous research, PSE and DFD muscle tissue are primarily determined based on an L* value of \geq 53 and \leq 46 (Dadgar et al., 2012a, 2012b). Previous studies exposing broilers to cold T (below -13° C) have found L* values consistent with DFD muscle tissue (Dadgar et al., 2011, 2012a); however, studies exposing broilers to hot T (above 29°C) resulted in no effects on breast muscle L* values (Holm and Fletcher, 1997), though exposure D varied for all of the previously

Table 3. Thigh muscle characteristics of white-feathered layer pullets during simulated transport with 5 temperature (T)/relative humidity (RH) combinations (-15°C uncontrolled RH, 21°C 30%RH, 21°C 80%RH, 30°C 30%RH, and 30°C 80%RH) for a 4 or 8 h duration (D).

Parameter		P_	Duration		<i>P</i> _	Interaction					
	-15	21/30	21/80	30/30	30/80	value	4 h	8 h	value	P-value	SEM^1
Initial weight (g)	61.03	60.91	61.43	58.48	59.23	0.33	60.47	59.96	0.62	0.50	0.545
Drip loss (%)	0.75	0.49	0.78	0.85	0.67	0.12	0.74	0.67	0.44	0.17	0.120
Initial pH	6.39	6.38	6.21	6.33	6.32	0.28	6.33	6.32	0.97	0.39	0.040
Final pH	6.16	6.28	6.05	5.95	6.13	0.15	6.09	6.13	0.63	0.87	0.053
Lightness $(L^*)^2$	49.84	51.73	50.72	50.07	50.47	0.16	50.59	50.54	0.92	0.82	0.257
Redness $(a^*)^{2'}$	$4.95^{\mathrm{a,b}}$	4.16^{b}	$4.43^{\mathrm{a,b}}$	5.43^{a}	$4.85^{\mathrm{a,b}}$	0.02	4.93	4.60	0.12	0.40	0.173
Yellowness $(b^*)^2$	-3.85^{b}	$-2.56^{\mathrm{a,b}}$	$-2.07^{\rm a}$	$-2.85^{\mathrm{a,b}}$	$-2.96^{\mathrm{a,b}}$	0.05	-2.68	-3.03	0.27	0.61	0.207

^{abc}Differences considered significant when P < 0.05.

¹Pooled standard error of mean.

²Color replications obtained for 2 replications only.

mentioned studies. In the current study, the T/RH combinations did not result in any impact on the L* values of either the breast or thigh muscle. Additionally, inconsistent T/RH effects were found on muscle tissue color, with the breast and thigh muscle having a higher a^* in pullets exposed to the 30/30 treatment and a lower a^{*} in pullets exposed to the 21/30 treatment, and the thigh muscle having a higher b^* in pullets exposed to the 21/30treatment and a lower b^* in pullets exposed to the -15 treatment. Changes in muscle tissue color are closely correlated with the muscle pH, but the results found for muscle tissue color in the current study do not follow the same pattern as observed for muscle pH. A lower pH would result in denaturation of proteins in the muscle resulting in a lighter muscle color (higher L^*) (Pearson, 1994). Finally, pullets exposed for the 8 h D had a lower breast muscle b^{*}, compared with those exposed for the 4 h D; however, little is known regarding the impact of transportation D on pullet muscle color. The results for muscle tissue color in the current study may be confounded as color values were only obtained for 2 replications.

Drip loss, thaw loss, and cook loss were not affected by the T/RH exposure conditions. These factors, which are determinates of the muscle water-binding capacity, are directly correlated with the muscle pH (Pearson and Young, 1989). With no effects observed in either the breast or thigh muscle pH, it would be expected that no significant differences in the drip, thaw, or cook loss of either the breast or thigh muscles would be noted. The current study found no differences in drip loss (%)relation to T/RH treatment. Dadgar et al. (2010) also found no effects on the muscle drip loss when broilers were exposed to either hot $(25^{\circ}C, 46\% \text{ RH})$ or cold $(-7^{\circ}C, 76\% \text{ RH})$ conditions. Although significant differences in cook loss of the breast were observed, the authors noted that these differences did not follow any specific trend based on the exposure conditions (Dadgar et al., 2010). Holm and Fletcher (1997) found a lower cook loss in broilers exposed to 29°C for 12 h, compared with those exposed to either $18^{\circ}C$ or $7^{\circ}C$ for the same D. Dadgar et al. (2011) found a significantly lower thaw and cook loss in broilers exposed to temperatures below 0°C but noted a higher drip loss in birds exposed to T below -14° C. Differences in results observed in these studies, as opposed to the current study, may be because of a number of factors, including differences between broilers and layers, differences in exposure conditions and D, as well as no initial weights being indicated in the previously mentioned studies.

An effect of D was observed on thaw loss, with breast muscles from pullets exposed for the 4 h D having a greater thaw loss (%) compared with those exposed for the 8 h D. Thaw loss can be correlated with muscle pH, but no impacts of exposure D were found for muscle pH. Additionally, no effects were noted for either drip loss or cook loss. Little is known regarding the effects of transportation D on muscle thaw loss, independent of transportation conditions. Therefore, it is difficult to draw conclusions on the effect of transportation D on the thaw loss of the breast muscle tissue.

Overall, the results of this study support the hypothesis that live shrink will increase as a result of heat exposure and as a result of a longer transportation D. However, the results in this study suggest that pullets cope well with transportation stressors, preventing physiological changes to the muscle tissues. Despite previous research showing the effects of environmental conditions on the muscle tissue characteristics of broilers and turkeys, the results found in the current study are minimal and inconsistent. Pullets in the current study needed energy to thermoregulate against exposure to hot conditions; however, thermoregulatory mechanisms employed were adequate to allow the pullets to cope with the environmental conditions. Therefore, exposure to the various T/RH conditions had a minimal impact on the well-being of pullets in this study. A D of up to 8 h also had little impact on the welfare of pullets in the current study.

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DISCLOSURES

The authors confirm 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, affi liations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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