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Abstract

The impact of crating density and prevailing weather conditions during transit on preslaughter losses, physiological characteristics, and meat quality in broilers was investigated. A total of 900 35-day-old Ross 308 male broilers with an average body weight of 1,860 ± 17.458 g (mean ± SEM) in summer and 1,864 ± 17.454 g in winter were allotted to one of six groups arranged in a 3 × 2 factorial arrangement according to the three different crating densities (low: 0.039 m^2/bird; medium: 0.031 m^2/bird; high: 0.026 m^2/bird) and two different weather conditions (low: -1 ℃ and high: 30 ℃). Birds stocked at medium density recorded lower ($p < 0.05$) body weight loss compared to the low density group; and demonstrated higher ($p < 0.05$) lactate levels along with lower ($p < 0.05$) respiration rates when compared to the high crating density group. Extreme conditions of low crating density under low air temperature and high crating density under high air temperature led to higher ($p < 0.001$) body weight loss and glucose concentration compared to low crating density under high air temperature. In conclusion, both excessively high and low crating densities are not conducive to reducing preslaughter losses and blood stress indicators. Broiler transportation under high crating density in low air temperatures and low crating density in high air temperatures is recommended.

Keywords: broiler, crating density, stress, transportation, weather, welfare

Introduction

Transportation of poultry from the farm to the slaughterhouse is an inevitable process in poultry meat production [1]. However, the transportation process exposes the birds to various stressors including catching, crating density, vehicle vibration influenced by the driver's driving skill, road quality, and the transportation environment encompassing temperatures, and wind, among others [2,3]. These factors have been reported to lead to physical injury in broilers; trigger shifts in biochemical and physiological conditions; disrupt normal homeostasis, and ultimately diminish the broiler meat quality and yield [4-6]. The levels of several specific plasma metabolites, including cortisol, glucose, and lactate, have been proposed as indicators reflecting stress levels [7-9]. Elevated levels of plasma cortisol, glucose, and lactate can serve as indicators of physical strain or stress resulting in the previously mentioned undesirable effects on meat quality and yield [10].

The poultry industry is therefore increasingly focused on finding strategies to mitigate transport-related stress through different interventions including reduced transportation duration, lower crating density, and dietary feed additive supplementation before transportation to reduce stress [11-14]. Certain welfare standards have been specified in various jurisdictions to indicate the timeframes for transporting broilers. The rules stipulated in [15] recommend that the duration between the loading of the final batch of broilers and broiler arrival at the abattoir should not exceed 8 hours. Additionally, the duration between the departure of the birds from the farm to their arrival at the processing plant should be no longer than 4 hours. Moreover, there is a guideline concerning the maximum weight of birds per square meter of tray floor area, with a limit set at 57 kilograms per square meter. In Korea, the appropriate crating density is 160-210 cm^2/kg for 1-2 kgs of broiler live weight, and there is an allowance for increasing the crating density in hot weather and reducing it in cold weather within 20% of the area required for transportation [16]. As an important aspect of transportation, the crating density influences the bird's ability to adapt to environmental fluctuations during transportation. Crating density is dependent upon factors like weather conditions, the cumulative weight of live birds, and the age of the birds that are selected for transport [17,18]. Opting for higher crating densities can lower the average transport costs per bird. Reduced densities provide more space for resting and greater chances for broilers to regulate their body temperatures through behavioral adjustments. Conversely, excessive space per bird might elevate the risk of physical injuries due to a higher risk of abrasion. However, these densities must be carefully considered in terms of the stipulated animal welfare guidelines.

Previous studies have reported that broilers transported at higher crating densities exhibited severe body weight loss and a decline in both physical appearance and functional properties during the summer season (27.2-33.6 ℃, 52.7-62.9%). In contrast, low crating density adversely impacted meat quality by leading to increased weight loss and stress in winter (3.6-9.5 ℃, 63.3-78.8%) [18-20]. Another study by [21] examined the impact of various crating densities and seasonal variations [low air temperature (-9 ℃, 60%), high air temperature (27 ℃, 80%)] on preslaughter losses, meat quality, and physiological characteristics in broilers. However, the crating density employed was notably lower than that commonly used. Despite these insights, there are few studies on the interaction between crating density and weather conditions for preslaughter losses, meat quality, and physiological attributes in broiler chickens. Consequently, the objective of the present experiment was to investigate the effect of different crating densities and weather conditions on the preslaughter losses, physiological traits, and meat quality in broiler chickens.

Materials and Methods

The Animal Ethics Committee of Chungnam National University, Daejeon, Republic of Korea, approved the protocols used in this experiment (approval number: 202206A-CNU-083)

Birds, experimental design, and treatments

Before transportation, all birds were housed in Chungnam National University's experimental farm which had 104 battery cages (76 × 60 × 40 cm^3) that housed six birds until transportation and were managed according to the Ross 308 broiler management guideline [22]. A total of 900 Ross 308 male broilers, aged 35 days with an average body weight of 1,860 ± 17.458 g (mean ± SEM) in summer and 1,864 ± 17.454 g in winter, were picked at random after 4 h of feed withdrawal before catching. Following the Japanese method, the birds were obtained from the cages and transported securely holding their wings against the handler's body using both hands [23]. The birds were transported in the truck (capacity 18 crates) in iron crates having dimensions of 1.00 m (length) × 0.78 m (width) × 0.26 m (height). To reduce the effect of microclimate in the crates, the location of the crates was randomly arranged and each treatment was replicated 6 times and the samples were collected randomly from all locations of the crates. The transportation's distance was 20 km for 40 min at an average speed of 30-50 km/h during the early morning from 8:00 a.m. The experiment was performed using a completely randomized design in a factorial arrangement with the experimental factors being the three different crating densities [20 birds (0.039 m^2/bird), 25 birds (0.031 m^2/bird), 30 birds (0.026 m^2/bird) per crate]; and the two different weather conditions [low air temperature (-1 ℃, 47%), high air temperature (30 ℃, 40%)]. Transportation was carried out in summer and winter by using the same truck following the same transportation route was maintained in both periods.

Transportation losses

Body weight loss (g) in transit was measured as the difference between all broilers' weight before transportation and the final body weight (g) from all crates upon arrival at the destination after transportation [21].

Carcass traits and sample collection

Carcass traits were measured as soon as transportation was completed. One bird was selected based on closeness to the mean body weight of the birds in the respective crate, and the resulting weight was recorded as the live body weight. Blood samples were collected from the brachial vein into a vacutainer coated with lithium heparin (BD Vacutainer, BD, Franklin Lakes, NJ, USA) before euthanizing the birds. The birds were then euthanized by cervical dislocation for the evaluation of some carcass characteristics. The dressing percentage with giblets (heart, gizzard, and liver) was determined as a function of the live weight of the birds. The breast meat was then separated and weighed to measure its relative to the total carcass weight. The breast meat of broilers was then collected for meat quality analyses [24].

Physiological responses

Collected blood samples were centrifuged (LABOGENE 1248R, Gyrozen, Daejeon, Korea) at 3,000 × g for 10 min at 4 ℃ and the plasma was separated and stored at -80 ℃ (UniFreez U 400, DAIHAN Scientific, Wonju, Korea) until analysis. Cortisol concentrations were determined from the plasma with a cortisol ELISA kit (CUSABIO, Wuhan Huamei lotech Co., Ltd., Wuhan, China) used in accordance with the manufacturer's instructions. Lactate concentration was determined by lactate assay kit (Sigma Aldrich, Co., Burlington, USA) using the manufacturer's instructions. Briefly, glucose was determined from the collected plasma using a glucose assay kit (Asan Pharmaceutical Co. Ltd., Seoul, Republic of Korea), following the manufacturer's instructions.

After finishing transportation, the respiratory frequency was measured as the number of breaths per minute using three randomly selected broilers per crate observed by the camera (GoPro Hero 8, San Mateo, CA) for 1 minute [25].

Physicochemical traits

The pH values of the breast meat were monitored immediately after sample collection. An aliquot (9 mL) of distilled water was added to 1 g of muscle, followed by homogenization (T25 basis, IKA-Werke GmbH & Co. KG, Germany) for 30 seconds. The homogenate was centrifuged at 2,090 × g (ScanSpeed 1580R, Labogene ApS, Lillerød, Denmark) for 10 min and the supernatant was filtered through filter paper (No. 4, Whatman, Maidstone, UK). The pH of the filtrate was measured using a pH meter (SevenEasy, Mettler-Toledo Intl. Inc., Schwerzenbach, Switzerland).

The CIE (Commission Internationale de l'Eclairage) lightness (L*), redness (a*), and yellowness (b*) of broiler breast meat were determined using a spectrophotometer (CM-3500d, Minolta Inc., Tokyo, Japan). Measurements were taken perpendicularly to the surface of the broiler breast meat with a 30 mm diameter of illumination area at two different locations per sample. The results were analyzed in the SpectraMagic software (SpectramagicTM NX, Konica Minolta Inc., Tokyo, Japan).

For the water holding capacity (WHC) measurements, the 2 g of raw broiler breast meat sample weighed exactly was placed on cotton wool, and added to a centrifuge tube. The weight of the meat after centrifugation at 2,090 × g (ScanSpeed 1580R, Labogene ApS, Lillerød, Denmark) for 10 min was measured and compared to the initial meat weight. The moisture content of meat was determined by drying 2 g of samples placed in aluminum dishes for 3 h at

110 ℃. The remaining moisture (%) present in the meat after centrifugation was expressed as the WHC [26].

To measure the cooking loss, the breast meat of the broiler was weighed and vacuum packaged and cooked for 20 min in a water bath at 80 ℃ until the internal temperature reached 70 ℃. The cooked breast meat of broilers was cooled at room temperature (20 ℃) for 30 min. After removal of the vacuum bag, the surface moisture of the breast meat of the broiler was removed with paper towels, and the cooked breast meat of the broiler was weighed. The cooking loss was calculated as the difference between the weight of raw breast meat and cooked breast meat.

Statistical analysis

The data obtained from the experiment were analyzed using the general linear model (GLM) procedure for two-way ANOVA to evaluate the main effects (the crating density and weather) in SPSS (Version 26; IBM SPSS 2019). In terms of transportation loss and respiratory frequency measurements, the experimental unit was defined as the crate. For carcass traits, meat quality, and blood metabolites, selected individual birds were considered as the experimental unit. Statistical significance was determined at a significance level of $p < 0.05$. Whenever treatment effects were found to be significant ($p < 0.05$), the means were further analyzed and compared using Tukey's multiple-range test procedures implemented in SPSS software.

Results

The broilers used in the experiment were transported according to the appropriate transport density specified in Korean Law [16], so the mortality rate did not occur.

Transportation losses and carcass traits

The results of the transportation losses and carcass traits of the broiler during the transportation are shown in Table 1. The group with medium crating density exhibited a lower ($p < 0.05$) body weight loss compared to the low crating density group. Additionally, the group exposed to higher air temperatures demonstrated a markedly reduced ($p < 0.001$) body weight loss when compared to the group exposed to lower air temperatures. Notably, there was an interaction ($p < 0.001$) observed between crating density and weather for body weight loss. However, there is no significant difference ($p > 0.05$) in carcass traits among the treatments.

Physiological responses

The interaction ($p < 0.05$) between crating density and the weather conditions yielded significant differences in cortisol, glucose, and lactate levels (Table 2). Birds transported under lower air temperatures exhibited higher ($p < 0.05$) glucose and lactate values compared to those transported under higher air temperatures. Furthermore, the lactate level in birds transported under medium crating density was found to be higher ($p < 0.05$) than that observed in birds transported under high crating density conditions.

The impact on the respiratory frequency of broilers between crating density and weather during transportation is shown in Table 3. The high crating density group exhibited an elevated ($p < 0.05$) respiratory frequency in comparison to the other groups. Additionally, broilers transported under high air temperatures demonstrated a higher ($p < 0.001$) respiratory frequency than those transported under low air temperatures. The interaction between crating density and weather conditions yielded significant differences in respiratory frequency ($p < 0.05$).

Physicochemical traits

Birds subjected to the low air temperature exhibited higher ($p < 0.001$) pH, and WHC in comparison to those in the high air temperature (Table 4). Conversely, the high air temperature group demonstrated higher ($p < 0.001$) L* values compared to the low air temperature group. Remarkably, there was an interaction observed between crating density and weather for b* ($p < 0.001$).

Discussion

This research aimed to evaluate the effects of different crating densities and prevailing weather conditions on preslaughter losses, meat quality, and physiological responses in broiler chickens. In the current study, broilers with low and high crating density exhibited weight loss levels increased by 43.32% and 20.36%, respectively compared to those under medium crating density. The increased body weight loss observed in broilers subjected to lower crating density might be linked to a comparatively heightened struggle, possibly stemming from the provision of more space during transit [20]. In the results of this experiment with respect to weather conditions, broilers exposed to low air temperatures showed a weight loss increase of 73.58% compared to the high air temperatures. Furthermore, body weight loss showed an interaction between crating density and weather conditions wherein broilers subjected to high crating density in high temperatures experienced substantial body weight loss, while those exposed to low crating density in low temperatures also exhibited significant body weight loss. Previous studies observed that broilers with high crating density showed high weight loss levels in summer (27.2-33.6 ℃, 52.7-62.9%) and broilers with low crating density showed higher weight loss in winter (3.6-9.5 ℃, 63.3-78.8%), which is consistent with this current study [18,20]. However, [21] reported no significant difference in body weight loss based on the season and interaction between crating density and weather conditions [low air temperature (-9 ℃, 60%), high air temperature (27 ℃, 80%)], which contradicts the findings of our experiment. For the carcass traits, the results from [18] align with the findings of this study, indicating no significant difference based on crating density. Conversely, [20] revealed no difference in carcass percentage among different crating densities, but broilers exposed to high crating density exhibited a relatively higher breast meat percentage compared to those in low crating density conditions. The variability in outcomes can likely be attributed to the differing crating densities and environments employed in each respective experiment.

Chickens have a tendency to undergo physiological alterations as an adaptive response when exposed to stressful environments [25]. Elevations in hormones or metabolites such as cortisol, glucose, and lactate, which act as markers for evaluating stress levels in broilers, can be triggered by various forms of stress [12,27,28]. In this current study, we observed that broilers transported at lower crate densities in low air temperatures exhibited significantly elevated cortisol levels when compared to those transported at higher crate densities under low air temperatures. These findings suggest that broilers subjected to lower crate density and lower temperatures may experience heightened psychological or physiological stress. Similarly, the glucose levels demonstrated an interactive effect between crate density and weather conditions in the current investigation wherein broilers transported in high-density crates during hot weather displayed elevated glucose levels, and conversely, those in low-density crates during cold weather also manifested increased glucose levels. The body weight losses followed a similar pattern to the stress markers cortisol and glucose, suggesting a potential association with stress [29]. In contrast to our findings, the research by [20] reported that broilers subjected to high transport density exhibited elevated glucose levels compared to those with low transport density during the winter season. The variability in research outcomes may be attributed to variations in factors such as transportation duration, loading density, and temperature, which can differ significantly depending on the transportation distances employed in the respective experiments [28]. In times of stress, animals undergo a metabolic process in which substances like glycogen, glucose, and glucose-6-phosphate are transformed into lactate. This lactate buildup subsequently reduces the overall pH level, consequently influencing the meat color and WHC of meat [27,30]. In this present study, we observed that broilers transported at medium crate densities in low air temperatures exhibited significantly elevated lactate levels when compared to other treatments except for low crate densities in low air temperatures. We suggest that this elevation resulted from the struggling activity of broilers during the transport.

Respiratory frequency is a commonly assessed variable employed to gauge and infer the physiological state of broilers [31]. Chickens, lacking sweat glands, resort to spreading their wings to enhance air contact and panting as mechanisms to regulate body temperature in high-temperature conditions [32]. Elevated respiration rates indicate a physiological response to high temperatures, often characterized as respiratory alkalosis [33]. In this study, the patterns observed in respiratory frequency corroborated those found in the stress marker glucose, indicating that high crating density in transit during the summer exerts a physiological impact on chickens.

pH serves as a crucial indicator closely associated with meat characteristics in chickens. pH of broiler meat is influenced by the muscle glycogen content prior to slaughter and the speed at which glycogen is transformed into lactic acid post-slaughter. Lower pH values are associated with reduced WHC of meat, leading to increased cooking loss, drip loss, and decreased tenderness [34,35]. [13] carried out investigations involving various crating densities (10, 12, and 15 birds per crate) during the summer, but they found no statistically significant difference in pH associated with crating density. Likewise, [19] reported that no significant pH differences were observed across different crating densities (10, 12, and 15 birds per crate) during the winter. These results are consistent with those in this present study.

Water-holding capacity affects color and tenderness and is one of the most important functional characteristics of raw meat [34]. Furthermore, meat color serves as a key indicator of its freshness and is a crucial sensory attribute influencing consumer purchasing decisions [34,36]. Alterations in meat color are contingent upon the degradation of muscle proteins, a process influenced by the final pH and the rate at which pH decreases [37]. In this current study, transportation at high temperatures resulted in lower pH levels, reduced WHC, and increased lightness compared to transportation at low temperatures. When animals experience stress, the autonomic sympathetic nervous system releases catecholamines, leading to a significant influx of calcium ions (Ca^{2+}) into the sarcoplasm [38]. This, in turn, amplifies postmortem utilization and glycogenolysis in muscles, ultimately resulting in a lower final pH [13,21]. Consequently, based on the findings of this present study, it can be inferred that higher stress levels in high temperatures accelerate the reduction in pH, suggesting that transportation in hot weather leads to a lower final pH. Furthermore, broilers exhibiting lower pH values align with previous research results indicating increased lightness due to reduced water-holding capacity, muscle relaxation, and protein degradation [21,39,40]. Nevertheless, the findings regarding the assessment of stress on seasonal meat quality and stress-related effects on blood metabolites in this experiment do not correspond, suggesting the necessity for further research to clarify this discrepancy.

Conclusion

While we observed that medium crating density led to higher lactate levels, regardless of the season, this was accompanied by lower body weight loss and reduced respiration rates. Furthermore, irrespective of crating density, transportation in summer resulted in lower levels of stress indicators such as glucose and lactate, but it led to a decline in the meat quality due to decreased pH and water-holding capacity. Additionally, during broiler transportation, it was observed that lower temperatures combined with lower crating density and higher temperatures combined with high crating density were associated with increased body weight loss, cortisol levels, and glucose levels. As a result, taking into account the outcomes of this study, it is advisable to employ a medium crating density regardless of the season. Moreover, considering both the season and crating density, it is recommended to conduct broiler transportation under high crating density in low air temperatures and low crating density in high air temperature conditions.

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Tables

Table 1. Influence of crating density and weather conditions on live bird weight losses and carcass traits of broilers during transportation.

Crating density[1]	Weather[2]	Body weight loss (g)	Dressing ratio (%)	Relative breast meat weight (g)
Low		82.95^{c}	88.72	31.64
Medium	Low	42.95^{b}	90.00	30.37
High		40.28^{b}	89.60	29.54
Low		20.27^{a}	89.95	28.96
Medium	High	29.06^{ab}	90.00	29.03
High		46.40^{b}	89.90	30.11
SEM		1.724	0.143	0.297
Main effect				
Crating density				
Low		51.61^{b}	89.33	30.30
Medium		36.01^{a}	90.00	29.70
High		43.34^{ab}	89.75	29.83
SEM		2.987	0.248	0.515
Weather				
Low		55.39	89.44	30.52
High		31.91	89.95	29.36
SEM		2.439	0.203	0.420
p-value				
Crating density		0.004	0.176	0.688
Weather		<0.001	0.088	0.063
Crating density × Weather		<0.001	0.205	0.097

[1]Crating density: Low = 20 birds/crate (0.039 m^2/bird), Medium = 25 birds/crate (0.031 m^2/bird), High = 30 birds/crate (0.026 m^2/bird).

[2]Weather: Low = low air temperature (- 1 ℃), High = high air temperature (30 ℃).

SEM: standard error of the mean.

a-c Values with different superscripts in the same row were significantly different ($p < 0.05$).

Table 2. Influence of crating density and weather conditions on blood metabolites of broilers during transportation.

Crating density[1]	Weather[2]	Cortisol (ng/mL)	Glucose (mg/dL)	Lactate (ng/µL)
Low		36.07^{b}	204.84^{c}	4.26^{bc}
Medium	Low	30.83^{ab}	194.54^{bc}	4.46^{c}
High		23.21^{a}	184.39^{bc}	3.93^{b}
Low		27.48^{ab}	122.35^{a}	1.52^{a}
Medium	High	28.83^{ab}	149.55^{ab}	1.54^{a}
High		31.97^{ab}	202.37^{c}	1.50^{a}
SEM		0.939	4.974	0.034
Main effect				
Crating density				
Low		31.77	163.59	2.89^{ab}
Medium		29.83	172.04	3.00^{b}
High		27.59	193.38	2.71^{a}
SEM		1.626	8.615	0.059
Weather				
Low		30.04	194.59	4.21
High		29.43	158.09	1.52
SEM		1.327	7.034	0.048
p-value				
Crating density		0.208	0.056	0.006
Weather		0.748	0.001	<0.001
Crating density × Weather		0.003	0.001	0.019

[1]Crating density: Low = 20 birds/crate (0.039 m^2/bird), Medium = 25 birds/crate (0.031 m^2/bird), High = 30 birds/crate (0.026 m^2/bird).

[2]Weather: Low = low air temperature (- 1 °C), High = high air temperature (30 °C).

SEM: standard error of the mean.

a-c Values with different superscripts in the same row were significantly different ($p < 0.05$).

Table 3. Influence of crating density and weather on respiratory frequency of broilers during transportation.

Crating density[1]	Weather[2]	Respiratory frequency (count/min)
Low		66.25[a]
Medium	Low	64.00[a]
High		64.50[a]
Low		158.00[b]
Medium	High	162.25[bc]
High		178.50[c]
SEM		1.515
Main effect		
Crating density		
Low		112.13[a]
Medium		113.13[a]
High		121.50[b]
SEM		2.624
Weather		
Low		64.92
High		166.25
SEM		2.142
p-value		
Crating density		0.041
Weather		<0.001
Crating density × Weather		0.022

[1]Crating density: Low = 20 birds/crate (0.039 m^2/bird), Medium = 25 birds/crate (0.031 m^2/bird), High = 30 birds/crate (0.026 m^2/bird).

[2]Weather: Low = low air temperature (- 1 ℃), High = high air temperature (30 ℃).

SEM: standard error of the mean.

a-c Values with different superscripts in the same row were significantly different ($p < 0.05$).

Table 4. Influence of crating density and weather conditions on physicochemical traits of broilers during transportation.

Crating density[1]	Weather[2]	pH	WHC (%)	Cooking loss (%)	L*	a*	b*
Low	Low	6.12	68.97	24.43	51.01	6.83	17.70^{ab}
Medium	Low	6.08	72.18	23.62	51.26	7.03	19.17^{b}
High	Low	6.16	72.76	23.26	51.70	6.41	17.73^{ab}
Low	High	5.99	63.88	22.91	54.97	7.12	18.33^{ab}
Medium	High	5.89	64.27	22.10	54.65	7.11	17.26^{a}
High	High	5.86	63.55	21.85	55.24	7.12	17.30^{a}
SEM		0.023	0.682	0.509	0.283	0.108	0.157
Main effect							
Crating density							
Low		6.05	66.43	23.67	52.99	6.97	18.01
Medium		5.98	68.23	22.86	52.95	7.07	18.22
High		6.01	68.16	22.55	53.47	6.77	17.51
SEM		0.040	1.181	0.882	0.490	0.188	0.271
Weather							
Low		6.12	71.31	23.77	51.32	6.75	18.20
High		5.91	63.90	22.29	54.95	7.12	17.63
SEM		0.033	0.965	0.720	0.400	0.153	0.222
p-value							
Crating density		0.472	0.484	0.655	0.709	0.510	0.175
Weather		<0.001	<0.001	0.156	<0.001	0.097	0.073
Crating density × Weather		0.360	0.460	0.999	0.911	0.492	0.006

[1]Crating density: Low = 20 birds/crate (0.039 m^2/bird), Medium = 25 birds/crate (0.031 m^2/bird), High = 30 birds/crate (0.026 m^2/bird).

[2]Weather: Low = low air temperature (- 1 °C), High = high air temperature (30 °C).

SEM: standard error of the mean.

a-b Values with different superscripts in the same row were significantly different ($p < 0.05$).