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JAST (Journal of Animal Science and Technology) TITLE PAGE

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ARTICLE INFORMATION	Fill in the information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Effect of different crate material types for transit on production, physiological characteristics, and welfare of broilers during the summer season.
Running Title (within 10 words)	Effect of pre-slaughter transport factors of broilers
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This research was carried out with the support of "Cooperative Research Program for Agriculture Science and Technology Development (Project No. RS-2021-RD010100 [PJ016214])" Rural Development Administration, Republic of Korea.
Acknowledgments	Not applicable.
Availability of data and material	Upon a reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Yu M, Heo JM Data curation: Yu M Formal analysis: Yu M, Chathuranga NC Methodology: Yu M Software: Yu M, Chathuranga NC Validation: Yu M, Heo JM Investigation: Yu M, Chathuranga NC, Oketch EO, Hong JS, Park H Writing - original draft: Yu M, Chathuranga NC Writing - review & editing: Yu M, Chathuranga NC, Oketch EO, Hong JS, Park H, Heo JM
Ethics approval and consent to participate	This study was approved by the Animal Ethics Committee of Chungnam National University, Daejeon, Republic of Korea (approval number: 202206A-CNU-081).

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9 **Abstract**

10 The current study investigated the impact of using iron and plastic crates during summer
11 transportation on production, physiological characteristics, and welfare of broiler chickens. A
12 total of 160 Ross 308 male broilers were randomly selected from a battery-caged house at 35
13 days of age. Their average body weight was 1866.62 ± 36.048 g (mean \pm SEM). Broilers were
14 crated into fixed iron crates with 1.00 m (length) \times 0.78 m (width) \times 0.26 m (height) and plastic
15 crates with 0.82 m (length) \times 0.57 m (width) \times 0.29 m (height) dimensions at 173 cm²/kg
16 densities. Afterward, they were transported in the early morning at an average speed of 30-50
17 km/h for 40 minutes, completing a total distance of 20 km. Body weights were recorded before
18 and after completing the journey. Following the weighing of birds, blood samples were collected
19 for blood metabolite (cortisol, glucose, and lactate) analysis. Cervical dislocation was performed
20 to euthanize broilers followed by breast and drumstick collection. Dressing, drumstick, and
21 breast meat were calculated as percentages whereas respiratory frequencies were measured as the
22 number of breaths per minute. Collected breast meat samples were utilized to analyze
23 physiochemical parameters such as pH, color (CIE L*, a*, b*), water holding capacity, and
24 cooking loss. Results from skin temperature assessments showed higher temperatures ($P < 0.05$)
25 in broilers that were loaded into iron crates, both before (iron, 41.23 ± 0.61 °C; plastic, $39.25 \pm$
26 0.06 °C) and after (iron, 43.53 ± 0.72 °C, and plastic, 41.63 ± 0.13 °C) completing the journey.
27 However, total skin temperature change was not significantly affected. Importantly, stress-
28 indicating blood metabolite analysis revealed that glucose and lactate levels were lower ($P <$
29 0.05) in broilers transported in plastic crates. Nevertheless, cortisol levels remained unaffected
30 by crate materials. Furthermore, transit losses, carcass characteristics, and physiochemical
31 properties were also unaffected despite the dissimilar crate types. In conclusion, the study
32 revealed that plastic is the more advantageous crating material compared to iron. Besides, plastic

33 crates ensure meat quality and animal welfare, as evidenced by blood metabolite levels and skin
34 temperature after transit.

35 **Keywords:** broiler, crate, meat quality, stress, transportation, welfare

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Introduction

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39 The global poultry meat industry, representing approximately 40% of total meat
40 production heavily relies on transportation. Notably, chickens have constituted a staggering 87%
41 of poultry that transit upon reaching market weight [1]. However, concerns have arisen regarding
42 broiler transportation and its impact on production, meat quality, food safety, and animal welfare
43 [2]. This process involves pre-transit, transit, and post-transit phases, during which broilers
44 encounter various stressors disrupting their physiological equilibrium. These stressors include
45 health conditions, microclimate, crating densities, trailer design, vibration, noise, and feed and
46 water withdrawal periods [3-5]. Such stressors can compromise productivity and meat quality [6],
47 with seasonal changes further exacerbating challenges, particularly in coping with temperature
48 fluctuations [7, 8].

49 Notwithstanding, producers adopt different strategies to mitigate economic and quality
50 losses during different phases of broiler transit [9], including temperature regulation in
51 microclimates [10], optimizing journey length [7], crating densities [11], ventilation [12], and
52 managing feed and water withdrawal periods [11] as crucial factors. However, these strategies
53 must prioritize animal welfare, encompassing physical and mental states, emotional and
54 cognitive aspects, avoidance behavior, biochemistry, and responses to stimuli [13, 14]. Stressful
55 environments can significantly impact broiler productivity and health by activating the
56 hypothalamo-pituitary-adrenocortical cascade and triggering hormonal secretions [15, 16].
57 Hence, plasma or serum corticosteroid levels serve as acute stress indicators when proper blood
58 drawing methods are followed [17].

59 Stress hormones such as corticosterone and cortisol trigger rapid energy production in
60 animals [18], increasing the demand for glucose and elevating blood glucose levels [19]. In
61 situations where oxygen supply is inadequate for aerobic metabolism, muscles undergo

62 anaerobic respiration, converting glucose into lactate. This accumulation of lactate in the
63 bloodstream indicates stress in birds [20]. Stressors also elevate plasma corticosteroid levels due
64 to heightened secretion of adrenocorticotrophic hormone and corticotropin-releasing hormone.
65 However, cortical cell function and numbers may also be altered by dietary protein restrictions as
66 well [21].

67 The World Organization for Animal Health acknowledges the crucial role of maintaining
68 appropriate animal welfare measures during transportation and recognizing its significant impact
69 on meat quality [2]. European Council regulation (EC No 1/2005) complements this stance by
70 aiming to prevent animal suffering and promote welfare regulations for European operators. This
71 regulation covers various aspects, including transport preparation, responsibilities, competence,
72 equipment standards, and documentation requirements [22]. Additionally, countries such as the
73 USA, Australia, and the Republic of Korea have implemented regulations prioritizing crate
74 design and construction within the respective regions [23, 24]. However, [25] identified four
75 major risk factors associated with animal transportation across different jurisdictions: climatic
76 conditions, animal fitness, stocking densities, and transit durations.

77 The selection of crate materials for broiler transportation is diverse globally due to factors
78 such as operational scale, cost, technology, durability, and environmental impact. Recently,
79 plastic crates have been among commonly used, while balancing convenience with ecological
80 considerations. However, researchers have traditionally overlooked crate materials as a major
81 concern. Emerging factors like environmental impact, hygiene, and local regulations should now
82 shape these decisions. Thus, the current study aims to explore the effects of different crate
83 materials on broiler production, stress levels, meat quality, and welfare, providing insights to
84 improve transportation practices, especially during the summer season.

85

86

Materials and Methods

87 The Animal Ethics Committee of Chungnam National University, Daejeon, Republic of
88 Korea, approved the protocols used in this experiment (approval number: 202206-CNU-081)

89

90 **Birds, experimental design, and treatments**

91 Before transportation, all birds were housed in Chungnam National University's
92 experiment farm which had 48 battery cages ($76 \times 61 \times 46 \text{ cm}^3$) that housed six birds until
93 transportation and were managed according to the Ross 308 broiler management guideline [26].
94 A total of 160 Ross 308 male broilers were used at 35 days of age with an average body weight
95 of $1,866.62 \pm 36.048 \text{ g}$ (mean \pm SEM). They were randomly selected after 4 h of feed
96 withdrawal before catching. Afterward, birds were taken out from the cages and transported
97 securely by holding their wings against the handler's body using both hands (Japanese method)
98 [27]. The birds were transported in different types of crates as follows: an iron crate having
99 dimensions of 1.00 m (length) \times 0.78 m (width) \times 0.26 m (height); a plastic crate having
100 dimensions of 0.82 m (length) \times 0.57 m (width) \times 0.29 m (height) with 4 replicates per crate type.
101 The birds were placed in crates based on optimal crating density suggested by the Korean
102 transportation standards [28] of an average of $173 \text{ cm}^2/\text{kg}$. The transportation's distance was 20
103 km for 40 min at an average speed of 30-50 km/h during the early morning from 8:00 a.m.

104

105 **Transportation losses**

106 Body weight loss (g) in transit was measured as the difference between all broilers'
107 weight before transportation and the final body weight (g) from all crates upon arrival at the
108 destination after transportation [29].

109

110 **Carcass traits and sample collection**

111 Once birds arrived at the destination, carcass trait measurements and sample collection
112 were taken place. Two birds were selected based on closeness to the mean body weight of the
113 birds in the respective crate, and the resulting weight was recorded as the live body weight.
114 Blood samples were collected from the brachial vein into a vacutainer coated with lithium
115 heparin (BD Vacutainer, BD, Franklin Lakes, NJ, USA) before euthanizing the birds. The birds
116 were then euthanized by cervical dislocation for the evaluation of some carcass characteristics.
117 The dressing percentage with giblets (heart, gizzard, and liver) was determined as a function of
118 the live weight of the birds. The breast meat was then separated and weighed to measure its
119 relative to the total carcass weight. The breast meat of broilers was then collected for meat
120 quality analyses [29].

121

122 **Physiological responses**

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

132 After finishing transportation, the respiratory frequency was measured as the number of
133 breaths per minute using three randomly selected broilers per crate observed by the camera
134 (GoPro Hero 8, San Mateo, CA) for 1 minute [30].

135

136 **Physicochemical traits**

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

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

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

155 110 °C. The remaining moisture (%) present in the meat after centrifugation was expressed as the
156 WHC [31].

157 To measure the cooking loss, the breast meat of the broiler was weighed vacuum
158 packaged and cooked for 20 min in a water bath at 80 °C until the internal temperature reached
159 70 °C. The cooked breast meat of broilers was cooled at room temperature (20 °C) for 30 min.
160 After removal of the vacuum bag, the surface moisture of the breast meat of the broiler was
161 removed with paper towels, and the cooked breast meat of the broiler was weighed. The cooking
162 loss was calculated as the difference between the weight of raw breast meat and cooked breast
163 meat.

164

165 **Skin temperature measurements**

166 The body surface temperature of broilers within individual broiler cages was assessed utilizing a
167 portable thermal imaging camera (IRay T3PRO, manufactured by Shandong, China) both before
168 and after transportation. The measurements were then averaged to derive a representative value
169 for each cage. Furthermore, temperature differentials pre- and post-arrival were quantified by
170 converting them into absolute values, facilitating rigorous analysis of the thermal dynamics
171 experienced by the broilers during transport [32].

172

173 **Statistical analysis**

174 The statistical analysis of the data was performed using SPSS (Version; IBM SPSS
175 2019). The data obtained from the experiment except for the skin temperature were analyzed by
176 independent sample t-test. The data of skin temperature were analyzed by a two-way ANOVA
177 model to evaluate the main effects (types of crate and transportation) followed by Tukey's
178 multiple range analysis with each crate as the experimental unit. In terms of transportation loss

179 and respiratory frequency measurements, the experimental unit was defined as the crate. For
180 carcass traits, meat quality, and blood metabolites, selected individual birds were considered as
181 the experimental unit. Statistical significance was determined at a significance level of $P < 0.05$.

182

183 **Results**

184 The broilers used in the experiment were transported according to the appropriate
185 transport density specified in Korean Law [28], so the mortality did not occur regardless of the
186 two crate material types.

187

188 **Transportation losses and carcass traits**

189 The results of the transportation losses and carcass traits of the broiler using different
190 types of crates during transportation are shown in Table 1. There was no significant difference (P
191 > 0.05) in body weight loss and carcass traits such as dressing ratios, relative breast meat, and
192 drumstick weights between the treatments.

193

194 **Physiological responses**

195 The results of broiler blood metabolites, after transporting with different crate types are
196 shown in Figure 1. Broilers transported in plastic crates had reduced glucose and lactate contents
197 ($P < 0.05$) compared with those in iron crates. However, plasma cortisol levels remained
198 unaffected ($P > 0.05$) despite the different types of crates used during transportation. The impact
199 of different crate materials on broiler chicken respiration after transportation is shown in Figure 2.
200 There was no significant difference ($P > 0.05$) in respiratory rate between the two treatments.

201

202 **Physicochemical traits**

203 The results of the physicochemical characteristics of broilers related to different crate
204 types during transportation are shown in Table 2. The meat quality parameters such as pH, water
205 holding capacity, cooking loss, and meat color (L^* , a^* , b^*) were not significantly different ($P >$
206 0.05) related to the two treatments.

207

208 **Skin temperature evaluation**

209 The skin temperature of transported broilers in crates made of different materials during
210 transportation in summer is presented in Table 3. The interaction between transportation and
211 crate type did not show any significant effect in the current study ($P > 0.05$). However,
212 transportation did affect the increase in total shipment temperature after transportation ($p <$
213 0.001), while crate type demonstrated higher ($P < 0.01$) temperatures in fixed iron crates
214 compared to plastic crates.

215

216 **Discussion**

217 The current study intended to evaluate the impact of different crating materials used
218 during transportation on the production, physiological characteristics, and welfare of broilers in
219 the summer season. The broilers used in the current study were transported in compliance with
220 the transport density regulations stipulated by Korean Law [28], which could result in non-
221 mortality during transportation.

222 Iron possesses high heat conducting and heat transferring properties as a structural
223 material. These characteristics enhance the heat absorption from the outer environment and
224 convey it from highly heated areas to low-heated areas through conductivity. Ultimately, this
225 process increases the total surface temperatures in particular areas [33, 34]. In comparison,
226 plastic has excellent insulating capabilities with inherited heat resistance properties which can be

227 used as a structural material in many industries of the world [35]. In our study, crates made of
228 iron have been observed to elevate the microenvironment temperature, as evidenced by the rise
229 in the skin temperatures of broilers. Conversely, broilers housed in plastic crates, under similar
230 conditions, exhibited lower skin temperatures. Since body surface temperature serves as a
231 common indicator of thermal comfort or stress in broiler chickens [36, 37], it is possible that iron
232 crates contribute to increased temperatures within the crate space due to radiation reflection and
233 high thermal absorptivity. In contrast, plastic crates exhibit lower temperatures, due to
234 their better thermal insulation properties against heat.

235 Typically, the plasma glucose levels in an avian species are 150% to 200% greater than
236 those found in mammals with comparable body mass [38]. Hence, its concentration directly
237 affects the metabolism of poultry. According to [39], there is a significant impact on glucose
238 metabolism in chickens related to their stress regulations. Particularly, glucose and triglycerides
239 are recognized as major metabolites that play crucial roles in biochemical and physiological
240 functions, providing the energy needed to fuel these processes [40]. This glucose concentration
241 can vary based on several factors in poultry including, physiological status, age, gender, energy
242 intake, and ambient temperature [39]. In relevance, the glucose level increment in broilers
243 transported in iron crates could be due to the stress, caused by the type of crating material and
244 macroenvironment (in summer) temperatures.

245 While transportation, broilers experience acute stress conditions (such as bruising or
246 broken wings) from hard surfaces like iron crates and they could lead to increased
247 gluconeogenesis and glucose mobilization within the animal body. Additionally, this sudden
248 introduction of broilers into an excessive heat and humidity environment (in summer) from a
249 relatively constant environment can exhibit a detrimental effect on them [27]. Consequently,
250 elevated microclimate temperatures can further exacerbate these physiological processes in

251 broilers, potentially resulting in augmented glucose levels in broiler plasma [41]. Similarly,
252 lactate level increment in metal-crated broilers could also be due to the aforementioned stress
253 factors similarly glucose level fluctuations.

254 The current results on broiler transport and welfare, emphasize the importance of further
255 research into the effects of crate materials on broiler health and the economic and practical
256 benefits for the industry. In conclusion, the study underscores the importance of crate material
257 choice in broiler transportation while revealing the plastic crate as the advantageous crate
258 material type compared to the iron that can ensure meat quality and animal welfare in terms of
259 blood metabolite changes and skin temperature changes after loading.

260

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Funding sources

262 This research was carried out with the support of “Cooperative Research Program for
263 Agriculture Science and Technology Development (Project No. RS-2021-RD010100
264 [PJ016214])” Rural Development Administration, Republic of Korea.

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Tables

386 **Table 1.** Live weight loss and carcass traits as affected by different materials of crates during
387 transportation in summer.

Items	Iron	plastic	SEM	<i>P</i> -value
Body weight loss (g)	32.15	26.46	1.737	0.102
Dressing ratio ¹ (%)	89.58	89.72	0.238	0.788
Relative breast meat weight ² (%)	28.03	28.23	0.604	0.878
Relative drumstick weight ³ (%)	10.53	10.41	0.155	0.713

388 ¹(carcass weight/Live body weight) × 100

389 ²(breast meat weight/carcass weight) × 100

390 ³(drumstick weight/carcass weight) × 100

391

ACCEPTED

392 **Table 2.** Physicochemical traits of chicken breast meat under different materials of crates during
393 transportation in summer.

Items	Iron	Plastic	SEM	<i>P</i> -value
pH	5.92	5.96	0.035	0.588
WHC (%)	63.13	67.22	2.019	0.335
Cooking loss (%)	23.13	21.42	0.966	0.402
L*	53.45	52.85	0.411	0.476
a*	6.74	7.20	0.287	0.440
b*	16.34	17.19	0.250	0.095

394 WHC: Water Holding Capacity
395

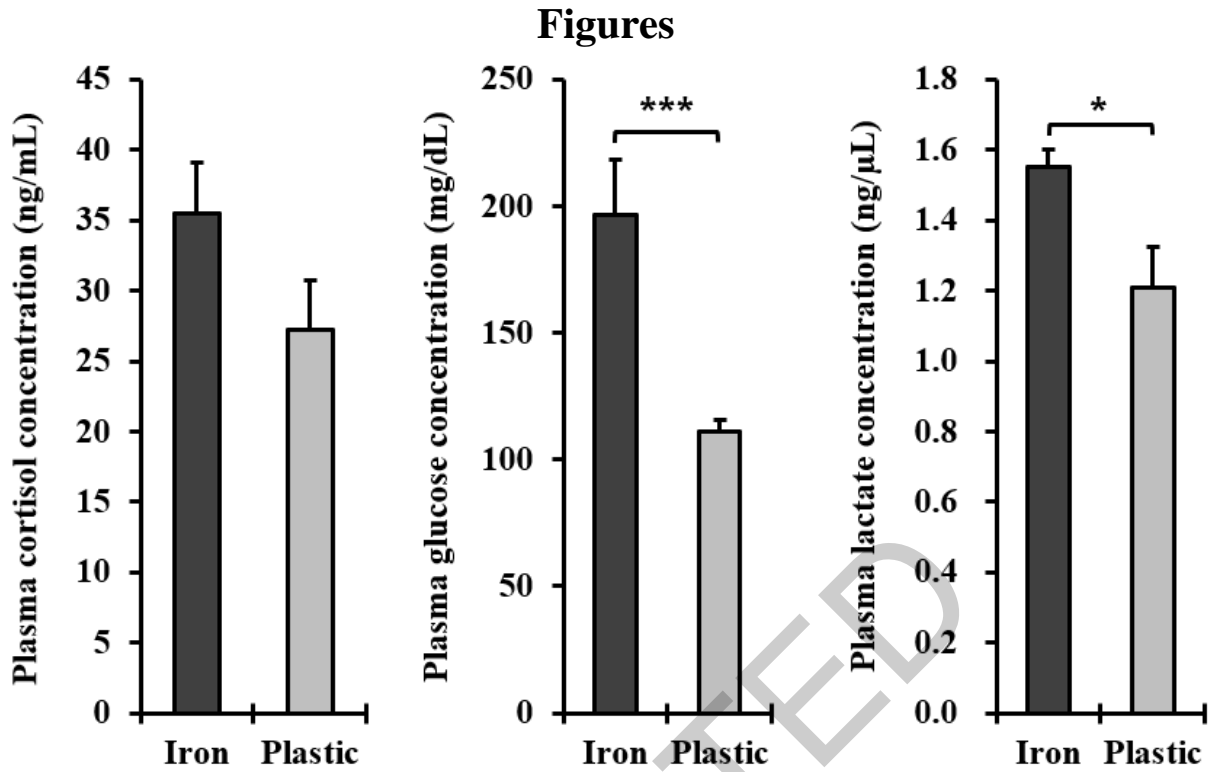
ACCEPTED

396 **Table 3.** Skin temperature of broilers under different materials of crates and transportation in
 397 summer

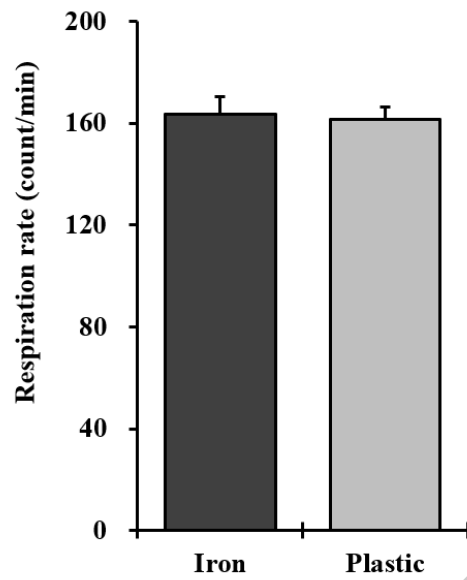
Transportation	Type of crate	Skin temperature (°C)
X	Iron	41.23
	Plastic	39.25
O	Iron	43.53
	Plastic	41.63
SEM		0.239
Main effect		
Transportation		
X		40.24
O		42.58
SEM		0.341
Type of crate		
Iron		42.38
Plastic		40.44
SEM		0.383
<i>P</i> -value		
Transportation		<0.001
Type of crate		0.002
Transportation × Type of crate		0.939

398 SEM: standard error of the mean.

399



401
 402 **Figure 1.** Blood metabolites of transported broilers under different materials of crates during
 403 transportation in summer. The values in the histogram are the means \pm SEM (n = 8). * Indicates a
 404 significant difference of $p < 0.05$ and *** indicates a significant difference of $p < 0.01$.
 405



406

407 **Figure 2.** Respiration rate of transported broilers under different materials of crates during
408 transportation in summer. The values in the histogram are the means \pm SEM (n = 4).

ACCEPTED