Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2020) 44: 418-426
© TÜBİTAK
doi:10.3906/vet-1905-85

Effect of transportation distance and crating density on preslaughter losses and blood biochemical profile in broilers during hot and humid weather

Faisal HUSSNAIN[1,*], **Athar MAHMUD**[1], **Shahid MEHMOOD**[1], **Muhammad Hayat JASPAL**[2]

[1] Department of Poultry Production, University of Veterinary and Animal Science, Lahore, Pakistan

[2] Department of Meat Science and Technology, University of Veterinary and Animal Science, Lahore, Pakistan

Received: 26.05.2019 • **Accepted/Published Online:** 28.01.2020 • **Final Version:** 06.04.2020

Abstract: Transportation of broilers from different localities to the processing plants is a critical step in the farm to fork chain, and any disturbance in this chain may result in worst consequences. Keeping this scenario in view, a study was conducted to determine the live losses at different transportation distances (~80, 160, 240 km) and crating densities (10, 12, 15 birds/crate) during hot and humid summer. The uniform weight birds were picked from a farm and placed in plastic crates (0.91 m × 0.55 m). The results showed a significant increase in body weight loss and dead on arrival (%) with the increase in transportation distance and crating density in broiler. The long journey (240 km) was also associated with significantly lower carcass and breast yield, and significantly higher catalase activity and uric acid concentration. An increase in crating density above 10 birds/crate resulted in a significant decrease in carcass and thigh yield in broiler. Similarly, placing 15 birds/crate showed significantly higher serum catalase activity and protein levels. Physical injuries, bruises, serum triiodothyronine (T3) and thyroxine (T4) remained unaffected from either transportation distance or crating density. Thus, transportation below 80 km and crating density below 12 birds/crate during hot and humid weather may be considered as less stressful for broilers.

Key words: Crating, carcass characteristics, metabolites, shrinkage, summer, transportation

1. Introduction

Transportation of broilers from farms located at variable distances to a centrally located processing plant as per welfare guidelines is a challenging task. Since, the world is facing the scenario of global warming (0.128 ± 0.026 ºC per year), the environment in Pakistan is also rapidly changing and extreme weather events are becoming more prominent [1,2]. The modern broiler breeds are more prone to heat stress, and their inability of thermoregulation under environmental extremes has adverse effects that may range from mild discomfort to death [3,4]. Broilers are transported in open sided trucks having only have passive ventilation linked with the movement of the truck, which results in poor airflow and heterogeneous distribution of air creating a thermal core inside the crates [5,6], especially when the truck stops or reduces speed owing to some unavoidable circumstances. Hence, the distance, ambient temperature or season of the transportation, and duration of birds being inside crates combine to develop a stressful microclimate around the birds [7,8].

The economic losses associated with the above mentioned stressors during transportation have become a potential cause of concern [9]. Mortality ranging from 0.14% to 2.09% for distances between 15 km and 300 km was reported in European countries with relatively less severe temperatures than the Asian subcontinent [10–12]. Not only the mortality but also the body weight loss and carcass downgrading are reported to increase during transportation [13]. The extent of body weight loss depends on geographical region and environmental conditions. For example, a loss of 2.36% was reported after 300 km of travel in Poland [14], while another study reported 6.02% loss with 400 km of transportation in Turkey [11]. Contrarily, a study in the hot and humid environment of Malaysia reported nonsignificant effects of 3 h of transportation on mortality and body weight loss [15]. Similarly, it was reported that up to 5 h of transportation had no effect on carcass yield and downgrading percentage [10].

The distance of transportation interacts with the number of birds in crates at different ambient temperatures to affect the bird's welfare [16]. The crating density has a decisive role in the developing microclimate inside the crate and truck due to inadequate ventilation and heat exchange rate [17]. Lower than normal crating density can

* Correspondence: faisal.hussnain@uvas.edu.pk

be beneficial from a welfare perspective but it increases the number of vehicles required and can also cause economic losses due to physical injuries [18], while higher than normal crating density can result in death due to fighting, trampling, and suffocation [19]. Nijdam et al. [20] reported 1.1% increase in mortality rate with every extra bird.

The preslaughter stressors also cause significant changes in bird physiological status and regulatory systems, which can be observed by estimating biochemical indices of the birds after slaughtering [8,21]. Studies regarding effects of transportation on physiological response are few and contradictory. Some studies have reported significant increase in blood protein, uric acid, and glucose contents [22,23] while others have reported a significant decrease in blood glucose and uric acid contents with the increase in transportation distance [24], and in some cases no effect of transportation on blood glucose contents [25].

There are many studies investigating the effect of transportation distance and crating densities on live losses during transportation and blood biochemical changes, but most of them were conducted in European countries or countries with relatively less severe ambient temperature as compared to Pakistan. Limited or no information is available regarding optimal crating density during summer season transportation in hot and humid conditions. Keeping this in view, the present study was planned to determine the effect of different transportation distances and crating densities on live losses, carcass characteristics, and blood biochemical profiles of broilers during hot and humid summer.

2. Materials and methods

2.1. Birds and their treatments

The study was conducted at the Department of Poultry Production, University of Veterinary and Animal Sciences, Lahore, Pakistan. In this study, Ross-308 broilers (age 35 days, body weight 1900–2050 g) were picked after applying 4 h of feed withdrawal before catching. The birds were transported in commercial trucks (capacity 144 crates) in loose plastic crates (Engi Plastic Industries Pvt. Ltd., Lahore, Pakistan) having dimensions of 0.91 m (length) × 0.55 m (width) × 0.30 m (height). A total of 270 birds were used in this study. The birds were placed in plastic crates (tagged) under three different crating densities [10 birds (0.050 m^2/bird), 12 birds (0.042 m^2/bird), and 15 birds (0.033 m^2/bird) per crate] and were subjected to three transportation distances. The transportation distances were categorized as short (~80 km), medium (~160 km), and long (~240 km). Each treatment was replicated six times and tagged crates were filled with 5 individually tagged birds after weighing them at the farm and the remaining untagged (5, 7, and 10, respectively) birds to complete three crating densities (i.e., 10, 12, or 15 birds per crate). To minimize the effect of the truck's microenvironment, tagged crates were equally distributed in the front, middle, and rear sections of the truck. The procedures and bird handling protocols used in this study were approved by the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore (Approval No: DR/916-2017).

2.2. Climatic indices

The experiment was conducted during extremely hot and humid weather (August) conditions in central Punjab, Pakistan. The birds were transported in the vicinity of districts Kasur and Lahore, Punjab, Pakistan. Birds subjected to transportation distances of 160 and 240 km first traveled in laps of 40 km from the farm and were then transported to a slaughtering facility located 80 km away from the farm, while the birds transported for 80 km were directly sent to the slaughtering facility. The temperature and relative humidity were measured at various distances and times using a digital weather tracker (Kestrel 4500 NV, Nielsen-Kellerman, USA) and remained in the range of 27.2–33.6 °C and 52.7%–62.9%, respectively.

2.3. Parameters

2.3.1. Preslaughter losses

The body weight loss (BWL) percentage during transportation was measured as the difference of weight at the farm (g) and weight (g) at the ultimate destination. The dead on arrival (DOA) percentage was calculated by dividing the number of birds found dead to the total birds in crates.

Every single bird from each crate was observed to record any incidence of physical injuries and the proportion was calculated from total birds. Similarly, bruises on wings, breast, and legs were individually observed, and their proportion with the total number of birds slaughtered per replicate was calculated.

2.3.2. Carcass characteristics

Upon reaching the slaughtering facility, 10 birds from each treatment were randomly picked and slaughtered manually. After bleeding, the birds were defeathered and eviscerated. The carcass percentage was calculated as hot eviscerated carcass with neck and without giblets. The percentage of cut-up parts was calculated relative to carcass weight.

2.3.3. Blood sampling and serum collection

Upon reaching the slaughtering facility, the tagged birds were separated from other birds, weighed, and slaughtered manually. To reduce the effect of stay before slaughtering, all the birds were divided among 6 slaughtering stations, and all treatments were slaughtered at the same time. The blood was collected from the jugular vein of 10 birds from each treatment in non-EDTA coated vacutainers. The vacutainers were then centrifuged at 3000 rpm for 10 min to separate the serum. The serum was then carefully separated with a sterilized pipette into labeled Eppendorf tubes. Serum samples were stored at –20 °C for analysis. The samples were maintained at 3–8 °C while handling.

2.3.4 Blood metabolites and hormonal assays

The samples were analyzed for serum total proteins (g/dL) using a Tron immunoassay kit (Paris, France), and serum glucose (mg/dL) and uric acid (mg/dL) using a Human immunoassay kit (Human Gesellschaft für Biochemica und Diagnostica GmbH, Wiesbaden, Germany) with a spectrophotometer (UV/VIS Spectrophotometer AMV 09 Tlead Int. Co. Ltd., Qingdao, Shangdong, China) at the kit's recommended wavelength.

The estimations of serum triiodothyronine (T3) and thyroxine (T4) were made using BioCheck enzyme immune assay test kits (Catalog Number: BC-1005 and BC-1007). The catalase activity was estimated using the methodology of Hadwan and Abed [26]. Briefly, 4 test tubes (sample, control test, standard, and blank) were prepared. The sample tube contained 100 µL of serum and 1000 µL of hydrogen peroxide (20 mM), the control test tube contained 100 µL of serum and 1000 µL of distilled water, the standard tube contained 100 µL of distilled water and 1000 µL of hydrogen peroxide, and the blank tube contained 1100 µL of distilled water. All these tubes were vortexed and incubated at 37 °C for 3 min, and after that, 4000 µL of ammonium molybdate (32.4 mmol/L) was added to all tubes to stop the reaction. After that, changes in absorbance were recorded at 374 nm against the reagent blank.

2.4. Statistical analysis

The data collected were analyzed by two-way analysis of variance (ANOVA) technique under a completely randomized design assuming transportation distance and crating density as a main effect. The significant means were separated using Tukey's HSD test, considering significance level of $P \leq 0.05$, with the help of SAS 9.4 [27].

3. Results and discussion

The results showed that the broilers transported up to 240 km showed significantly higher ($P < 0.0001$) body weight loss (BWL) followed by those transported for 160 km and least in those transported up to 80 km (Table 1). The longer journeys were associated with extended fasting and water deprivation periods along with more exposure to stress related to transportation [28,29], which resulted in increased BWL [30]. Accordingly, other studies have also reported a significant increase in BWL with the increase in transportation distance [14,31], and the impact increases in hot weather conditions [32]. In the present experiment, average BWL (%) in birds subjected to 80, 160, and 240 km transportation was 3.76%, 5.69%, and 6.80%, respectively. Such higher values are not desirable and cause considerable losses to transporters and poultry industry. The BWL (%) values in the present study were in accordance with the findings of Aral et al. [11], who reported 6.63% BWL

Table 1. Effect of transportation distance and crating density on preslaughter losses[1].

Treatments	BWL (%)	DOA (%)	Physical injuries (%)	Breast bruises (%)	Wing bruises (%)
Transportation distance (km)					
80 km	3.76 ± 0.16^{c}	2.00 ± 0.70^{b}	1.11 ± 1.11	4.44 ± 2.64	5.56 ± 3.23
160 km	5.69 ± 0.17^{b}	6.277 ± 1.79^{ab}	1.11 ± 1.11	8.89 ± 4.21	3.33 ± 2.45
240 km	6.80 ± 0.18^{a}	10.22 ± 2.12^{a}	4.44 ± 2.10	6.67 ± 2.48	0 ± 0
Crating densities (birds/crate)					
10 birds/crate	4.89 ± 0.22^{b}	3.33 ± 1.11^{b}	4.44 ± 2.10	10.00 ± 3.96	6.67 ± 3.35
12 birds/crate	5.04 ± 0.21^{b}	5.00 ± 1.36^{b}	2.22 ± 1.54	7.78 ± 3.81	2.22 ± 2.22
15 birds/crate	6.33 ± 0.23^{a}	10.17 ± 2.33^{a}	0 ± 0	2.22 ± 1.54	0 ± 0
P-value					
Distance	<0.0001	0.0021	0.2042	0.6462	0.2408
Density	<0.0001	0.0091	0.1219	0.2460	0.1248
Interaction	0.6135	0.6232	0.7068	0.7004	0.5526

$^{a–c}$ Means in a row with no common superscript differ significantly at $P \leq 0.05$.
[1]Values are least square mean ± standard error.
BWL: Body weight loss; DOA: Dead on arrival.
Crating density: Per crate 10 birds (0.050 m^2/bird), 12 birds (0.042 m^2/bird), and 15 birds (0.033 m^2/bird).

after a transportation period of >10 h in Turkey. Similarly, Arikan et al. [13] compared ≤50 km, 51–150 km, and ≥151 km transportation distances and reported 259.40 g, 307.35 g, and 350.14 g BWL in each category, respectively. Among different crating densities, transportation under the highest crating density (15 birds/crate) resulted in significant ($P < 0.0001$) increase in BWL compared to transportation at medium and low crating densities (Table 1). Furthermore, nonsignificant differences were observed for BWL at 10 and 12 birds/crate density. Data regarding the effect of crating density on BWL are scarce. Delezie et al. [18] compared the BWL between two crating densities in Belgium and reported nonsignificant differences.

The results showed significantly higher ($P = 0.0021$) DOA (%) in broilers transported up to 240 km followed by those transported up to 160 and 80 km (Table 1). Similarly, the birds transported at high crating density (15 birds/crate) showed significantly ($P = 0.0091$) higher DOA as compared to the rest of the treatments. Higher mortalities in long-distance transported broilers at higher crating density could be due to inability of birds to thermoregulate the hyperthermia produced in crates due to accumulation of metabolic heat, and moisture due to panting of birds [33,34]. In accordance with the findings of the present study, other studies [11,31] have also reported increase in DOA with the increase in transportation distance. The highest DOA observed in the present study, i.e. 10.22%, was relatively higher as compared to previously reported rates of 0%–2.09% in many studies [10,12,31], although some studies reported a relatively higher DOA, i.e. 7.7%, in Turkey [13] and 15.8% in the United Kingdom [35]. The higher DOA in the present study could be attributed to the relatively extreme environment in Pakistan as compared to other studies.

This study also revealed that neither transportation distance nor the crating densities and their interaction showed any significant effect ($P > 0.05$) on physical injuries or breast and wing bruises (Table 1). Similarly, no bruising was observed on leg regions of the birds. Moran and Bilgili [36] also reported no effect of transportation of broilers for bruises on wings and back. Usually, it is considered that the fewer the birds in a crate, the more space for birds, and during jerks and jolts of transportation, there will be relatively higher chances of bruising and injuries [19]. However, the present study showed that reducing crating density up to 10 birds/crate did not increase the injuries or bruises percentage (Table 1). Some studies reported average bruises (%) ranges from 0.022% to 25% in broilers due to multiple risk factors [20,37,38].

The present study showed that the carcass (%) decreased significantly ($P < 0.0001$) with the increase in transportation distance above 80 km (Table 2). Similarly, significantly ($P = 0.0352$) higher carcass (%) was observed in the birds transported at lower crating density, i.e. 10 birds/crate, followed by those transported at 12 and 15 birds/crate density (Table 2). The reduction in carcass (%) with the increase in distance could possibly be attributed to the shrinkage of carcass, as the birds remained off feed 4 h before being caught and up until slaughtering such that the total withdrawal time reached 8–11.5 h; Mazanowski

Table 2. Carcass characteristics as affected by transportation distance and crating density[1].

Treatments	Carcass (%)	Wing (%)	Breast (%)	Thigh (%)	Drumstick (%)	Back (%)
Transportation distance (km)						
80 km	64.21 ± 0.30^{a}	8.84 ± 0.06	32.83 ± 0.32^{a}	15.82 ± 0.11	13.26 ± 0.09	26.39 ± 0.29^{c}
160 km	63.18 ± 0.32^{b}	8.67 ± 0.08	32.08 ± 0.29^{a}	15.67 ± 0.13	13.45 ± 0.09	27.65 ± 0.32^{b}
240 km	62.32 ± 0.25^{b}	8.90 ± 0.10	30.49 ± 0.26^{b}	15.49 ± 0.18	13.50 ± 0.06	29.90 ± 0.24^{a}
Crating densities (birds/crate)						
10 birds/crate	63.71 ± 0.37^{a}	8.83 ± 0.09	31.79 ± 0.32	15.51 ± 0.13^{b}	13.40 ± 0.07	28.24 ± 0.29^{a}
12 birds/crate	63.35 ± 0.27^{ab}	8.82 ± 0.08	32.07 ± 0.28	16.02 ± 0.16^{a}	13.42 ± 0.08	27.18 ± 0.31^{b}
15 birds/crate	62.66 ± 0.25^{b}	8.76 ± 0.08	31.53 ± 0.37	15.45 ± 0.12^{b}	13.38 ± 0.09	28.52 ± 0.42^{a}
P-value						
Distance	<0.0001	0.1284	<0.0001	0.2460	0.0951	<0.0001
Density	0.0352	0.7909	0.4253	0.0094	0.9272	0.0012
Interaction	0.0003	0.5238	<0.0001	0.0806	0.5201	<0.0001

$^{a-c}$ Means in a row with no common superscript differ significantly at $P \leq 0.05$.
[1]Values are least square mean ± standard error.
Crating density: Per crate 10 birds (0.050 m^2/bird), 12 birds (0.042 m^2/bird), and 15 birds (0.033 m^2/bird).

[39] reported a significant decrease in carcass weight where weight loss during transportation exceeded 3%, and in the present study weight loss ranged between 3.76% and 6.80% (Table 1). Similarly, Doktor and Połtowicz [25] also reported significantly higher dressing percentage in birds slaughtered without any transportation as compared to those having 2.5 h of transportation. Among different cut-up parts' yields, a significant decrease in breast (%) was observed after 160 km of transportation, while a gradual increase with increase in transportation distance was observed in back (%). Wings, thighs, and drumsticks (%) showed nonsignificant (P > 0.05) differences among different transportation distances (Table 2). Among different crating densities, the birds crated at 12 birds/crate crating density showed significantly higher thigh (%) (P = 0.0094) and lower back (%) (P = 0.0012) as compared to those kept at 10 and 15 birds/crate density (Table 2). These findings might be attributed to a reduction in the share of viscera and other inedible portions up until 80 km of traveling, as Taylor et al. [40] reported that out of 4.2% total body weight loss during transportation 1.8% was due to the emptying of the intestines and excreta. Thus, the reduction in intestinal weight during transportation may also increase the relative share of cut-up parts. However, Doktor and Połtowicz [25] reported no effect of transportation on cut-up carcass parts. There is a lack of reported data regarding the effect of different crating densities in summer season on cut-up parts yield.

The results showed nonsignificant differences (P > 0.05) among different transportation distances for serum T3 and T4 contents (Table 3). However, numerically the data showed an overall decreasing trend in T3 and T4 concentrations with the increase in transportation distance. Previous studies have reported a decrease in plasma T3 and T4 activity with an increase in temperature during heat stress [41,42]. Joiner and Huston [43] reported a smaller thyroid size at high environmental temperatures and suggested that thyroid activity and subsequently metabolic rate might be reduced at hot temperatures. Results also showed nonsignificant differences (P > 0.05) among different crating densities for T3 and T4 levels (Table 3). Similarly, Delezie et al. [18] reported no effect of different crating densities on T3 concentration but also reported a significant decrease in serum concentration at higher crating density. Our results also showed a decrease in T4 concentration at higher crating density but the difference was nonsignificant.

A significant increase (P < 0.0001) in serum catalase concentration was observed in birds transported up to 240 km as compared to those transported for 80 and 160 km (Table 3). Similarly, significantly higher (P = 0.0144) serum catalase activity was observed in birds placed at 15 birds/crate density as compared to the rest of the treatments (Table 3). Catalase is an antioxidant enzyme and catalase activity can be used as a measure to assess the oxidative stress on the living organism [44]. During heat stress, the production of free radicals increases, and the body responds with the increase in catalase activity as a mechanism to control oxidation in cells [45,46]. No study had been reported on serum catalase activity in response

Table 3. Physiological response under different transportation distances and crating densities[1].

Treatments	T3 (ng/mL)	T4 (µg/dL)	Catalase (ng/mL)	Glucose (mg/dL)	Total protein (g/dL)	Uric acid (mg/dL)
Transportation distance (km)						
80 km	2.13 ± 0.22	7.49 ± 0.51	0.73 ± 0.05^{b}	221.00 ± 5.12^{a}	6.11 ± 0.13	7.48 ± 0.14^{b}
160 km	1.79 ± 0.19	6.85 ± 0.60	0.86 ± 0.05^{b}	165.83 ± 3.90^{b}	5.91 ± 0.21	8.25 ± 0.30^{ab}
240 km	1.62 ± 0.12	6.90 ± 0.52	1.32 ± 0.12^{a}	136.34 ± 2.78^{c}	5.73 ± 0.22	8.89 ± 0.35^{a}
Crating densities (birds/crate)						
10 birds/crate	1.82 ± 0.20	7.43 ± 0.42	0.85 ± 0.09^{b}	179.23 ± 9.46	6.29 ± 0.25^{a}	7.99 ± 0.23
12 birds/crate	1.83 ± 0.18	7.17 ± 0.40	0.90 ± 0.07^{b}	171.77 ± 8.36	5.90 ± 0.15^{ab}	8.25 ± 0.35
15 birds/crate	1.88 ± 0.20	6.65 ± 0.74	1.16 ± 0.13^{a}	172.18 ± 7.00	5.55 ± 0.15^{b}	8.37 ± 0.30
P-value						
Distance	0.1830	0.6589	<0.0001	<0.0001	0.3746	0.0035
Density	0.9775	0.5914	0.0144	0.3241	0.0293	0.6300
Interaction	0.7546	0.3493	<0.0001	<0.0001	0.2608	0.0958

[a–c] Means in a row with no common superscript differ significantly at P ≤ 0.05.
[1]Values are least square mean ± standard error.
Crating density: Per crate 10 birds (0.050 m^2/bird), 12 birds (0.042 m^2/bird), and 15 birds (0.033 m^2/bird).

Table 4. Economic evaluation of broiler transportation for variable distances at different crating densities during extreme hot and humid weather in terms of Rupees (Rs.) in Pakistani currency (PKR) and US dollars (US $).

	Description of the most important cost related factors									
	Distances	80 km			160 km			240 km		
	Crating densities	10	12	15	10	12	15	10	12	15
	Fuel used (liters)	14.55	14.55	14.55	29.09	29.09	29.09	43.64	43.64	43.64
	Fuel price (Rs.PkR)	1018.18	1018.18	1018.18	2036.36	2036.36	2036.36	3054.55	3054.55	3054.55
A	Fuel cost (Rs.PKR) per kg live weight	0.39	0.33	0.26	0.79	0.65	0.52	1.18	0.98	0.79
	Live weight losses % on the bases of distance and densities	3.13	3.27	4.88	5.35	5.36	6.36	6.19	6.48	7.74
	Cost: Per kg liveweight (Rs.PKR)	125	125	125	125	125	125	125	125	125
B	Loss in terms of Rs.PKR	3.91	4.09	6.10	6.69	6.70	7.95	7.74	8.10	9.68
	Mortality %	1.00	1.67	3.33	2.00	4.17	12.67	7.00	9.17	14.50
	Weight loss on the bases of mortality	0.02	0.03	0.06	0.04	0.08	0.23	0.13	0.17	0.26
C	Loss in terms of Rs.PKR	2.25	3.76	7.49	4.50	9.38	28.51	15.75	20.63	32.63
	Carcass %	64.51	64.21	63.90	64.15	63.29	62.12	62.47	62.54	61.95
	Cost of meat at the rate of Rs. 220/kg	255.46	254.27	253.04	254.03	250.63	246.00	247.38	247.66	245.32
D	Loss in terms of Rs.PKR on the basis of carcass shrinkage	0.00	1.19	2.42	1.43	4.83	9.46	8.08	7.80	10.14
A+B+C+D	Total loss in terms of Rs.PKR on per kg basis	6.56	9.36	16.27	13.40	21.57	46.45	32.74	37.52	53.22
	Loss in terms of US $ @ Rs 160 per US $	0.04	0.06	0.10	0.08	0.13	0.29	0.20	0.23	0.33

to transportation distance and crating densities. The higher crating density and longer transportation distance in the present study combined to create heat stress in birds, which resulted in increased catalase activity. Lin et al. [42] also reported a significant increase in oxidative stress as a response to heat stress in broilers.

Results of the present experiment showed a significant ($P < 0.0001$) decrease in blood glucose concentration with the increase in transportation distance (Table 3). Probably, an increase in panting at high temperature with the increase in transportation length resulted in a decrease in metabolic rate and reduction in blood glucose level [41,47]. Furthermore, depletion of glycogen due to no access to feed in the long journey can also result in a reduction of blood glucose levels [48]. Similarly, Vošmerová et al. [24] also reported a decrease in blood glucose when transportation distance increased above 130 km, while the differences were nonsignificant up to 130 km. Accordingly, several studies have reported a decrease in blood glucose concentration particularly in birds subjected to long term transportation stress [23,49]. On the other hand, nonsignificant differences ($P = 0.3241$) were observed among different crating densities for blood glucose concentration (Table 3).

Serum total protein contents showed nonsignificant differences among different transportation distances ($P = 0.3746$), whereas significant ($P = 0.0293$) decreases in total protein contents were observed with each level of increase in crating density of birds (Table 3). These findings can be justified with the fact that an increase in crating density results in accumulation of more heat inside the crate, and heat stress in birds has been associated with an increase in corticosterone [50], which was reported to be a proteolytic enzyme resulting in decrease in protein and increase in creatinine, urea, and uric acid levels [51]. In accordance with the findings of the present study, some other studies [24,52] also reported a decrease in serum protein contents in birds subjected to transportation stress.

The serum uric acid concentration showed a significant ($P = 0.0035$) increasing trend with the increase in transportation distance (Table 3). Uric acid is considered as a potent scavenger of free radicals in humans and poultry [53,54]. Not only a potent antioxidant, uric acid has also been reported as the primary product of nitrogen metabolism in birds, and it is a reliable indicator of oxidative stress and tubular function of the kidneys [55]. The birds transported for 240 km had the highest stress levels, and it was reported that stress in poultry affects metabolism and results in increased in uric acid levels [56]. In accordance with the present study, an increase in the concentration of uric acid with the increase in transportation distance was also reported [18,22,24] in birds subjected to transportation. In the present study, different crating densities showed nonsignificant differences ($P = 0.6300$) for uric acid contents (Table 3). Likewise, Delezie et al. [18] also reported no effect of different crating densities during transportation on blood uric acid level.

Economic analysis showed an increase in losses with the increase in transportation distance and crating density (Table 4). Broiler transportation for 80 km at 10 birds per crate resulted in the lowest losses, while transportation for 240 km with 15 birds per crate resulted in huge losses. Although an increase in crating density resulted in a reduction in per bird fuel cost, the body weight loss, mortality, and carcass shrinkages due to stress associated with higher transportation distance and crating density resulted in huge financial losses. Thus, transportation for 80 km with 10 or 12 birds per crate seems ideal, while in the case of longer transportation distance (160 km), a crating density of only 10 birds per crate resulted in lower losses, while transportation of 240 km is not feasible at all during the hot summer season.

In conclusion, the current study demonstrated that transportation of broilers especially in summer (hot and humid) is associated with economic losses due to high live body weight shrinkage and dead on arrival rates. The transportation of broilers for 80 km with 10 or 12 birds per crate had minimum negative effects. The physiological response also showed induction of oxidative stress in birds above 160 km of transportation and crating density of 12 birds/crate or higher. Hence, it is recommended to have a relatively lower crating density with the lowest possible distances for optimum bird welfare and carcass characteristics in the hot and humid season.

Acknowledgments
The authors want to acknowledge Dr. Muhammad Imran (Institute of Biochemistry and Biotechnology, UVAS) and Mohsin Ali (Department of Physiology, UVAS) for their contributions in measuring blood biochemical profiles and physiological responses.

References

1. Solomon S, Qin D, Manning M, Chen Z, Marquis M et al. (editors). IPCC. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC). Cambridge, UK: Cambridge University Press; 2007.
2. Zahid M, Rasul G. Thermal classification of Pakistan. Atmospheric and Climate Sciences 2011; 1 (4): 206-213. doi: 10.4236/acs.2011.14023

3. Hunter RR, Mitchell MA, Matheu C. Mortality of broiler chickens in transit–correlation with the thermal micro-environment. In: Proceedings of the 6th International Symposium; Louisville, Kentucky, USA; 2001. pp. 542-549.

4. Yalçin S, Özkan S, Türkmut L, Siegel PB. Responses to heat stress in commercial and local broiler stocks. 1. Performance traits. British Poultry Science 2001; 42: 149-152. doi: 10.1080/00071660120048375

5. Simões GS, Oba A, Matsuo T, Rossa A, Shimokomaki M et al. Vehicle thermal microclimate evaluation during Brazilian summer broiler transport and the occurrence of PSE (pale, soft, exudative) meat. Brazilian Archives of Biology and Technology 2009; 52: 195-204. doi: 10.1590/S1516-89132009000700025

6. Spurio RS, Soares AL, Carvalho RH, Silveira V Jr, Grespan M et al. Improving transport container design to reduce broiler chicken PSE (pale, soft, exudative) meat in Brazil. Animal Science Journal 2016; 87: 277-283. doi: 10.1111/asj.12407

7. Mitchell MA, Kettlewell PJ. Welfare of poultry during transport: a review. In: Proceedings of 8th Poultry Welfare Symposium; Cervia, Italy; 2009. pp. 90-100.

8. Kannan G, Heath JL, Wabeck CJ, Souza MCP, Howe JC et al. Effects of crating and transport on stress and meat quality characteristics in broilers. Poultry Science 1997; 76: 523-529. doi: 10.1093/ps/76.3.523

9. Chauvin C, Hillion S, Balaine L, Michel V, Peraste J et al. Factors associated with mortality of broilers during transport. Animal 2011; 5 (2): 287-293. doi: 10.1017/S1751731110001916

10. Bianchi M, Petracci M, Cavani C. Effects of transport and lairage on mortality, liveweight loss and carcass quality in broiler chickens. Italian Journal of Animal Science 2005; 4 (Suppl. 2): 516-518. doi: 10.4081/ijas.2005.2s.516

11. Aral Y, Arıkan MS, Akın AC, Kuyululu CYK, Güloğlu SC et al. Economic losses due to live weight shrinkage and mortality during the broiler transport. Ankara Üniversitesi Veterinerlik Fakültesi Dergisi 2014; 61 (3): 205-210.

12. Vecerek V, Grbalova S, Voslarova E, Janackova B, Malena M et al. Effects of travel distance and the season of the year on death rates of broilers transported to poultry processing plants. Poultry Science 2006; 85: 1881-1884. doi: 10.1093/ps/85.11.1881

13. Arikan MS, Akin AC, Akcay A, Aral Y, Sariozkan S et al. Effects of transportation distance, slaughter age, and seasonal factors on total losses in broiler chickens. Brazilian Journal of Poultry Science 2017; 19 (3): 421-428. doi: 10.1590/1806-9061-2016-0429

14. Sowińska J, Wójcik A, Pomianowski JF, Chorąży Ł, Mituniewicz T et al. Effects of different variants of pre-slaughter transport on body weight loss and meat quality in broiler chickens. Medycyna Weterynaryjna 2013; 69 (7): 420-423.

15. Ulupi N, Aryani SS, Evni FT, Nugraha R. Effects of transportation duration on broiler chicken physiology and performance factors. International Journal of Poultry Science 2018; 17: 197-204. doi: 10.3923/ijps.2018.197.204

16. Whiting TL, Drain ME, Rasali DP. Warm weather transport of broiler chickens in Manitoba. II. Truck management factors associated with death loss in transit to slaughter. Canadian Veterinary Journal 2007; 48: 148-154.

17. Vieira FMC, Silva IJO, Barbosa Filho JAD, Vieira AMC. Reducing pre-slaughter losses of broilers: crating density effects under different lairage periods at slaughterhouse. Journal of Animal Behaviour and Biometeorology 2013; 1 (1): 1-6. doi: 10.14269/2318-1265.v01n01a01

18. Delezie E, Swennen Q, Buyse J, Decuypere E. The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. Poultry Science 2007; 86: 1414-1423. doi: 10.1093/ps/86.7.1414

19. Elrom K. Handling and transportation of broilers-welfare, stress, fear and meat quality. Part IV: Handling of broilers. Israel Journal of Veterinary Medicine 2000; 55: 121-125.

20. Nijdam E, Arens P, Lambooij E, Decuypere E, Stegeman JA. Factors influencing bruises and mortality of broilers during catching, transport, and lairage. Poultry Science 2004; 83: 1610-1615. doi: 10.1093/ps/83.9.1610

21. Voslarova E, Chloupek P, Vosmerova P, Chloupek J, Bedanova I et al. Time course changes in selected biochemical indices of broilers in response to pre transport handling. Poultry Science 2011; 90: 2144-2152. doi: 10.3382/ps.2011-01473

22. Yalçin S, Özkan S, Oktay G, Çabuk M, Erbayraktar Z et al. Age-related effects of catching, crating, and transportation at different seasons on core body temperature and physiological blood parameters in broilers. Journal of Applied Poultry Research 2004; 13: 549-560. doi: 10.1093/japr/13.4.549

23. Zhang L, Yue HY, Zhang HJ, Xu L, Wu SG et al. Transport stress in broilers: I. Blood metabolism, glycolytic potential, and meat quality. Poultry Science 2009; 88: 2033-2041. doi: 10.3382/ps.2009-00128

24. Vošmerová P, Chloupek J, Bedanova I, Chloupek P, Kruzikova K et al. Changes in selected biochemical indices related to transport of broilers to slaughterhouse under different ambient temperatures. Poultry Science 2010; 89: 2719-2725. doi: 10.3382/ps.2010-00709

25. Doktor J, Połtowicz K. Effect of transport to the slaughterhouse on stress indicators and meat quality of broiler chickens. Annals of Animal Science 2009; 9 (3): 307-317.

26. Hadwan MH, Abed HN. Data supporting the spectrophotometric method for the estimation of catalase activity. Data in Brief 2016; 6: 194-199. doi: 10.1016/j.dib.2015.12.012

27. SAS Institute. SAS User's Guide: Statistics, Release 9.4. Cary, NC, USA: SAS Institute; 2013.

28. Mitchell MA, Kettlewell PJ. Physiological stress and welfare of broiler chickens in transit: solutions not problems! Poultry Science 1998; 77: 1803-1814. doi: 10.1093/ps/77.12.1803

29. Savenije B, Lambooij E, Gerritzen MA, Venema K, Korf J. Effects of feed deprivation and transport on preslaughter blood metabolites, early postmortem muscle metabolites, and meat quality. Poultry Science 2002; 81: 699-708. doi: 10.1093/ps/81.5.699

30. Warriss PD, Wilkins LJ, Brown SN, Phillips AJ, Allen V. Defaecation and weight of the gastrointestinal tract contents after feed and water withdrawal in broilers. British Poultry Science 2004; 45: 61-66. doi: 10.1080/0007166041668879

31. Elsayed MA. **Effects of length of shipping distance and season of the year temperature stress on death rates and physiological condition of broilers on arrival to slaughterhouse. Journal of Nuclear Technology in Applied Science 2014; 2 (4): 453-463.**

32. Holm CGP, Fletcher DL. Antemortem holding temperatures and broiler breast meat quality. Journal of Applied Poultry Research 1997; 6 (2): 180-184. doi: 10.1093/japr/6.2.180

33. Simmons JD, Lott BD, May JD. Heat loss from broiler chickens subjected to various air speeds and ambient temperatures. Applied Engineering in Agriculture 1997; 13: 665-669. doi: 10.13031/2013.21645

34. Balnave D. Increased utilization of sensible heat loss mechanisms in high temperature, high humidity conditions. World's Poultry Science Journal 1998; 54: 69-72. doi: 10.1079/WPS19980006

35. Warriss PD, Bevis EA, Brown SN, Edwards JE. Longer journeys to processing plants are associated with higher mortality in broiler chickens. British Poultry Science 1992; 33: 201-206. doi: 10.1080/00071669208417458

36. Moran ET Jr, Bilgili SE. Influence of broiler live haul on carcass quality and further processing yields. Journal of Applied Poultry Research 1995; 4: 13-22. doi: 10.1093/japr/4.1.13

37. Farsaie A, Carr LE, Wabeck CJ. Mechanical harvest of broilers. Transactions of the American Society of Agricultural and Biological Engineers 1983; 26: 1650-1653. doi: 10.13031/2013.33818

38. Ekstrand C. An observational cohort study of the effects of catching method on carcass rejection rates in broilers. Animal Welfare 1998; 7: 87-96.

39. Mazanowski A. Czynniki wpływające na efektywność odchowu kurcząt brojlerów. Cz. IV (Profilaktyka odchowu). Polskie Drobiarstwo 1997; 5: 2-4 (in Polish).

40. Taylor NL, Fletcher DL, Northcutt JK, Lacy MP. Effect of transport cage height on broiler live shrink and defecation patterns. Journal of Applied Poultry Research 2001; 10 (4): 335-339. doi: 10.1093/japr/10.4.335

41. Sahin K, Sahin N, Kucuk O. Effects of chromium, and ascorbic acid supplementation on growth, carcass traits, serum metabolites, and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). Nutrition Research 2003; 23: 225-238. doi: 10.1016/S0271-5317(02)00513-4

42. Lin H, Decuypere E, Buyse J. Acute heat stress induces oxidative stress in broiler chickens. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 2006; 144 (1): 11-17. doi: 10.1016/j.cbpa.2006.01.032

43. Joiner WP, Huston TM. The influence of high environmental temperature on immature domestic fowl. Poultry Science 1957; 36: 973-978. doi: 10.3382/ps.0360973

44. Cadenas E, Davies KJA. Mitochondrial free radical generation, oxidative stress and aging. Free Radical Biology and Medicine 2000; 29 (3-4): 222-230. doi: 10.1016/S0891-5849(00)00317-8

45. Dröge W. Free radicals in the physiological control of cell function. Physiological Reviews 2002; 82: 47-95. doi: 10.1152/physrev.00018.2001

46. Ismail IB, Al-Busadah KA, El-Bahr SM. Oxidative stress biomarkers and biochemical profile in broilers chicken fed zinc bacitracin and ascorbic acid under hot climate. American Journal of Biochemistry and Molecular Biology 2013; 3: 202-214. doi: 10.3923/ajbmb.2013.202.214

47. Hilmann PE, Scott NR, Van Tienhoven A. Physiological responses, and adaptations to hot, and cold environments. In: Yousef MK (editor). Stress Physiology in Livestock. Boca Raton, FL, USA: CRC Press; 1985. pp. 1-71.

48. Warriss PD, Kestin SC, Brown SN, Knowles TG, Wilkens LJ et al. Depletion of glycogen stores and indices of dehydration in transported broilers. British Veterinary Journal 1993; 149 (4): 391-398. doi: 10.1016/S0007-1935(05)80078-8

49. Suchy P, Bedanova I, Vecerek V, Voslarova E, Pistekova V et al. Effect of transport stress and floor space reduction on selected biochemical indices in common pheasant (*Phasianus colchicus*). European Poultry Science 2007; 71: 56-61.

50. Kataria N, Kataria AK, Gahlot AK. Ambient temperature associated variations in serum hormones and interrelated analytes of broiler chickens in arid tract. Slovenian Veterinary Research 2008; 45: 127-133.

51. Khan WA, Khan A, Anjuman AD, Rehman ZU. Effects of induced heat stress on some biochemical values in broiler chicks. International Journal of Agriculture and Biology 2002; 4: 74-75.

52. Sarkar P, Ghosh S, Batabyal S, Chatterjee S. Biochemical stress responses of broiler chickens during transport. Indian Journal of Animal Research 2013; 47 (1): 29-34.

53. Becker BF. Towards the physiological function of uric acid. Free Radical Biology and Medicine 1993; 14 (6): 615-631. doi: 10.1016/0891-5849(93)90143-I

54. Simoyi MF, Van Dyke K, Klandorf H. Manipulation of plasma uric acid in broiler chicks and its effect on leukocyte oxidative activity. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 2002; 282 (3): 791-796. doi: 10.1152/ajpregu.00437.2001

55. Hartman S, Taleb SA, Geng T, Gyenai K, Guan X et al. Comparison of plasma uric acid levels in five varieties of the domestic turkey, *Meleagris gallopavo*. Poultry Science 2006; 85 (10): 1791-1794. doi: 10.1093/ps/85.10.1791

56. Siegel HS, Van Kampen M. Energy relationships in growing chickens given daily injection of corticosterone. British Poultry Science 1984; 25: 477-485. doi: 10.1080/00071668408454889