Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2020 / v.22 / n.2 / 001-010

http://dx.doi.org/10.1590/1806-9061-2019-1169

Original Article

Effect of Broiler Crating Density and Transportation Distance on Preslaughter Losses and Physiological Response During the Winter Season in Punjab, Pakistan

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Keywords

Cold stress, crating density, shrinkage, transportation, winter.

Submitted: 20/August/2019
Approved: 19/April/2020

ABSTRACT

A study was conducted to determine the impact of crating density and transportation distance on losses and physiological response of broilers during the winter season. For this, ROSS-308 broilers were crated at three densities (i.e., 10, 12, and 15 birds/crate) in plastic crates and transported in three distances (i.e., 80, 160, and 240 km) during the winter season at 3.6 – 9.5°C temperature and 63.3 – 78.8% relative humidity. Results showed that body weight loss increased significantly with the increase in transportation distance and decrease in crating density, whereas dead on arrival, physical injuries and bruises were not different among different transportation distance and crating density treatments. Significant reduction in carcass and breast yield was observed with the increase in transportation distance, whereas an increase in crating density above 12 birds *per* crate increased breast yield. Birds transported for 240 km had the highest serum catalase activity and the lowest rectal body temperature compared to other treatments. A gradual increase in crating density resulted in the increase in thyroxine concentration and reduction in rectal temperature. Serum glucose, albumin and uric acid concertation increased significantly after 160km of transportation, but serum triglyceride contents decreased. Moreover, an increase in crating density also increased serum glucose and triglyceride. Results suggested that longer transportation distance during the winter season increased body weight shrinkage with a reduction in carcass and breast yield, and the impact of losses and stress was greater in treatments having a lower number of birds in crates.

INTRODUCTION

The journey of broilers from farms to the processing plant, after a relatively comfortable environment of a poultry house, is associated with multi-dimensional stressors (Gregory, 2010). Genetic improvements have enabled broiler to gain maximum weight at a relatively shorter time, but it also increased the sensitivity of the birds particularly for thermal variations (Burkholder *et al.*, 2008). The transportation of broilers has been reported to induce immediate stress in swine and poultry and the reaction of the animal to stress is dependent on the duration and intensity of the stress, their physiological status, and environment after stress (Brown *et al.*, 1998; Fazio & Ferlazzo, 2003). In the case of transportation stress, the intensity of stress depends on environment during transportation, length of transportation journey and duration of the bird being inside the crates (Yalçin *et al.*, 2004). In broiler, heat stress has always been a point of concern for the producers, but considerable data reveals that the cold stress is equally detrimental for the health and welfare of poultry (Aarif *et al.*, 2013; Phuong *et al.*, 2016). Transportation at low environmental temperature can cause hypothermia and can affect performance and welfare of broilers

(Warriss *et al.*, 2005; Qi *et al.,* 2017). The cold stress has been reported to activate the hypothalamic-pituitary-thyroid axis of the bird and increases corticosterone and cortisol concentration (Aarif *et al.*, 2013; Slota *et al.*, 2015).

The stress associated with transportation can cause dead-on-arrival (DOA) in broilers depending on transportation distance and ambient temperature (Elsayed, 2014). Contrarily, Vošmerová *et al.* (2010) reported more stress in short distance transported birds due to the lack of post crating recovery period. While Silva *et al.* (2011) did not find any effect of short distance transportation (up to 57 km) during the winter season on DOA. Not only mortality but an increase in the length of journey has been reported to increase body weight loss along with a significant increase in injuries, broken wings, and condemn carcass percentage and a decrease in the proportion of carcass cut up organs in broilers (Moran & Bilgili, 1995; Carlyle *et al.*, 1997; Bianchi *et al.*, 2005).

Broilers are usually transported to processing plants in loose crates and trucks without any environment control system. Welfare concerns arises with this system as the birds suddenly shifts from the nearly ideal environment of poultry farm to environmental extremes without proper protection (Weeks, 2014). The lack of controlled ventilation in open trucks hinders air flow and lead to building different temperature zones within a truck (Knezacek *et al.*, 2010). Crating density can by hypothesized as the major factor determining the microclimate of the vehicle and its effect varies with the length of journey in different seasons (Whiting *et al.*, 2007). Data regarding the effect of crating density on pre-slaughter losses is scarce. A few studies conducted regarding the effect of crating density has been associated with the changes in physiological response (Delezie *et al.*, 2007; Vieira *et al.*, 2013). So, crating density, in countries where winter temperature is low, need to be optimized. Since transportation stress cannot be eliminated in poultry production, therefore, the study was planned with the objective to assess preslaughter losses and physiological response in broilers at different transportation distances under different crating densities in the winter season.

MATERIALS AND METHODS

Birds and their treatments

The experiment was conducted at the Department of Poultry Production, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. In this experiment, ROSS-308 broilers (straight run, body weight 1900 – 2050g, age: 35 days) were picked at random from a commercial broiler farm after 4 hours of feed withdrawal before catching. The birds were separated into nine treatment groups (03 distances × 03 crating densities). Transportation distance was categorized as short (80 km), medium (160 km), and long (240 km) under three crating densities: Low = 10 birds (0.050 m^2/ bird), Medium = 12 birds (0.042 m^2/ bird), and high = 15 birds (0.033 m^2/ bird) *per* crate. To reduce the effect of the truck microenvironment, each treatment was replicated 10 times with individually tagged birds in each crate placed at various locations in the truck. The birds were transported in commercial trucks (capacity 144 crates) carrying different crating densities in loose plastic crates (Engi Plastic Industries Pvt. Ltd., Sundar, Pakistan) having the dimensions of 0.91 m (length) × 0.55 m (width) × 0.31 m (height) at an average speed of 45-50 km/h during late night – early morning. The experimental birds were weighed and tagged on shanks before being placed in the crates. During growing phase, the birds were placed on litter floor (rice husk) in environmentally controlled commercial broiler farms (0.06 m^2/bird) under standard temperature and relative humidity conditions. The birds were fed with *ad-libitum* diets (strain specific broiler ration) and fresh water and light was available for 24 h. All the procedures and bird handling protocols were used according to guidelines and standards after approval from the Ethical Review Committee of UVAS, Lahore (Approval No: DR/916-2017).

Climatic indices

The experiment was conducted during the winter season and the birds were transported in the vicinity of the districts of Kasur and Lahore, Punjab, Pakistan. The temperature and relative humidity were recorded with the help of a digital weather tracker (Kestrel 4500 NV, Nielsen-Kellerman, USA). The range of temperature and relative humidity measured at various distances and time ranged from 3.6 – 9.5ºC and 63.3 – 78.8%, respectively. During the journey, there were patches of mild to severe fog at several places. The journeys to be traveled were of 240 km, 160 km, and 80 km and started at 12:25 AM, 02:45 AM, and 04:30 AM, respectively, and all the trucks reached the slaughtering facility at almost 07:00 AM.

Parameters studied

Transportation losses

Body weight loss (%) during the transportation was measured as the difference in bird weight at the farm (g) and final body weight (g) measured at the ultimate destination. The dead-on-arrival (DOA) percentage for each group was calculated by dividing the number of birds found dead by the total of birds in the experimental crates. To calculate physical injuries (%), every single bird from each crate was observed and physical injuries (if any) were recorded and the proportion was calculated from total birds. Similarly, bruises on wings, breast, and legs (if any) were visibly observed and their proportion with the total number of birds slaughtered *per* replicate was calculated.

On arrival at the slaughtering facility, 3 tagged birds from each replicate were randomly picked and slaughtered manually with a knife. After bleeding, the birds were defeathered and eviscerated. Dressing (%) was calculated as hot carcass with neck and without giblets. The percentage of abdominal fat pad was calculated as weight abdominal fat relative to live body weight (g) while breast (%) was calculated relative to carcass weight.

Physiological Response

On arrival at the slaughtering facility, rectal body temperature was recorded randomly from each treatment with the help of digital thermometer (NB-100, Ossmax, Germany). The tagged birds were separated from the other birds weighed and slaughtered manually. To reduce the effect of stay before slaughtering, all the birds were divided into 6 slaughtering stations and all treatments were slaughtered at same time randomly. The blood was collected from the jugular vein of 10 birds from each treatment in plain vacutainers. Vacutainers were then centrifuged for 10 minutes at 3000 rpm and serum was carefully separated with a sterilized pipette into labeled Eppendorf tubes. Serum samples were stored at -20 °C for later blood biochemical analysis. The samples were maintained at 3-8 °C while handling.

The estimation of serum triiodothyronine (T3), and thyroxine (T4) were made using the enzyme immune assay test kit of BioCheck, Inc. (Catalog #: BC-1005 and BC-1007). Whilst, the Cortisol was measured with the help of ELISA Kit of Calbiotech Inc. (Catalog #CO103S). The catalase activity was estimated using the methodology of Hadwan & Abed (2016). Briefly, 4 test tubes (sample, control test, standard and blank) were prepared. The sample tube contained 100 µl serum and 1000 µl hydrogen peroxide (20mM), the control test tube contained 100 µl serum and 1000 µl distilled water, the standard tube contained 100 µl distilled water and 1000 µl hydrogen peroxide, and the blank contained 1100 µl distilled water. All these tubes were vortexed and incubated at 37 °C for 3 min, after that 4000 µl ammonium molybdate (32.4mmol/l) was added to stop the reaction. Later changes in absorbance were recorded at 374 nm against the reagent blank.

The samples were analyzed for serum total proteins (g/dL) and albumin (g/dL) contents using immunoassay kit of Tron® (3-4 Rue De Montolembert, Paris, France); while serum glucose (mg/dL), triglycerides (mg/dL), and uric acid (mg/dL) were analyzed using Human® (Human Gesellschaft für Biochemia und Diagnostica mbH, Wiesbaden, Germany) kits with spectrophotometer (UV/VIS Spectrophotometer AMV 09 Tlead Int. Co. Ltd., Qingdao, Shangdong, China) at kit recommend wavelength.

Statistical analysis

The data collected were analyzed by two-way analysis of variance (ANOVA) technique under Completely Randomized Design assuming transportation distance and crating density as the main effect. The significant means were separated using Tukey's HSD test (Tukey, 1953), considering significance level at $p \leq 0.05$, with the help of SAS 9.4 (SAS Institute, 2013).

RESULTS AND DISCUSSION

Transportation of animals is a necessary step in meat processing value chain. Along with other preslaughter handling factors, however, transportation can impact bird welfare and, in some cases, lead to economic losses (Vieira *et al.*, 2011). There was a significant increase in BWL (%) with the increase in transportation distance (Table 1). The average BWL (%) in broilers traveled for 80, 160, and 240 km was 1.99, 2.55, and 3.65%, respectively. Longer transportation times are associated with longer fasting periods in broilers (Savenije *et al.*, 2002; Delezie *et al.*, 2007) and higher exposure to stressful conditions especially in terms of thermal variations, crating, acceleration, vibration, noise, feed and water deprivation and social disruption (Kannan *et al.*, 1997; Mitchell & Kettlewell, 1998; Fletcher, 2002). Similarly, Bianchi *et al.* (2005) investigated the effect of broiler transportation for ≤3.5 h, 3.5-5 h, >5 h in Bologna, Italy from September 2002 to May 2003 (321 flocks) and reported 0.24, 0.41, 0.45% BWL. The study reported an average BWL of 321 flocks, so, the BWL in that study was relatively lower than in the present study, moreover, higher BWL in the present study could be associated with the lower temperature than the previous study, poor road conditions, differences in the length of transportation and differences in the

Table 1 – Live bird losses and carcass yield as affected by transportation distance and crating density.

Treatments	BWL (%)	DOA (%)	Breast bruises (%)	Wing bruises (%)	Carcass (%)	Breast (%)	Abdominal Fat (%)
Transportation Distance (Km)							
80 km	1.99±0.12[c]	0.00±0.00	2.22±1.54	0.00±0.00	65.40±0.11[a]	28.79±0.32[a]	1.83±0.08
160 km	2.55±0.14[b]	0.33±0.33	3.33±1.86	1.11±1.11	64.77±0.10[b]	28.32±0.37[ab]	1.72±0.10
240 km	3.65±0.13[a]	0.94±0.53	6.67±2.48	3.33±1.86	64.20±0.13[c]	27.51±0.32[b]	1.60±0.07
Crating densities (birds/crate)							
Low	3.24±0.15[a]	1.00±0.56	6.67±2.48	3.33±1.86	64.94±0.13	27.40±0.33[b]	1.65±0.11
Medium	2.96±0.14[a]	0.28±0.28	4.44±2.10	1.11±1.11	64.75±0.11	28.25±0.31[ab]	1.76±0.07
High	1.98±0.12[b]	0.00±0.00	1.11±1.11	0.00±0.00	64.69±0.13	28.97±0.36[a]	1.74±0.08
p-value							
Distance	<0.0001	0.1754	0.2742	0.1634	<0.0001	0.0220	0.1591
Density	<0.0001	0.1326	0.1230	0.1634	0.2516	0.0040	0.6132
Interaction	0.0974	0.5915	0.9818	0.6204	<0.0001	0.3971	0.5331

[a-c] Means in a column 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: Low = 10 birds/crate (0.050 m^2/ bird), 12 birds/crate (0.042 m^2/ bird), and 15 birds/crate (0.033 m^2/ bird)

crating densities in both studies. BWL (%) remained comparable ($p>0.05$) in birds transported at low and medium crating density, while a significant decrease in BWL (%) was observed in birds transported at high crating densities (15 birds/crate) compared to other treatments. Higher BWL (%) in birds crated at lower crating densities (i.e., 10 birds *per* crate) could be attributed to relatively higher struggle and enhanced metabolic activity to cope with lower environmental temperature (Dadgar *et al.*, 2012). Other groups, however, have demonstrated that more birds in a crate generated enough metabolic heat that they survived better in Canadian winter temperatures of -5, -10, and -15°C (Strawford *et al.*, 2011).

No differences in dead on arrival (DOA) and bruising percentage (breast and wing bruises) were observed among different transportation distance and crating density treatments (Table 1). These findings suggested that the stress associated with winter transportation was bearable by the birds. The birds transported for 240 km or those transported at low crating densities (10 birds *per* crate) showed 0.94 and 1% DOA, respectively; but the differences were not significant ($p>0.05$) among different treatments (Table 1). However, these little margins can result in significant variations in terms of profit margin. In accordance, Silva *et al.* (2011) reported no effect of 57 km transportation on DOA (%) during the winter season in Londrina, Brazil, while Chauvin *et al.* (2011) also reported no effect of transportation on mortality rates in France at an average 13 °C temperature. High mortality in birds transported at low crating density suggested that the DOA during the winter season was more associated with the chilling effect in birds, produced due to hypothermia, in trucks having lower number of birds *per* crate (Watts *et al.*, 2011). Similarly, another study reported that mortality at very low ambient temperature (below -14 °C) was higher if the birds were placed at low (<40 kg/m^2) crating density as compared to the birds at higher (40-45 kg/m^2) crating density in Canada (Caffrey *et al.*, 2017). Fewer birds *per* crate translate to more space for birds, which could result in relatively higher chances of bruising and injuries during jerks and jolts of transportation (Elrom, 2000). The bruising (%) in the present study remained comparable to that of Jacobs *et al.* (2017), who reported 3.65 % bruised breast and wings and 1.38 % bruised leg in birds after transportation. However, some studies also reported bruises % ranges from 0.022 to 25% in broilers (Farsaie *et al.*, 1983; Ekstrand, 1998).

Broilers transported for 80 km had the highest carcass and breast (%) while significant reduction was observed with each level increase in transportation distance (Table 1). Similarly, breast (%) was increased significantly with the increase in crating density but abdominal fat (%) was neither affected ($p>0.05$) by different transportation distance nor by different crating densities (Table 1). A higher dressed carcass (%) in birds transported for short distances and subsequent decrease with the increase in transportation distance could be due to a reduction in the share of viscera and other inedible portions for 80 km traveling (Table 1). Kim *et al.* (2007) reported a significant decrease in the share of small intestine after 3 hours of feed withdrawal. Similarly, in the 80 km treatments, to cope with the cold environment the metabolism gets faster and resulted in faster emptying of the intestine as compared to the rest of the treatments; thereby, increasing the relative share of the dressed carcass. While the further reduction in carcass (%) with an increase in distance could possibly be attributed to shrinkage of the carcass as the birds were feed 4 hours before catching and up till slaughtering the total withdrawal time reached to 8 – 11.5 hours. In accordance, Kim *et al.* (2007) also reported a significant decrease in carcass yield after 6 hours of the feed withdrawal period. Similarly, Mazanowski (1997) also reported a significant decrease in carcass weight when weight loss during transportation exceeds 3% and weight loss in the present study for broiler transported for 160 and 240 km were 2.55, and 3.65%, respectively. Although the percentage of different losses during transportation was less; these small values can combine to cause severe economic losses to the processor as well as severely affect animal welfare (Ali *et al.*, 2008; Chauvin *et al.*, 2011). Data regarding the effect of crating density on carcass characteristics is scarce and no study has been reported on this aspect.

Rectal body temperature was found to be higher in birds transported for 80 and 160 km while a significant decrease was observed when transportation distance increased up to 240 km (Table 2). The finding could be associated with relative decrease in the rate of metabolism with the length of the journey and the increase in fasting period (Delezie *et al.*, 2007). Broilers with slower metabolism were unable to regulate their body temperature, particularly those subjected to prolonged exposure (as in longer transportation) to a low ambient temperature of up to -18°C (Watts *et al.*, 2011). Similarly, Hunter *et al.* (1999) reported 2.1°C and 10°C drop in rectal temperature of the birds exposed to 12°C and from 0 to 4°C ambient temperature during transportation. Accordingly, Dadgar *et al.* (2012) reported a decrease in body temperature in birds exposed to low ambient temperature (i.e. -15, -12, -9, and +22 °C). Results also showed that the crating of broilers at high crating density (15 birds *per* crate) resulted in higher rectal temperature compared to those transported at low and medium crating densities (Table 2). Similar findings were also previously reported by Delezie *et al.* (2007), but the study was conducted during the hot season and no such study was previously conducted during the winter season.

Results regarding physiological response showed that broiler transportation during the winter season did not affect (p>0.05) serum triiodothyronine (T3) and serum thyroxine (T4) contents (Table 2). Whereas, a significant (p<0.05) increase in serum catalase concentration was observed in the broilers transported for 240 km as compared to other treatments (i.e., 80 and 160 km). Similarly, serum T3 and catalase contents were not affected by different crating densities, but serum T4 concentration decreased significantly (p<0.05) with the increase in crating density (Table 2). Catalase is an antioxidant enzyme, and its activity is a measure to assess the oxidative stress on the living organism (Cadenas & Davies, 2000). Higher catalase activity in longer transported broilers could be due to increase in production of free radicals in birds subjected to longer stress periods (Ismail *et al.*, 2013). No study has been conducted till date regarding serum catalase activity in response to transportation distance and crating densities. Similarly, Doktor & Połtowicz (2009) reported no effect of broiler transportation on T3 concentration compared to non-transported birds. Different crating densities had significant differences in T4 contents, while T3 contents and catalase activity

Table 2 – Physiological response and blood metabolites under the influence of transportation distance and crating densities.

Treatments	Rectal temp. (°F)	T3 (ng/mL)	T4 (µg/dL)	Catalase (kU/L)	Glucose (mg/dL)	Total Protein (g/dL)	Albumin (g/dL)	Uric Acid (mg/dL)	Triglyceride (mg/dL)
Transportation distance (km)									
80 km	105.19±0.11[a]	3.29±0.30	3.17±0.12	0.08±0.010[b]	189.22±2.45[ab]	4.46±0.19	2.67±0.11[b]	4.98±0.12[b]	111.31±2.55[a]
160 km	105.00±0.08[a]	2.80±0.18	3.70±0.14	0.08±0.004[b]	185.60±2.79[b]	4.87±0.23	2.75±0.08[b]	5.20±0.15[b]	105.65±1.79[a]
240 km	104.58±0.10[b]	3.12±0.24	3.56±0.14	0.11±0.009[a]	196.07±3.77[a]	5.25±0.23	3.33±0.15[a]	5.81±0.17[a]	99.16±2.09[b]
Crating densities (birds/crate)									
Low	104.70±0.09[b]	2.99±0.27	3.52±0.15[a]	0.09±0.009	179.67±1.24[c]	4.62±0.19	2.82±0.09	5.47±0.15	98.04±1.87[c]
Medium	104.91±0.12[ab]	3.04±0.26	3.49±0.13[ab]	0.08±0.007	190.68±2.38[b]	4.77±0.18	2.94±0.13	5.38±0.19	105.66±2.31[b]
High	105.16±0.09[a]	3.18±0.22	3.41±0.14[b]	0.09±0.010	200.54±3.72[a]	5.20±0.30	2.98±0.18	5.13±0.13	112.42±1.99[a]
p-value									
Distance	<0.0001	0.3825	0.0169	0.0381	0.0123	0.0573	0.0008	0.0007	<.0001
Density	0.0050	0.8643	0.8284	0.7216	<.0001	0.1827	0.6295	0.2638	<.0001
Interaction	0.6605	0.7236	0.1476	0.6708	0.0984	0.8964	0.7742	0.8551	0.2614

[a-c] Means in a column with no common superscript differ significantly at $p \leq 0.05$.
[1]Values are least square mean ± standard error
T3: Triiodothyronine, T4: Thyroxine, temp: temperature
Crating density: Low = 10 birds/crate (0.050 m^2/ bird), 12 birds/crate (0.042 m^2/ bird), and 15 birds/crate (0.033 m^2/ bird).

were not affected by different crating densities (Table 2). It is well established that thyroid hormones status correlates with body weight and energy expenditure (Fox *et al.*, 2008). These hormones influence key metabolic pathways that control energy balance by regulating energy storage and expenditure (Cheng *et al.*, 2010). The activation of the thyroid will convert T4 into T3 to regulate metabolism (Mullur *et al.*, 2014). In accordance, the transportation of broiler at low crating density resulted in an increase in T4 concentration (Table 2) to help sustain body temperature during winter transportation.

Significantly higher glucose concentration in birds transported for 240 km compared to rest of the treatments were observed (Table 2). Birds transported for 80 km had higher glucose concentration than those transported for 160 km (Table 2). Blood glucose has been considered as cellular fuel and studies have reported an elevation in plasma glucose concentration with the increase in transportation length or stress (Kent & Ewbank, 1986), primarily due to glycogen breakdown in the liver (Mayes, 1996). Similarly, Yalçin & Güler (2012) also reported a significant increase in blood glucose concentration with the increase in transportation distance (65 – 165 km) in a comprehensive study of 27 flocks (eliminating the effect of seasons). In accordance, Scope *et al.* (2002) also reported a significant increase in blood glucose concentration in racing pigeons after 3 h transportation. An increase in the serum glucose contents was also observed with the increase in number of birds *per* crate (Table 2). The increase in blood glucose levels with the increase in crating density could be due to reduced utilization of glucose at higher crating densities (Zhao *et al.* 2012). The birds kept at higher crating densities have relatively higher microclimate temperature due to the accumulation of metabolic heat, thereby they showed less struggle for maintaining temperature at low ambient temperature i.e., up to -18^{o}C (Watts *et al.*, 2011). Similarly, some studies also reported a decrease in blood glucose at low ambient temperature (up to -14 °C) (Dadgar *et al.*, 2012) and during winter season (16-18 °C) as compared to summer season (28-32 °C) in Turkey (Yalçin *et al.*, 2004).

No differences (p>0.05) in serum total protein contents could be observed in birds after different transportation distance and under different crating densities (Table 2). In accordance with the present study, Sarkar *et al.* (2013) also reported no effect of transportation distance on total proteins contents when compared with a non-transported treatment group at 24±0.5 °C and 62% relative humidity. In a study on pheasants, Suchy *et al.* (2007) also reported non-significant difference in the total protein contents between low or high crating densities. While Voslarova *et al.* (2011) also reported no effect of different crating durations on total protein contents. Serum Albumin contents were higher (p<0.05) in birds transported for 240 km as compared to those transported for 80 and 160 km (Table 2). The increase in albumin contents could be due to dehydration associated with longer journeys (Ghanem *et al.*, 2008). Similarly, other studies (Yalçin *et al.*, 2004; Yalçin & Güler, 2012) have also reported an increase in blood albumin contents after an increase in transportation distance under various temperatures in Turkey.

Serum uric acid contents were significantly higher in birds transported for 240 km compared to those transported for 80 and 160 km. No difference in uric acid contents were observed between 80 and 160 km transportation. Similarly, uric acid contents were not affected in different crating densities (Table 2). Uric acid has been reported as one of the major antioxidants in body; an increase in its concentration can be attributed with stress (Simoyi *et al.*, 2002; Hartman *et al.*, 2006). While Sturkie (2000) suggested that the circulating uric acid is a measure for protein catabolism in birds. As it was already discussed that the low-temperature transportation triggers gluconeogenesis, which converts amino acids to glucose and produces uric acid as a waste product, thus resulting in an increase in the uric acid concertation in longer transported (240 km) broilers (Siegel & Van Kampen, 1984; Malheiros *et al.*, 2003). Reviews regarding the effect of winter transportation on uric acid concentration is scarce. Some studies reported an average increase in uric acid contents in different seasons and transportation under different crating densities was also reported in many studies previously (Yalçin *et al.*, 2004; Delezie *et al.*, 2007). Similarly, Elsayed (2014) also reported a significant increase in uric acid with transportation, but within transportation distances of 15 – 150 km the differences remained non-significant. Similarly, no effect of crating and transportation of broiler were observed on uric acid contents (Sarkar *et al.*, 2013).

Serum triglyceride (TGL) contents were found to be higher in birds transported for low and medium distances, whereas significant reduction in TGL levels was observed in longer (240 km) transported birds (Table 2). Moreover, the birds subjected to transportation with the low crating density had low levels of TGL as compared to those transported at higher stocking densities (Table 2). Probably, winter transportation triggers metabolic heat production to maintain body temperature and results in hypoglycemia and decrease in TGL contents with the increase in transportation distance (Strawford *et al.*, 2011; Vošmerová *et al.*, 2010; Elsayed, 2014). Moreover, the length of feed withdrawal due to an increase in the length of the journey also caused negative energy balance and stress in birds during transportation that triggers the glycogen breakdown (Nijdam *et al.*, 2005). In accordance with the present study, Vošmerová *et al.* (2010) also reported a significant increase in TGL contents from 10 to 70 km but after that, the TGL contents decreased significantly. The differences in both studies could be attributed to the use of three temperatures in previous studies while in the present study the birds were transported at low temperatures only. Similarly, studies conducted in India (Sarkar *et al.*, 2013) and Egypt (Elsayed, 2014) also reported a significant decrease in TGL contents in broiler subjected to transportation as compared to the control group. Moreover, Voslarova *et al.* (2011) also reported a significant decrease in triglyceride contents with the increase in crating time up to 2 h. However, the study was conducted at relatively higher temperatures than the present study. Most of the studies related to transportation distance and its effect on blood biochemical profile were conducted in countries with mild or hot temperature whilst the data regarding effect of different transportation distance and crating densities on blood biochemical profile during winter season is scarce.

The economic analysis showed transportation of 10 birds *per* crate for 240 km resulted in the maximum losses followed by those transported for 240 and 160 km with 12 and 10 birds *per* crate respectively (Table 3). Transportation for 80 km distance showed the least losses compare to those transported for 160 and 240 km. Regarding crating densities, placing 15 birds *per* crate for 160 and 240 transportation distance showed lower losses compared to placing 10 and 12 birds *per* crate for the same distance. However, in broiler transported for 80 km, keeping 10 birds *per* crate resulted in the least losses while losses increased with the increase in the number of birds *per* crate, but the difference between 12 and 15 birds *per* crate was marginal (Table 3). The table also showed that, excluding carcass (%), birds transported for 80 km with 15 birds *per* crate crating density showed the least losses during the winter season. These findings can be important from a middleman or transporter perspective, who only transport broilers from farm to processing plants in Pakistan.

Table 3 – Economic evaluation of broiler transportation for variable distances at different crating densities during winter season in terms of Rupees (Rs.) in Pakistani currency (PKR) and US dollars (US $).

	Treatments								
Distance (km)	80 km			160 km			240 km		
Crating densities (birds *per* crate)	15	12	10	15	12	10	15	12	10
Fuel used (Liter) ~approx.	16.00	16.00	16.00	32.00	32.00	32.00	48.00	48.00	48.00
Total Fuel cost (Rs. PKR)	1440	1440	1440	2880	2880	2880	4320	4320	4320
A: *Per* bird fuel cost (Rs. PKR)	0.37	0.46	0.56	0.74	0.93	1.11	1.11	1.39	1.67
Live weight losses (%)	1.56	2.05	2.35	1.71	2.92	3.01	2.68	3.91	4.37
Cost: *Per* kg liveweight (Rs. PKR)	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00	95.00
B: Loss in terms of shrinkage (Rs. PKR)	1.48	1.95	2.23	1.62	2.77	2.86	2.55	3.71	4.15
Mortality	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.83	2.00
Weight loss on the basis of mortality	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.04
C: Loss in terms of mortality (Rs. PKR)	0.00	0.00	0.00	0.00	0.00	2.25	0.00	1.87	4.50
Carcass %	64.78	65.09	66.34	64.96	64.92	64.44	64.32	64.25	64.03
Total Cost of meat (Rs. 180 *per* kg market rate) (Rs. PKR)	209.9	210.9	214.9	210.5	210.3	208.8	208.4	208.2	207.5
D: Losses on the basis of carcass shrinkage (Rs. PKR)	5.05	4.05	0.00	4.47	4.60	6.16	6.54	6.77	7.48
Total Losses (Rs. PKR) (A+B+C+D)	6.91	6.46	2.79	6.84	8.30	12.38	10.20	13.74	17.80
Loss in terms of US$ @ Rs 155 *per* $	0.04	0.04	0.02	0.04	0.05	0.08	0.07	0.09	0.11

CONCLUSION

The current study demonstrated that transportation of broiler is associated with economic losses due to live body weight shrinkage, mortality and bruising of the carcass. 80 km transportation with 15 birds *per* crate resulted in lower live losses, however, distance up to 160 km during the winter season also showed comparable results. Transportation of broilers also resulted in a disturbance in blood metabolites levels, but it did not result in irregularity of body physiological homeostasis till 160 km of transportation.

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