SUMMARY
Transportation is a major component of the global commercial poultry production system and it can lead to various levels of stress in chickens, even under optimal conditions. It can cause harm ranging from slight disorders to death. Birds that die between loading at the farm and slaughtering are described as ‘dead on arrival’ (DOA). DOA is an important indicator of animal welfare and financial losses. It can be influenced by various factors such as ambient temperature, stocking density in crates, transport distance, lairage time in the holding barn and slaughter age. The aim of this survey was to determine the effects of some factors on the incidence of the phenomenon ‘dead on arrival’ in broiler chickens under commercial transport conditions. This survey was carried out in a commercial slaughterhouse on the basis of data for 4,062 transfers and 12,723,444 broilers under commercial conditions during 2018. The data related to slaughter age, transport distance, lairage duration, ambient temperature at the slaughterhouse and the incidence of DOA of broiler chickens was recorded by staff throughout the study. Slaughter age was divided into four groups (up to 39 days, 40 to 42 days, 43 to 44 days, 45 days or more); lairage duration was divided into five groups (up to 60 min, 61 to 120 min, 121 to 180 min, 181 to 240 min, 241 min or more); transport distance was divided into four groups (up to 15 km, 16 to 50 km, 51 to 101 km, 100 to 200 km) and ambient temperature at the slaughterhouse was divided into six groups (–5°C to 0°C, 0.1°C to 5°C, 5.1°C to 10°C, 10.1°C to 15°C, 15.1°C to 20°C and 20.1°C to 28°C).

Across the present study, the overall DOA rate was 0.389%. The effects of slaughter age, transport distance, lairage duration and temperature on the DOA rate were all significant (P<0.001). The DOA rate at ≤39 days slaughter age was higher than that of the other groups (P<0.001). The DOA rate for transport distance up to 15 km (0.448%) was higher than that for the other distance intervals (P<0.001). As the transport distance increased, the DOA rate usually increased (P<0.001). In addition, there was a positive relationship between DOA rate and lairage duration (P<0.001). As the lairage duration increased, the DOA rate increased. In addition, the DOA rate was highest (0.622%) at cold ambient temperatures (–5°C - 0°C) and lowest at 5.1°C to 10°C (0.334%).

In conclusion, the results of the current study regarding DOA rates clearly showed that short or long distance transport and long lairage duration were extremely detrimental to the health of broiler chickens. It is therefore important to avoid long or short distance transportation and long lairage duration, especially in adverse environmental conditions such as sub-zero and high temperatures. In addition, broiler chickens of up to 40 days of age were more susceptible to pre-slaughter stress than other ages. Therefore, more attention should be paid to the management of broilers in this age range in the pre-slaughter period.

KEY WORDS
Animal welfare, dead on arrival, broiler chicken, transport, ambient temperature.

INTRODUCTION
Transportation is a major element of the global commercial poultry production system, and can induce stress in chickens, even under optimal conditions, with harm ranging from slight stress to death. The death of broiler chickens may happen after loading at the farm, during transportation to the slaughterhouse or during lairage in a holding barn. The death of birds between the catching stage at the farm and unloading from their crates at the slaughterhouse is described as ‘dead on arrival’ (DOA), which is an important indicator of both animal health and welfare. Chickens in the DOA category also represent a financial loss to the producer because they are unacceptable for human consumption and are therefore condemned. The DOA rate may be affected by various factors such as ambient temperature, stocking density in crates, transport distance, lairage duration in holding barn and slaughter age. Extreme ambient temperatures and humidity are major reasons for mortality; 40% of DOA was associated with heat or cold stress. The effects of ambient temperature and relative humidity may vary according to season, stocking density in crates, design of vehicle and conditions in the holding barn. The DOA rate has been reported as 0.15% by Mitchell, by Petracci et al. as 0.35%, by Nijdam et al. as 0.46% and by Ritz et al. as 0.68%.

Earlier studies have investigated the effects of one or two factors on DOA in broiler chickens. In addition, these studies were carried out with small numbers of individuals in the groups. In contrast, the current study, which was carried out on an extremely large number of animals, investigated...
the effects of four factors on DOA rate simultaneously. In specific terms, the aim was to determine the effects of some factors on DOA in broiler chickens under commercial transport conditions.

MATERIALS AND METHODS

The survey was carried out in a commercial slaughterhouse and covered 4,062 transfers and 12,723,444 broilers under commercial conditions during 2018. The data related to the crating, transport and lairage at the slaughterhouse and the DOA rate of broiler chickens were recorded by staff during the course of the study. Feeding at the rearing farms was withdrawn 8 hours before the loading of all flocks. The Ross 308 broiler chickens were manually caught and loaded into plastic crates on the trailer by three staff members. The dimensions of the crates were length (80 cm) × width (45 cm) × height (30 cm). Stocking densities in the crates for all transfers were within the range recommended by the FAWC42. The type of the trailers was similar. The top, front and rear of the trailers were closed but the sides were open throughout the year. The trailers were transported by road to the slaughterhouse after loading. The broiler chickens were transported from different locations to the slaughterhouse (latitude 41°03’ N, longitude 36°05’ E and 514 m above sea level) in the city of Samsun, Turkey. The slaughtering period started at 11:30 p.m. and finished at 8:00 a.m., according to the workload. The vehicles were weighed on arrival at the slaughterhouse. The total weight of the broiler chickens was divided by their total number to obtain the mean live weight of a broiler chicken. When the vehicles loaded with broilers arrived at the slaughterhouse, they were parked in a holding barn for lairage. The lairage duration was different for each truck. The holding barn capacity was 6 trucks and the fans worked at maximum capacity during the summer period. A data logger (Testo 174H) was placed in the holding barn and the data were collected at 1 h intervals for one year. The vehicle was driven to the unloading area of slaughterhouse for ante-mortem inspection after the lairage period. After that, the crates were manually unloaded from the trailer and the broiler chickens were placed on a shackle line. At this stage, the number of dead broiler chickens was recorded as the mortality rate per transfer.

In order to evaluate the independent effects of the four factors on the mortality of the broiler chickens, the slaughter age was divided into four groups (up to 39 days, 40 to 42 days, 43 to 44 days, 45 days or more); lairage duration was divided into five groups (up to 60 min, 61 to 120 min, 121 to 180 min, 181 to 240 min, 241 min or more); transport distance was divided into four groups (up to 15 km, 16 to 50 km, 51 to 101 km, 100 to 200 km) and the ambient night temperature was divided into six groups (–5°C to 0°C, 0.1°C to 5°C, 5.1°C to 10°C, 10.1°C to 15°C, 15.1°C to 20°C and 20.1°C to 28°C). The numbers of transfers, the numbers of transported broiler chickens and the numbers of dead broiler chickens were recorded and the mortality percentages were calculated for these intervals.

Statistical analyses

The data were analysed with the Proc GENMOD procedure of SAS43. The model included the fixed effects of slaughter age, lairage duration, transport distance and ambient temperature. The Tukey’s multiple comparison test procedure was used to assess differences between means.

RESULTS

The data for some pre-slaughter parameters or the broiler chickens in the current study in Table 1. The overall DOA rate in the present study was 0.389%. The effects of slaughter age, transport distance, lairage duration and temperature on the DOA rate were significant (P<0.001). The mean DOA rate by slaughter age is given in Fig. 1. The DOA rate at ≤ 39 days slaughter age was significantly higher than that of the other slaughter age groups (P<0.001). The mean DOA rates for the transport distance intervals are presented in Fig. 2. The DOA rate for transport distance up to 15 km (0.448%) was significantly higher than that for other transport distance intervals (P<0.001). As the transport distance increased, the DOA rate generally increased. The mean DOA rates for lairage duration are given in Fig. 3. There was a positive relationship between DOA rate and lairage duration. As the lairage duration increased, the mean DOA rate increased significantly (P<0.001). The mean DOA rate by temperature is presented in Fig. 4. The DOA rate was highest (0.622%) at cold ambient temperatures (–5°C - 0°C) and lowest (0.334%) in the temperature range from 5.1°C to 10°C in present study.

DISCUSSION

DOA rate

The DOA rate is an important indicator of both animal welfare and financial losses. Therefore, the DOA level is a major factor in the economics of the broiler industry. The DOA rate was reported as 0.35% in Italy44, 0.37% in the Czech Republic44, 0.41% in Turkey45 and 0.46% in the Netherlands46. A similar result (0.389%) was also found in the current study. These rates were below the maximum level recommended by the EU which is less than 0.5%. On the other hand, the DOA rate was reported as 0.68% in the United States47. The differences in the DOA rate between studies may be due to differences in ambient temperature and humidity, feed withdrawal duration, stocking density, airflow characteristics during transport and/or lairage duration.

Table 1 - Means (± SEM) for some pre-slaughter conditions.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slaughter age (d)</td>
<td>41.79 ± 0.058</td>
</tr>
<tr>
<td>Stocking density (m²/bird)</td>
<td>0.046 ± 0.001</td>
</tr>
<tr>
<td>FAWC value (m²/bird)</td>
<td>0.037 ± 0.001</td>
</tr>
<tr>
<td>Transport distance (km)</td>
<td>55.76 ± 0.771</td>
</tr>
<tr>
<td>Lairage duration (min)</td>
<td>143.21 ± 1.146</td>
</tr>
<tr>
<td>Ambient temperature (°C)</td>
<td>10.74 ± 0.093</td>
</tr>
<tr>
<td>Ambient humidity (%)</td>
<td>87.09 ± 0.203</td>
</tr>
<tr>
<td>Pre-slaughter live weight (kg/bird)</td>
<td>2.346 ± 0.005</td>
</tr>
<tr>
<td>DOA rate (%)</td>
<td>0.389 ± 0.010</td>
</tr>
</tbody>
</table>
Slaughter age
Caffrey et al.\(^1\) reported that the mortality rate in a study in Canada was lower at older ages (more than 40-45 days) than for younger ages (33-37 days of age) and the difference was significant. They also stated that the mortality rate was higher for the 33-37 days slaughter age period than for the 38-39 days period. Chauvin et al.\(^17\) stated that the mortality rate in a study in France was higher at \(\leq 40\) days of age than at older ages (41-47 days of age) and the difference was significant. In the present study, the DOA percentage at \(\leq 39\) days slaughter age was higher than for the other slaughter age groups and the difference was significant (\(P<0.001\)). The results obtained in the current study were similar to those reported for the two earlier studies. Older birds have higher body weight and greater feather coverage of the body. This issue appears to be related (at least in part) to surface to volume ratio; the smaller an animal is, the higher is its surface to volume ratio and hence its propensity to lose body heat. The body form of older birds better insulates them against cold weather conditions. That means they can better maintain their body temperature in cold weather conditions and therefore have higher a survival rate than younger birds\(^18\). In addition, the significant effect of age of broiler chickens on DOA was also accordance with previous studies\(^3,17,16,20,12,2\).

Transport distance
Vecerek et al.\(^6\) stated that the DOA rate in a study in Czech Republic increased from 0.247\% to 0.862\% as the transport distance increased from 150 km to 300 km. Warriss et al.\(^25\) analysed the relationship between mortality in broiler chickens and distance of transport to slaughterhouse in a study in the UK. They reported that the DOA rate was 1.81 times higher when broiler chickens were transported for more than 4 h than less than 4 h (0.283\% vs 0.156\%). Aral et al.\(^15\) found in a study in Turkey that the mortality rate rose in broiler chickens from 0.29\% to 0.46\% when the transport duration increased from less than 2 h to longer than 10 h. Separately, Vecerek et al.\(^14\) found that the mean DOA percentage was 2 times higher when broilers were transported less than 100 km when compared with more than 300 km. Cockram\(^26\) stated that in a study in Canada the quality of transport was more important than transport duration for the welfare of broiler chickens. In the present study, the DOA rate increased as the transport distance increased, except for a distance up to 15 km. Overall, there was a positive and significant relationship between DOA rate and transport distance (\(P<0.001\)). This result is in accordance with the results of previous studies\(^6,14,13,30\). If transport takes a long time, broiler chickens deplete the glycogen store in their muscles and this
situation results in fatigue. Thus, broiler chickens may die due to sudden death syndrome, congestive heart failure or generalized circulatory collapse\(^2\). Therefore, Nijdam et al.\(^3\) suggested that slaughterhouses should be built within 2 h travel distance from farms in order to decrease the DOA rate. However, it seems more logical to build farms near the slaughterhouse because the slaughterhouse would normally be more capital intense.

Vecerek et al.\(^1\) reported that high mortality rate in broiler chickens is not only related to long distance transport but also to short distance transport. They found that the DOA rate for transport distance up to 50 km was higher than that for broiler chickens transported for distances from 51 to 100 km. In the present study, the DOA rate for transport distance up to 15 km (0.448%) was higher than that for 16-50 km (0.350%) or 51-100 km (0.390%). The result produced by this study was accordance with the result reported by Vecerek et al.\(^1\). Longer distance transport provides relatively adequate time for the recovery of broiler chickens from loading. Therefore, broiler chickens need at least one hour transport duration\(^2,3\).

**Lairage duration**

Lairage duration can be defined as the period between the arrival of the broiler chickens at the holding area in the slaughterhouse and their slaughter. An appropriate lairage period after transport decreases thermal stress and contributes to broiler welfare. Lairage also assists animals to adjust to their new environment. Lairage reduces the effects of physiological stress before slaughter and thus improves meat quality\(^4\). Most researchers have recommended a lairage duration of less than 2 h. Warriss et al.\(^5\) proposed a maximum lairage duration of 1 h in a holding barn, but if the conditions in the holding area were good, this time can be extended to 2 h. Hunter et al.\(^6\) found that the optimal lairage duration period was up to 2 h, if sufficient ventilation and appropriate thermal conditions were supplied. Bayliss and Hinton\(^7\) investigated the effect of lairage duration on the DOA rate. They concluded that the DOA percentage significantly increased if the lairage duration was 4 h. Vieira et al.\(^8\) studied three different lairage durations (between 1 h and 2 h, 3 and more than 3 h) and reported that the mean DOA rate was 0.33% and it decreased by about 0.1% as the lairage duration increased from below 1 hour to 3 hours. They concluded that optimal lairage duration was between 1 and 2 hours when the ambient temperature was below 21°C.

A different study reported that liver glycogen was depleted to maintain body temperature when the ambient temperature was high or low, such as under summer or winter conditions. If this situation persists, the animal may die. Therefore, the recommended lairage duration was between 1 h and 2 h. Chauvin et al.\(^9\) studied 403 broiler chicken flocks and reported that the DOA rate increased significantly in broiler chickens lairaged for 260 min or more before slaughter. In the present study, the DOA rate increased as lairage duration increased, especially after 2 h, which is in accordance with the results of previous studies\(^2,3,5,6,14\).

**Temperature**

Thermal stress is a main reason for mortality in broiler chickens and it causes economic losses in most countries. Ritz et al.\(^1\) evaluated broiler DOA in the UK by necropsy and concluded that 40% of DOA was associated with heat or cold stress. Warriss et al.\(^5\) investigated the relationship between temperature and DOA rate due to transport from farms to a commercial slaughterhouse. They found average mortality rates of 0.10%, 0.13%, 0.26% and 0.66% for temperature ranges of 14-17°C, 17-19.9°C, 20-22.9°C and 23-27°C, respectively. The DOA rate was very high for the 23°C to 27°C interval in their study. Petracci et al.\(^15\) investigated the relationship between DOA rate and season. They found DOA rates of 0.47%, 0.28%, 0.35% and 0.32% for summer, autumn, winter and spring, respectively. The DOA rate was highest in summer. In another study, DOA records were investigated by Vecerek et al.\(^16\) who studied the relationship between DOA percentage and ambient temperature. They found that the DOA rate was highest (about 0.80%) at ambient temperatures between -6°C and -3.1°C. This temperature interval was the lowest in their study. Vosmerova et al.\(^17\) noted that broiler chickens transported in cold weather were very stressed and further stated that their plasma corticosterone levels when transported at lower ambient temperatures (-5°C to 5°C) were higher than in the higher temperature intervals. Vecerek et al.\(^16\) and Caffrey et al.\(^18\) analyzed the relationship between mortality rate and season in the Czech Republic and Canada, respectively. They found that the DOA percentage was higher in both summer and winter months than that in spring and autumn. Nijdam et al.\(^1\) reported a significant association between ambient temperature and DOA rate. They stated that if the ambient temperature was relatively high (>15°C) or low (<5°C), the DOA percentage increased. In the present study, the DOA rate was highest (0.622%) at low ambient temperatures (-5°C to 0°C) and also very high (0.576%) at high ambient temperatures (20.1°C - 28°C). On the other hand, the DOA rate was lowest when the temperature ranged from 5.1°C to 10°C (0.334%). These results are in agreement with those reported by previous studies\(^1,2,5,6,12\). Increased DOA rates in summer and winter are most likely related to the more extreme ambient temperatures in those seasons, when high or low temperatures adversely affect the welfare of transported broiler chickens. If the weather conditions are very hot, cold or humid, broiler chickens may not be able to maintain thermoregulation and may die from hyperthermia or hypothermia\(^2,7\).

**CONCLUSIONS**

In conclusion, the results for the DOA rates in the present study clearly showed that very short and long distance transport and long lairage duration were harmful to broiler chickens. Birds did not have enough time to recover after very short distance transport. Long distance transport and long lairage time, or both, could have led to decreased blood glucose levels, overfatigue and even death in broiler chickens. It is especially important to avoid long distance transport and long lairage duration under adverse environmental conditions such as low and high temperatures as broiler chickens struggle to maintain their body temperature in the optimum range and are therefore more vulnerable to premature death. In addition, broiler chickens of up to 40 days of age were more susceptible to pre-slaughter stress than other ages. This may again reflect a lesser ability of younger broilers to maintain their body temperature in the optimum range when un-
der stress. More attention should be paid to this age range in the pre-slaughter period because younger broiler chickens are more negatively affected by cold ambient conditions. Therefore, in addition to these measures, stocking density in crates of broiler chickens of up to 40 days of age should be increased in cold weather conditions to reduce the possibility of hypothermia.

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