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Original Article

The Effect of Space Allowance During Pre-Slaughter Transport of European Quail Breeders on Welfare, Carcass Traits and Meat Quality

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ABSTRACT

The objective was to evaluate whether space allowance during pre-slaughter transport of European breeding quail affects welfare, carcass traits and meat quality. A total of 248 quails (body weight = 344.90 ± 2.09 g) were used. During pre-transport, the quails were fasted for 5 hours and 30 minutes. The poultry were caught and placed in plastic transport crates with an area of 0.40 m^2. The treatments consisted of a different space allowance in each crate: 22, 28, 34 or 40 quails per crate. Immediately upon arrival at the slaughterhouse, the quails were stunned. During bleeding, blood samples were collected. The carcasses were individually weighed. The meat quality was measured. Data were analysed using the effects of space allowance during transport of quails and pickup truck as dependent variables. Death on arrival increased as the space allowance in transport crates decreased (p=0.08). Glucose and uric acid levels were not significantly different between groups ($p \geq 0.14$). The cold carcass weight presented the maximum value when the crates contained 31 quails (p=0.03). The meat pH, intensity of red, intensity of yellow and cooking losses were not different between groups ($p \geq 0.12$). The meat lightness value presented the maximum value when the crates contained 31 quails (p=0.04). In view of the above, the number of European breeding quails transported in crates must not exceed 30 animals.

INTRODUCTION

Animal welfare is often related to living, transport, and slaughter conditions (Lehmann & Christoph-Schulz, 2022). Pre-slaughter operations should be adequately planned with a view to reducing negative effects on animal welfare (Saraiva *et al.*, 2020). The pre-slaughter period, in relation to the animals' growth period, is a short interval, but one with many variations and nuances. For example, catching, crating and transportation can cause severe stress (Nijdam *et al.,* 2005) and death on arrival (DOA) (Nijdam *et al.*, 2004; Vecerkova *et al.*, 2019; Saraiva *et al.*, 2021). The death of an animal is an extreme but useful measure of welfare (Welfare Quality® 2009).

Scientific information on pre-slaughter handling usually concerns broilers, which can result in inaccurate recommendations for other bird species. Even in the case of animals of the same species, there may still be details that need to be seen or revised for refinement in the elaboration of practical guides. Citing a similar case, the studies of Di Martino *et al.* (2017) and Vecerkova *et al.* (2019) discussed the pre-slaughter handling of end-of-lay hens. Although quail egg and meat production is growing rapidly, quail farming practices and welfare aspects are not well established (El Sabry *et al.*, 2022). In addition to issues related to welfare, pre-slaughter stressors can influence carcass weights and meat quality indicators, such as colour change (Ali *et al.*, 2008; Hussnain *et al.*, 2020).

One of the main criticisms surrounding welfare is the limited space for animals to move around (Bessei, 2018; Engel *et al.*, 2019). Specifically addressing the space allowance for animals in transport crates, space is routinely determined by age, mean live bird weight and crate dimensions, as seen in the Animal Transport Guides (2017). Allometric equations of the form area = kW2/3, where k = a constant and W = live weight, can be used to estimate the space an animal occupies because of its mass and presupposes that animals are of similar shape, both between species and in relation to changes over time (reviewed by Petherick & Phillips, 2009). Moreover, there may be adjustments in the number of animals according to the weather conditions to obviate suffocation/heat stroke and ensure there are sufficient birds within the crate to prevent lateral movement, which may result in physical injury to the birds (Bayliss & Hinton, 1990).

We did not find studies that make recommendations on the space allowance for road transport of quails, regardless of production category. In view of the above, the objective was to evaluate whether space allowance during pre-slaughter transport of European breeding quail affects welfare, carcass traits and meat quality.

MATERIALS AND METHODS

The Ethics Committee on Animal Experimentation and Welfare of the State University of Montes Claros approved this experiment, under protocol number 212/2020. The experiment was performed in Janaúba, Minas Gerais, Brazil, located at 15°43′ 47″ S latitude, 43°19′ 18″ W longitude, and 516 metres altitude.

Poultry

A total of 248 European breeding quail (*Coturnix coturnix coturnix*) of 365 days of age were used, distributed in a completely randomised design. These were spent quails at the end of their reproductive lifespan. The quails received a balanced diet (Table 1). Two days before slaughter, the quails were individually weighed and had a mean body weight (BW) of 344.90 ± 2.09 g (median = 343.50 g; first quartile = 323.25 g and third quartile = 365.00 g). In the pre-transport period, the quails were fasted for 5 hours and 30 minutes (Lopes, 2019; Silva *et al.*, 2022). Water was provided *ad libitum*. After fasting, the poultry were caught and placed in plastic transport crates with external dimensions of 0.75 × 0.56 × 0.28 m and internal dimensions of 0.74 × 0.54 × 0.24 m (standard crates for broiler chickens used in Brazil).

Table 1 – Ingredients and nutritional contributions of the experimental diet.

Ingredient	%
Cornmeal	54.123
Soybean meal	33.420
Soy oil	3.060
Calcitic Limestone	6.290
Dicalcium phosphate	1.854
Mineral and vitamin supplement[1]	0.500
Common salt	0.280
DL-methionine	0.203
L-threonine	0.270
Crude Protein	20.000
Metabolizable energy (kcal/kg)	2,900
Calcium	3.000
Phosphorus available	0.450
Sodium	0.150
Lysine[2]	1.300
Methionine + cystine[2]	0.826
Tryptophan[2]	0.249
Threonine[2]	1.090
Isoleucine[2]	0.859
Valine[2]	0.929

[1]Mineral and vitamin supplement (content per kg of product): vitamin A 2,000,000 IU; vitamin D_3 375,000 IU; vitamin E 3,750 mg; vitamin K_3 500 mg; vitamin B_1 250 mg; vitamin B_2 750 mg; vitamin B_6 500 mg; vitamin B_{12} 3,750 mcg; niacin 6,250 mg pantothenic acid 2,500 mg; biotin 10 mg; folic acid 125 mg; vitamin B_8 75,000 mg; selenium 45 mg; iodine 175 mg; iron 12,525 mg; copper 2,500 mg; manganese 19,500 mg; zinc 13,750 mg; avilamycin 15,000 mg; narasin 12,250 mg; butylated hydroxytoluene 500 mg; vitamin C 12,500 mg.

[2]Total amino acids.

Treatments

Guidance from the Animal Transport Guides (2017) and Petherick & Phillips (2009) were used to delimit the treatments. The Consortium established that poultry < 1.6 kg must be transported in an area of 180–200 cm²/kg, while Petherick & Phillips (2009) cited a space allowance (m²) equivalent to $0.027W^{0.67}$ (W = live weight) for poultry.

The quails were distributed into four treatments. The treatments consisted of different space allowances in each transport crate: greater individual space = 22 quails per crate with an internal area of 0.40 m²; intermediate space 1 = 28 quails per 0.40 m² crate; intermediate space 2 = 34 quails per 0.40 m² crate; and smaller individual space = 40 quails per 0.40 m² crate.

Transportation

The quails were transported, at the same time on the same day, in two pickup trucks, each with four transport crates, so that all groups were in both vehicles. The transport took 1.3 hours to cover 105 kilometres to a commercial slaughterhouse, considered a moderate distance (Silva & Vieira, 2009). In planning the experiment, information was sought from those responsible for the pre-slaughter transport of chickens, from eight slaughterhouses in Minas Gerais (MG), Brazil, concerning the average distance between the farm and industry. It was reported that most transports lasted 45–60 minutes. At the time of transport, the temperature and relative humidity observed were 29 ± 0.7 °C and 44 ± 2.2%, respectively. The median, first and third quartiles of temperatures (°C) for the years 2020 and 2021 in Janaúba, MG were 25 and 25, 22 and 22 and 28 and 29, respectively. The median and first and third quartiles of humidity (%) for the years 2020 and 2021 in Janaúba, MGwere 69 and 62, 53 and 46 and 83 and 79, respectively (Brazil 2022).

Lairage and slaughter

The DOA was measured (n = 248). The slaughterhouse had a humidity and ventilation control system in lairage. Immediately upon arrival, the quails were stunned using electrodes placed on their heads (n = 248). The stunning from the first quail to the last lasted 1.5 hours. The voltage (275 volts), electrical current (60 milliampere) and application time (4 seconds) used for stunning were studied by Tserveni-Gousi *et al.* (1999). Bleeding was inducted with a cut to the jugular veins and carotid arteries. During bleeding, blood samples were collected from the quails into two collection tubes, one without anticoagulant and the other with anticoagulant (Ethylenediaminetetraacetic acid). On the same blood collection day, the tubes were centrifuged at 3000 rotations per minute for 10 minutes to extract serum and blood plasma. Glucose (n = 55) and uric acid (n = 46) levels were measured with a spectrophotometer using commercial kits (supplied by Bioclin, https://www.bioclin.com.br/). The chemiluminescence method was used to analyse corticosterone (n = 25). The standard = RAND function in Microsoft Excel was used for randomisation of the blood samples.

The quails were scalded at 60 °C for 30 seconds and plucked. The poultry were eviscerated, and the feet and heads removed to obtain hot carcasses. The carcasses were individually weighed (n = 190) and cooled in a tank at 0 °C for 15 minutes. The edible organs (heart–liver–gizzard set) of each quail were also weighed (n = 181). After dripping (5 minutes), the carcasses were weighed (n = 190).

Meat quality measures

The meat traits of 80 quails (n = 20 from each group), were measured in an acclimatised laboratory. The standard = RAND function in Microsoft Excel was used for randomisation of the carcasses. The carcasses were weighed without the head. The ultimate pH was measured by direct insertion of an electrode into the left pectoralis major muscle of the birds (Berri *et al.*, 2001) in the longitudinal direction of the muscle until reaching the central part (Ramos & Gomide, 2017). Parallel to pH, electrical conductivity was also measured by direct insertion of an electrode into the muscle. For this, three technical replicates of each sample were used.

The colour of the left pectoralis major muscle was determined on the ventral side of each sample (Narinc *et al.* 2013), using a Hunter Lab EZ Scan portable spectrophotometer (Illuminant A, 45/0 LAV, 2.54 centimetre diameter aperture, 10° observer) (CIE; L*, a* and b*). Four technical replicates of each sample were used. To determine water-holding capacity, measurements of the pectoralis major muscle, the filter-paper press method and cooking loss techniques were used, employing the methods mentioned by Hamm (1986). For the filter-paper pressing technique, 5-gram meat cubes (left pectoralis major muscle) were used between two circular filter papers. These papers were placed between two glass plates and pressed with a weight of 5 kg for 5 minutes (Hamm, 1986; Sanfelice *et al.*, 2010). Two technical replicates of each sample were used.

To determine cooking loss, we used the right pectoralis major muscle without skin and an electric grill preheated to 140 °C, according to the methodology described by Ramos & Gomide, 2017. The muscle, wrapped in aluminium foil, was heated until an internal temperature of 50 °C was reached and then turned. When the sample reached 82 °C, it was removed from the grill. Afterwards, the sample was cooled for 30 minutes at 15 °C. Cooking loss was determined by recording the sample weights before and after cooking. The analysis of water activity was measured directly using an Aqualab series 3TE instrument.

Statistical analysis

The type of variables, and Shapiro–Wilk and Bartlett tests were used to verify whether the data met analysis of variance assumptions of normality and variance homoscedasticity (Table 2). Data were analysed using the effects of space allowance during transport of quails and pickup truck as dependent variables. RStudio linear model procedures were used. Orthogonal polynomial contrasts were used to test the linear and quadratic effects of treatments on dependent variables. The effect of vehicle was not significant for dependent variables (p>0.10); therefore, it was excluded from the model. The model was accepted when the T value of the estimates was less than 0.10 and agreed with the normality assumption. At this time, the adjusted R-squared (R^2), root mean squared error (RMSE), mean absolute percentage error (MAPE) and mean absolute error (MAE) of the model were verified in the 'Metrics' package. If we had considered one quail as an experimental unit, the sum of squares would be too high and R^2 would be compromised. Therefore, quails contained in the same transport crate were considered the experimental unit. Considering that coefficient of variation values were low, the restricted number of replications did not interfere in treatment comparisons and therefore, the conclusions were acceptable. Then, model validation through the bootstrap technique was used ('Caret' package). Training parameters to 1000 bootstrap samples were configured. At this time, the R^2, RMSE and MAE of the model were verified. The number of dead quails, as they did not assume a normal distribution, was evaluated using the non-parametric Kruskal–Wallis test. In this case, one quail was considered an experimental unit. For DOA, p<0.10 was considered statistically significant.

RESULTS

DOA increased as available space allowance in transport crates decreased (0.00%, 1.79%, 1.47% and 5.00% for crates with 22; 28; 34 and 40 quails, respectively) (p=0.0788). There was no variation in corticosterone results between groups of quail (Table 2). Glucose and uric acid levels were not significantly different between groups (p≥0.14). The weights of the hot and cold carcasses presented maximum values when the transport crates contained 31 or 30 quails, respectively (p≤0.08). The cold carcass weight without head also presented the maximum value when the transport crates contained 31 quails (p=0.03). Edible organs, carcass yields and water absorption in chilling were no different between groups (p≥0.13).

Table 2 – Normality and variance homoscedasticity tests, and regression model performance measures.

	Hom	NVar	NM	adR^2	RMSE	MAE	MAPE	R^2	RMSE	MAE
Gluc	0.53	0.74	-	-	-	-	-	-	-	-
UA	0.07	0.47	-	-	-	-	-	-	-	-
HCW	0.84	0.93	0.24	0.30	4.33	4.01	1.56	0.67	6.73	6.29
CCW	0.65	0.95	0.68	0.37	3.70	3.29	1.20	0.69	6.12	5.71
HCWwh	0.14	0.55	0.72	0.52	3.32	2.44	0.98	0.71	7.03	6.57
WLG	0.39	0.18	-	-	-	-	-	-	-	-
HCY	0.35	0.59	-	-	-	-	-	-	-	-
CCY	0.99	0.75	-	-	-	-	-	-	-	-
WC	0.28	0.58	-	-	-	-	-	-	-	-
pH	0.56	0.90	-	-	-	-	-	-	-	-
L*	0.95	0.60	0.10	0.46	0.53	0.51	1.32	0.70	1.03	0.96
a*	0.06	0.24	-	-	-	-	-	-	-	-
b*	0.23	0.48	-	-	-	-	-	-	-	-
Aw	0.08	0.54	-	-	-	-	-	-	-	-
CL	0.87	0.45	-	-	-	-	-	-	-	-

Hom – variance homoscedasticity; NVar – normality; NM – normality of model residuals; adR2 – adjusted R-squared; RMSE – root mean squared error; MAE – mean absolut error; MAPE – mean absolute percentage error; Glu – glucose; UA - uric acid; HCW – hot carcass weight; CCW – cold carcass weight; CCWwh – cold carcass weight without the head; WLG – heart–liver–gizzard; HCY – hot carcass yield; CCY – cold carcass yield WC – water absorption in chiller; L* - lightness; a* - intensity of red; b* - intensity of yellow; Aw - water activity; CL - cooking loss.

The meat pH, intensity of red (a*), intensity of yellow (b*), water activity and cooking losses were not significantly different between groups (Table 3) (p≥0.12). The meat lightness (L*) value presented the maximum value when the transport crates contained 31 quails (p=0.04).

The statistical models presented precision (MAPE) below 1.56% and an adjusted R^2 between 0.30 and 0.54 (Table 4). The results of the model validations showed satisfactory values. The models presented an R^2 between 0.67 and 0.71. The prediction error result of model configurations was also acceptable; with a deviation of less than 7 g for the variables linked to carcass weights. There were probably no outliers, due to the proximity between RMSE and MAE.

Table 3 – Blood variables, carcass traits and weight of the edible organs (heart–liver–gizzard set) of quails subjected to different space allowances in crates during transport.

	Number of quails per transport crate				CV (%)	T Value	
	22	28	34	40		Linear	Quadratic
Cort (nmol/L)	13.8	13.8	13.8	13.8	0.00	-	-
Glu (mmol/L)	12.88	15.93	13.47	14.70	6.27	0.5441	0.439
UA (µmol/L)	533.70	435.32	462.86	476.08	9.64	0.4165	0.1444
HCW (g)[1]	252.63	262.13	260.28	252.57	2.36	0.931	0.0772
CCW (g)[2]	270.25	277.77	275.45	267.29	1.91	0.591	0.0641
CCWwh (g)[3]	245.95	253.21	254.01	243.23	1.86	0.731	0.0288
WLG (g)	14.53	15.89	16.91	15.59	7.80	0.3427	0.156
HCY %)	74.24	75.50	74.98	74.16	1.19	0.809	0.1342
CCY (%)	79.41	80.02	79.42	78.64	0.83	0.226	0.165
WC (%)	6.53	5.64	5.52	5.54	11.52	0.1613	0.331

Cort – corticosterone; Glu – glucose; UA - uric acid; HCW – hot carcass weight; CCW – cold carcass weight; CCWwh – cold carcass weight without the head; WLG – heart–liver–gizzard; HCY – hot carcass yield; CCY – cold carcass yield; WC – water absorption in chiller; CV – coefficient of variation. T value – gives you the P-value for the T-test.

.[1] $\hat{Y} = 148.48215 + 7.37535x - 0.11950x^2$

[2] $\hat{Y} = 178.68676 + 6.56744x - 0.10894x^2$

[3] $\hat{Y} = 138.14556 + 7.64472x - 0.12528x^2$

Table 4 – Meat traits of quails subjected to different space allowance in crates during the transport.

	Number of quails per transport crate				CV (%)	T Value	
	22	28	34	40		Linear	Quadratic
pH	5.87	5.85	5.81	5.85	1.56	0.721	0.5877
L* value[1]	38.00	39.43	39.23	37.99	1.93	0.949	0.0377
a* value	5.49	5.00	5.13	5.72	9.42	0.6241	0.1518
b* value	8.66	8.92	9.30	8.68	3.66	0.7382	0.1200
Aw	0.99	0.99	0.99	0.99	0.24	0.851	0.834
CL(%)	27.77	28.27	24.81	25.66	8.04	0.182	0.917

L* - lightness; a* - intensity of red; b* - intensity of yellow; Aw - water activity; CL - cooking loss; CV – coefficient of variation. T value – gives you the P-value for the T-test.

[1] $\hat{Y} = 21.78155 + 1.14641x - 0.01855x^2$

DISCUSSION

The DOA occurrence, by itself, advises against the transport of 40 quails (BW = 344.90 ± 2.09 g) in crates with internal dimensions of 0.74 × 0.54 × 0.24 m. Possibly, the high ambient temperature (29 °C) and low humidity (44%) contributed to the highest mortality of quails given the smaller space allowance. Petrucci *et al.* (2006) reported a DOA percentage of 1.62% during the summer in 19 spent hen slaughterhouses in Italy over 4 years. Saraiva *et al.* (2021) observed that a total of 1,156 (0.18%) birds were found DOA. Grilli *et al.* (2018) highlighted the importance of respecting the space allowance when transporting broilers to reduce the DOA percentage. Unlike in this work, Voslarova *et al.* (2006) found changes in the haematological profile of hand-reared pheasants when they were transported for 4 hours; they concluded that these birds require more available space than other poultry. Delezie *et al.* (2007) including feed deprivation, crating density (high vs. low reported higher levels of corticosterone in broilers transported with a smaller space allowance (0.0350 m²/Ross broiler and 0.0575 m²/broiler, transported for 1.5 hours). Engel *et al.* (2019), on the other hand, evaluating the effect of floor space on the physiological variables of caged laying hens, found no changes in corticosterone concentrations when animals had extra space; the authors suggested a possible physiological adaptation over time. Türkyilmaz & Fidan (2006) investigated the effects of regular physical contact on fear and stress reactions in broiler chickens and found no differences in corticosterone levels of birds; the mean values found were similar to those in this research (11.6 nmol/L).

Poultry, facing stress-causing agents, activate the breakdown of hepatic glycogen, carry out gluconeogenesis and consequently, increase blood glucose (Bejaei & Cheng, 2014; Yalçin *et al.*, 2004) which was not observed in this work under the effect of different space allowances for quail transport. Weight loss and uric acid levels can be used to determine dehydration status (Thrall *et al.*, 2015). Dehydration is defined as the reduction in total intra and/or extracellular liquids due to reduced liquid intake or increased liquid discharge (Çelik & Irak, 2020). The uric acid levels were no different between groups, but, possibly, the carcass weights were influenced by the animals' dehydration in the groups that were at the treatment extremes. Even after immersion chilling, the difference between the groups was maintained.

The effort to maintain balance may have resulted in greater loss of carcass weight from animals that were given the larger space allowance. Considering the cold carcass weight without the head, there was a 3.6% difference between the groups that had the larger space allowance (22 quails) and those with the maximum carcass weight (30.5 quails); however, there were no deaths when 22 quails were transported. Furthermore, the scientific texts that deal with the subject recommend a minimum space, as seen in the following excerpt quoted by the EFSA (2022): "The generic allometric equation 'space allowance (cm^2) = 290 × live weight (kg2/3)' can be used to calculate the minimum required floor space during transport for most types of birds to adopt a sitting position and have the possibility to shuffle around". According to the equation above, for the standard crates used in this experiment, up to 27.8 quails must be transported.

Frerichs *et al.* (2021) noted that stressors during transportation can influence weight loss by causing end-of-cycle hens to implement thermoregulatory mechanisms to cope, which quickly deplete the animals' limited energy reserves. Analysing carcass weight losses when the quails were given the smallest space allowance, the impact of high ambient temperature and low air humidity seems clear. Schwartzkopf-Genswein *et al.* (2012) noted that the microclimate within the trailer is the most important factor affecting broiler welfare during transportation, as heat and cold stresses are two major contributors to both death and overall transportation stress in broilers. Petherick & Phillips (2009) reported that one should not use linear relationships between the weight of the animals and space allowance, because there are species specificities and body changes over time that are not linear, which could influence technical recommendations. The same authors cited the allometric equation: area (m^2) = $KW^{0.66}$ (where K = 0.027 and W = live weight, kg) as suitable for animal transport; a recommendation compatible with the results of this work.

The pH of meat influences its colour and other quality traits (Lawrie, 2005; Kim *et al.*, 2014). However, the quail's meat was lighter even without changes in the ultimate pH. There was consistency in the results of the L* value and carcass weights; all these variables showed better values when 30 or 31 quails were transported in the crates. Hussnain *et al.* (2020) also found changes in the lightness of meat when evaluating crate densities during broiler transport. Despite the similarity of the ultimate pH values, there is a need for improvement in the pre-slaughter handling of quails since the values should be closer to 5.5 (Lawrie, 2005).

In view of the above, considering the details of this work, the number of European breeding quails transported in plastic crates, with internal dimensions of 0.74 × 0.54 × 0.24 m, must not exceed 30 animals.

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