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Technical Note

Impact of Pre-transport Feed Withdrawal on Welfare and Meat Characteristics of European Quails

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ABSTRACT

The objective of this study was evaluate whether pretransport feed withdrawal affects welfare, carcass, and meat characteristics of European quails. A total of 120 European quails were used, with an initial age of 15 days. Bird feed was removed before transport to the slaughterhouse at the following times, which characterised the different treatments: zero hours, three hours, six hours, nine hours and twelve hours. The transport took 54 minutes to cover 27 kilometres to a commercial slaughterhouse. The birds were slaughtered at 41 days of age. During bleeding, blood samples were collected. Blood glucose, total protein, albumin, uric acid, and corticosterone concentrations were measured. The carcasses and meat characteristics were measured. Poultry body weight decreased and blood glucose concentrations increased with the increase in feed withdrawal time. The treatments did not affect carcass weights. Carcass yields after three hours fasting were similar to those in the six hours and nine hours groups, indicating that gastrointestinal tracts were empty after the third hour. Meat quality was negatively affected (pH, lightness, water holding capacity, cooking loss) by the increase in feed withdrawal time; integrated parameters that characterise dark, firm, dry meat. Pre transport feed withdrawal time should be three hours to empty the gastrointestinal tract and minimise losses in meat quality of European quails. It is necessary to adjust feed withdrawal so that it does not exceed this time, since there is no technical justification for supporting it.

INTRODUCTION

Birds may be exposed to a variety of potential stressors during preslaughter. The adverse effects of these factors and their combinations may range from mild discomfort to death (Petracci *et al.*, 2006). One such factor is feed withdrawal prior to transport to the slaughterhouse (Petracci *et al.*, 2010). Feed is withdrawn from poultry prior to slaughter to reduce the potential of carcass contamination with crop and intestinal contents (Bilgili, 2002). However, when fasting is performed beyond necessary, it can bring about physiological changes caused by hunger and frustration (De Jong *et al.*, 2002); rapidly decreasing the energy supply, compromising animals' welfare (Savenije *et al.*, 2002) and also affecting meat quality (Ali *et al.*, 2018).

No specific research was found on the effects of pretransport feed withdrawal for European quails (*Coturnix coturnix coturnix*). For example, the legislation in Brazil determines that birds are fasted for a minimum period of six hours, while not exceeding a total of 12 hours. This total fasting time is determined based on the feed withdrawal time from the shed until the moment of stunning (Brazil, 2021), but there are no specifications for different bird species in the legislation.

Therefore, due to quails' specificities, this fasting can exceed the time needed to empty gastrointestinal tracts, thus affecting survival-related factors highlighted in the 'Five Domains' model (Mellor *et al.*, 2015), especially food restriction. In view of the above, the objective was to evaluate whether pretransport feed withdrawal affects the welfare, carcass, and meat characteristics of European quails.

MATERIALS AND METHODS

Animal care

The Ethics Committee on Animal Experimentation and Welfare of the State University of Montes Claros approved this experiment, under protocol number (213/2020).

Birds and housing

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. A total of 120 European quails were used (60 females and 60 males), housed in floor pens, with deep litter and an initial age of 15 days. Animal care staff and those who administered treatments were not aware of allocation groups to ensure that all animals in the experiment were handled, monitored, and treated in the same way. The experimental shed had fans and side curtains. Nutritional handling was conducted twice a day. The animals received water *ad libitum*. The diet used from 15 days until slaughter was formulated based on the nutritional information on feed composition presented by Rostagno *et al.* (2017) (Table 1). At 38 days of age, the birds were individually caught with cupped hands and weighed. They were then identified with a plastic ring for birds. The average body weight on that day was 220.83 ± 41.76 g.

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

Ingredients	%
Cornmeal	53.469
Soybean meal	41.954
Soy oil	1.016
Calcitic Limestone	1.072
Dicalcium phosphate	0.960
Mineral and vitamin supplement[1]	0.500
Starch	0.500
Common salt	0.265
L-threonine	0.265
Crude Protein	23.886
Metabolizable energy (kcal/kg)	2.900
Calcium	0.800
Phosphorus available	0.300
Sodium	0.140
Lysine	1.300
Methionine + cystine	0.731
Tryptophan	0.305
Arginine	1.612
Isoleucine	1.040
Valine	1.107

[1]Mineral and vitamin supplement (content per kg of product): vitamin A 2,000,000 IU; vitamin D3 375,000 IU; vitamin E 3,750 mg; vitamin K3 500 mg; vitamin B1 250 mg; vitamin B2 750 mg; vitamin B6 500 mg; vitamin B12 3,750 mcg; niacin 6,250 mg; pantothenic acid 2,500 mg; biotin 10 mg; folic acid 125 mg; vitamin B8 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.

Treatments

Birds' feed was withdrawn before transport to the slaughterhouse at the following times, which characterised the treatments: zero hours, three hours, six hours, nine hours, and twelve hours. The sample number of each group was 24 quails. During fasting, water was available *ad libitum*.

Pretransport and transport

When the twelve hours group completed this fasting time, all animals were individually weighed and placed in a poultry transport crate (73 cm x 54 cm x 28 cm). A draw was made to determine which animals inside each pen would be weighed first. This procedure lasted 31 minutes. Each transport crate received the birds from a floor pen. The quails were transported at the same time and day, in two pickup trucks, each one with five transport crates, so that all groups were present in both vehicles. The characteristics of the transport crate and the replication of the vehicle justify the sample number used. The transport took 54 minutes to cover 27 kilometres to a commercial slaughterhouse, which is considered an average distance (Silva & Vieira, 2009). In planning the experiment, information was sought from those responsible for the preslaughter of birds in eight slaughterhouses in Minas Gerais/Brazil, concerning the average distance between the farm and the industry. It was reported that most transports lasted between 45 and 60 minutes. The slaughterhouse had humidity and ventilation control system in lairage.

Slaughter, blood collection, and carcass measurements

The birds were slaughtered at 41 days of age. Upon arrival at the slaughterhouse, quails were desensitised

using electrodes placed on their heads. The voltage (275 volts), electrical current (60 milliampere), and application time (four seconds) used for stunning were studied by Tserveni-Gous*i et al.* (1999). This procedure lasted 43 minutes. A draw was made to randomise which animals from each transport crate would be slaughtered first. Bleeding occurred with a cut of the jugular veins and carotid arteries. During bleeding, blood samples were collected from the quails and stored in two collection tubes, one with and the other without anticoagulant (Ethylenediaminetetraacetic acid). On the same day of blood collection, the tubes were centrifuged at 3000 rotations per minute for ten minutes to extract serum and blood plasma. Blood glucose, total protein, albumin, and uric acid concentrations 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 = 6 from each group). As the blood volume collected per quail was small, the sample number for each blood parameter was 55 samples (n = 11 from each group), after excluding inappropriate samples. The standard = RAND function in Microsoft Excel was used for randomisation of blood samples.

Quails were then scalded at 60 °C for 30 seconds and plucked. The birds were eviscerated, and feet and heads removed to obtain hot carcasses. The carcasses were individually weighed and cooled in a tank at 0 °C for 15 minutes. The edible organs (heart–liver–gizzard set) of each quail was also weighed. After dripping (five minutes), the carcasses were weighed.

Meat quality measurements

The meat characteristics of 60 birds (n = 12 from each group) were measured in an acclimatised laboratory. The standard = RAND function in Microsoft Excel was used for the randomisation of the carcasses. The ultimate pH was measured by direct insertion of an electrode in the left *pectoralis major* muscle of the birds (Berr*i 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. Three technical replicates of each sample were used for this.

The left *pectoralis major* muscle colour was determined on the ventral side of each sample (Narinc *et al.*, 2013) using a HunterLab EZ Scan portable spectrophotometer (Illuminant A, 45/0 LAV, 2.54 centimetres diameter aperture, 10° observer) (CIE; L*, a* and b*). Four technical replicates of each sample were used. The filter-paper press method and cooking loss techniques were used for water holding capacity, measurements of the *pectoralis major* muscle, as described 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 five 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 and Gomide (2017). The muscle, wrapped in aluminium foil, was heated until an internal temperature of 50 °C was reached, after which it was turned over. 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 determination was performed by recording the sample weights before and after cooking. The analysis of water activity was measured directly using the Aqualab series 3TE instrument.

Statistical analyses

The 'outliers' package was used to detect points outside the curve in the sample data. Analysis of variance was performed using the General Linear Model procedure of RStudio software to test the effects of feed withdrawal time, floor pens, sex, and transport, as well as their interactions with body weight, blood variables, and carcass and meat characteristics. The body weight of birds at 38 days of age (birds not subjected to treatments) was included in the model as a covariate to assess the following variables: body weights after feed withdrawal and carcass characteristics. This covariate was not significant ($p \geq 0.05$). The effects of floor pens, sex, transport, and interactions were not significant for body weight, blood variables, and carcass and meat characteristics ($p \geq 0.05$).

When the model was significant ($p < 0.05$), orthogonal polynomial contrasts were used to test the linear and quadratic effects of treatment on body weight, blood variables, and carcass and meat characteristics. When both effects were significant (linear and quadratic) ($p < 0.05$), they were evaluated through ANOVA to determine if there was an increase in adjustment using the quadratic model ($p < 0.05$). When the model was significant ($p < 0.05$), means were also compared using Tukey's test ($p < 0.05$).

RESULTS

There were no quail deaths before stunning. Poultry body weight decreased linearly (Table 2), and blood glucose concentrations increased with the increase in feed withdrawal time. Corticosterone, total proteins, albumin, and uric acid were not significantly different among groups.

Table 2 – Body weight and blood variables of European quails subjected to different pretransport feed withdrawal times.

	Feed Withdrawal (h)					SEM	*p*-value	
	0	3	6	9	12		L	Q
BW (g)	231.7	231.3	226.9	213.3	212.1	33.43	0.013	0.046
Cort (ng/mL)	5.0	5.0	5.0	5.0	5.0	0.00	0.161	0.230
Glu (mg/dL)	321.4^{a}	358.4^{ab}	370.5^{ab}	398.3^{b}	396.7^{b}	43.05	<0.001	0.001
TP (g/dL)	2.4	2.9	2.7	2.3	2.5	0.80	0.624	0.660
Alb (g/dL)	1.2	1.2	1.3	1.2	1.2	0.38	0.950	0.846
UA (mg/dL)	9.1	11.4	11.3	8.9	11.2	2.22	0.291	0.217

Mean values followed by different letters were significantly different according to Tukey's test ($p<0.05$).

SEM. standard error of the means; BW. body weight; Cort. corticosterone; Glu. glucose; TP. total protein; Alb. albumin; UA. uric acid; L. linear effect of feed withdrawal time; Q. quadratic effect of feed withdrawal time.

The weights of hot and cold carcasses were not significantly different between groups (Table 3). The yields of hot and cold carcasses (Figure 1), and the weight of the edible organs showed quadratic behaviour. The highest yields of hot and cold carcasses were achieved when the animals reached 7.56 hours and 8.22 hours without access to feed, respectively. There was no difference between carcass yields in the three, six, nine, and twelve hours groups. The edible organs showed less weight 9.18 hours after feed withdrawal.

Table 3 – Carcass characteristics and weight of the edible organs (heart–liver–gizzard set) of European quails subjected to different pretransport feed withdrawal times.

	Feed Withdrawal (h)					SEM	*p*-value	
	0	3	6	9	12		L	Q
HCW (g)	173.5	177.4	176.1	170.4	170.4	30.30	0.509	0.736
CCW (g)	188.1	189.0	178.6	180.5	183.1	30.74	0.392	0.596
WLG (g)	13.0^{a}	12.2^{ab}	11.0^{b}	10.9^{b}	11.1^{b}	1.50	<0.001	<0.001
HCY (%)	75.8^{a}	78.0^{b}	79.0^{b}	78.8^{b}	78.7^{b}	2.01	<0.001	<0.001
CCY (%)	80.4^{a}	82.8^{b}	84.5^{b}	83.4^{b}	83.5^{b}	2.66	<0.001	<0.001

Mean values followed by different letters were significantly different according to Tukey's test ($p<0.05$).

SEM. standard error of the means; HCW. hot carcass weight; CCW. cold carcass weight; WLG. heart–liver–gizzard; HCY. hot carcass yield; CCY. cold carcass yield; L. linear effect of feed withdrawal time; Q. quadratic effect of feed withdrawal time.

Meat pH increased linearly and meat lightness (L*) decreased with the increase in feed withdrawal time (Table 4). The red and yellow intensities (a* and b*, respectively), and water activity of the meat were not significantly different between groups. The meat electrical conductivity and the cooking loss decreased, while the water holding capacity increased with feed withdrawal.

DISCUSSION

Different on-farm fasting times are used in meat-type quails, e.g. four and six hours in the study of Muniz *et al.* (2018) and Pasquetti *et al.* (2014), respectively; which raises doubts regarding the preslaughter handling of quails. Becker *et al.* (1985) reported a decrease in the body weight of Japanese quails after 12 hours of feed withdrawal. In this experiment, despite a linear decrease in body weight, there were no changes in carcass weights, the main commercial product. As the carcass weight did not change, the increase in carcass yield and its stabilisation after the third hour of fasting (Figure 1) was the result of gastrointestinal tract emptying.

Corticosterone, a glucocorticoid secreted during stress responses, has a range of actions that help birds respond to stressors (Hull *et al.*, 2007). Corticosterone concentrations found in this experiment were close to those presented by Satterlee & Jones (1997) for Japanese quails immobilised for four minutes. Post-treatment handling (weighing, transportation, and stunning) was the same for all groups and quails showed similar corticosterone values, possibly for these being unfamiliar activities.

Table 4 – Meat quality of European quails subjected to different pretransport feed withdrawal times.

	Feed Withdrawal (h)					SEM	*p*-value	
	0	3	6	9	12		L	Q
pH	5.9	5.9	6.1	6.0	6.1	0.21	0.009	0.035
EC (mV)	64.9^{a}	66.4^{a}	61.4^{ab}	59.6^{bc}	54.9^{c}	6.69	0.003	0.530
L*	44.4^{a}	43.2^{ab}	41.1^{ab}	42.2^{ab}	39.2^{b}	3.25	0.035	0.009
a*	5.4	4.8	5.3	4.6	4.0	1.87	0.231	0.415
b*	11.5	10.3	10.3	10.5	10.7	1.37	0.572	0.227
WHC (%)	12.0^{a}	13.5^{ab}	13.2^{ab}	13.5^{ab}	14.7^{b}	1.75	0.009	0.035
CL (%)	28.4^{a}	21.1^{b}	18.1^{b}	20.7^{b}	18.8^{b}	4.99	0.001	<0.001
Aw	0.9	0.9	0.9	0.9	0.9	0.00	0.371	0.624

Mean values followed by different letters were significantly different according to Tukey's test ($p<0.05$).

SEM. standard error of the means; EC. electric conductivity; L*. lightness; a*. intensity of red; b*. intensity of yellow; WHC. water holding capacity; CL. cooking loss; Aw. water activity; L. linear effect of feed withdrawal time; Q. quadratic effect of feed withdrawal time.

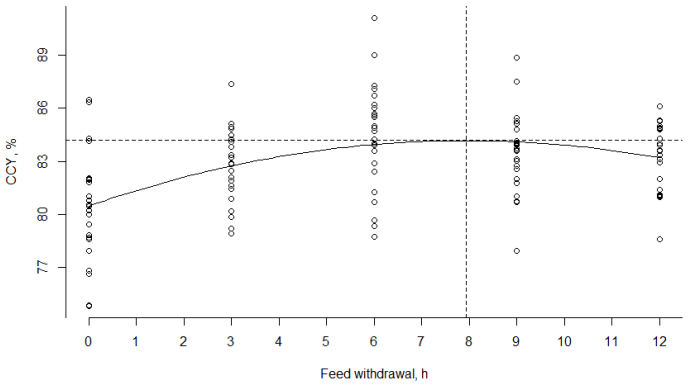

Figure 1 – Cold carcass yield (CCY) of European quails subjected to different pretransport feed withdrawal times.

When facing stress-causing agents, the birds, activate the breakdown of hepatic glycogen and gluconeogenesis, consequently increasing blood glucose (Yalçin *et al.*, 2004; Bejaei *et al.*, 2014), as seen in this experiment. However, other scientific studies have shown that glucose concentrations remained stable in different periods of broiler feed restriction during growth (De Jong *et al.*, 2003; Rajman *et al.*, 2006) or fasting for several days in Japanese quails (Sartori *et al.*, 1995); therefore, it is suggested that the glycaemic response is different in face of possible acute or chronic stressors.

Chicks (*Gallus gallus*) that have been fasting for two days (Yaman *et al.*, 2000) or due to food restriction during growth (Rajman *et al.*, 2006) have decreased plasma concentrations of total proteins and albumin. Possibly, these parameters did not change in this research due to the treatments being less intense than those presented above. Differing from this research, Yalçin *et al.* (2004) reported an increase in uric acid concentrations when they subjected broilers to preslaughter stressors.

The meat quality of the quails was affected by the increase in feed withdrawal time. No parameters were found in the scientific literature stipulating whether quail meat in this research may be characterised as dark, firm or, dry meat, such as the existing parameters for chicken (Sheard *et al.*, 2012; Jiang *et al.*, 2017). It should be noted that, with the increase in feed withdrawal time, meat showed increased pH values and water holding capacity and decreased losses due to cooking and electrical conductivity, in addition to becoming progressively darker. These integrated parameters characterise dark, firm and, dry meat (Zhang & Barbut, 2005). Similar to this work, Remignon *et al.* (1998) subjected Japanese quails to different stressor agents in the slaughter line and found an increase in pH and changes in water holding capacity.

Three hours of fasting were sufficient for gastrointestinal tract emptying and to minimise losses in meat quality of European quails. Therefore, it is necessary to adjust feed withdrawal so that it does not exceed this time, since there is no technical justification supporting it. Exceeding three hours of fasting in the pretransport of European quails compromises results according to the 'Five Domains' model for animal welfare assessment, as it incorporates negative welfare states. Technical recommendations on the subject must be tailored to each bird species.

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