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Impacts of pre-transport fasting time on blood parameters, carcass characteristics and meat quality of Japanese quails

Impactos do tempo de jejum pré-transporte nos parâmetros sanguíneos, características de carcaça e qualidade da carne de codornas japonesas

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Highlights

Blood glucose increased with increasing fasting time.
The longer the pre-transport fasting time, the lower the carcass weight.
Carcass quality was not affected by pre-transport fasting times.

Abstract

The objective of this research was to evaluate the effects of different fasting times before transport to the slaughterhouse on blood parameters, carcass characteristics and meat quality of Japanese quails. In total, 300 Japanese quails were used, with an average age of 14 months (discard age) and an initial body weight of 185.3 ± 7.3 g. The quails were distributed in a completely randomised design with six treatments and five replications of 10 birds each. The treatments consisted of different fasting periods on the farm: zero (control), 1 hour and 30 minutes, 3 hours, 4 hours and 30 minutes, 5 hours and 30 minutes and 7 hours. The quails showed higher blood glucose concentrations with increasing fasting time, but there was no increase in total proteins, albumin, lactate, creatine kinase, uric acid, globulin and the albumin/globulin ratio. The weight and yield of the hot carcass and the weight of the cold carcass decreased with increasing fasting time. Quails that fasted for 7 hours had lower hot carcass and cold carcass weights compared to quails that did not fast. There was no influence of fasting time on meat quality. A fasting period of 5 hours and 30 minutes before transportation is recommended to ensure proper emptying of the digestive tract and the maintenance of the carcass weight of Japanese quails.
Key words: *Coturnix coturnix japonica.* Food restriction. Pre-slaughter. Welfare.

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Resumo

O objetivo desta pesquisa foi avaliar os efeitos de diferentes tempos de jejum antes do transporte para o abatedouro sobre parâmetros sanguíneos, características de carcaça e qualidade da carne de codornas japonesas. No total foram utilizadas 300 codornas japonesas, com idade média de 14 meses (idade de descarte) e peso corporal inicial de 185,3 ± 7,3 g. As codornas foram distribuídas em delineamento inteiramente casualizado com seis tratamentos e cinco repetições de 10 aves cada. Os tratamentos consistiram em diferentes períodos de jejum na granja: zero (controle), 1 hora e 30 minutos, 3 horas, 4 horas e 30 minutos, 5 horas e 30 minutos e 7 horas. As codornas apresentaram maiores concentrações de glicose com o aumento do tempo de jejum, mas não houve aumento nas proteínas totais, albumina, lactato, creatina quinase, ácido úrico, globulina e relação albumina/globulina. O peso e o rendimento da carcaça quente e o peso da carcaça fria diminuíram com o aumento do tempo de jejum. Codornas que jejuaram por 7 horas tiveram menor peso de carcaça quente e carcaça fria em comparação com codornas que não jejuaram. Não houve influência do tempo de jejum na qualidade da carne. Recomenda-se um período de jejum de 5 horas e 30 minutos antes do transporte para garantir o adequado esvaziamento do trato digestivo e a manutenção do peso da carcaça das codornas japonesas.

Palavras-chave: *Comunicação coturnix japonica.* Restrição alimentar. Pré-abate. Bem-estar.

Introduction

Although the rearing of Japanese quails (*Coturnix coturnix japonica*) in Brazil is more directed towards egg production, most end-of-lay quails are sent to slaughter, making meat production also relevant to quail farming. Although different pre-slaughter fasting times have been evaluated in broiler chickens (Castro et al., 2008; Pereira et al., 2013; Ramão et al., 2011; Schneider & Gewehr, 2023), there are no studies on fasting times for Japanese quails.

Solid fasting is an indispensable pre-slaughter procedure for preventing contamination and damage to carcasses. Pre-slaughter fasting reduces the risks associated with the rupture of the viscera in the slaughterhouse (Bilgili, 2002). Fasting should last long enough to ensure the absence of feed particles in the digestive tract, and its length depends on the poultry species being studied (Genchev et al., 2008).

The total duration of the fast consists of the time involved in collecting the feed from the farm, transporting it and waiting in the cold storage facilities. In Brazil, Ordinance number 365, of July 16, 2021 (Ministério de Agricultura Pecuária e Abastecimento [MAPA], 2021), establishes that the fasting period for birds must not exceed a total of 12 hours. However, there are no specifications for different species of birds in the legislation. Therefore, the use of pre-slaughter fasting times based on studies in broiler chickens for quails may not be appropriate due to the particularities of the species. Fasting used for chickens can exceed the period necessary to empty the quail's gastrointestinal tract, leading to stress due to hunger, which goes against animal rights related to the "Five Domains" (Mellor, 2017).

In studies with meat quails (*Coturnix coturnix coturnix*), such as those by Pasquetti et al. (2014), J. D. T. Silva et al. (2012) and Vasconcelos et al. (2014), fasting periods of 8, 10 and 6 hours were used, respectively. However, according to Mir et al. (2017), fasting intervals between 8 and 12 hours can affect meat quality, significantly reducing the muscle energy stores used during postmortem metabolism, therefore accelerating the onset of *rigor mortis.*

A. A. Silva et al. (2022) investigated the effects of different periods of pre-transport fasting on (European) meat quails and concluded that a 3-hour fast is sufficient to preserve meat quality. However, the quails used belonged to a different species, highlighting the need for research with Japanese quails, which may present different responses due to their specific characteristics.

In this study, we evaluated the effects of different pre-transport fasting times on the blood biochemistry profile, carcass characteristics and meat quality of Japanese quails.

Material and Methods

Research on animals was conducted according to the Institutional Committee on Animal Use (191/2019).

Location, birds and experimental management

The experiment was conducted in the city of Janaúba, Minas Gerais, Brazil, at latitude 15°52'38"S and longitude 43°20'05" W. A total of 300 Japanese quails (*Coturnix coturnix japonica*) aged 14 months were used (at the end of posture/at discard age). The birds were housed in cages 90 cm wide, 25 cm deep and 15 cm high, with 10 birds per cage. Quails were fed a laying diet containing 220 g kg^{-1} crude protein and 2,720 kcal ME kg^{-1} (J. H. V. Silva & Costa, 2009) for 21 days for weight standardisation. Two days before slaughter, quails were weighed to obtain the average body weight before treatment (185.3 ± 7.3 g).

Experimental design and treatments

The quails were allotted to a completely randomised design with six treatments and five replicates. The treatments consisted of different pre-transport fasting times: zero (control), 1 hour and 30 minutes, 3 hours, 4 hours and 30 minutes, 5 hours and 30 minutes and 7 hours of fasting before transport to the slaughterhouse. During fasting, water was available *ad libitum.* The quails were weighed again after the corresponding fasting time to obtain the post-fasting weight.

Transport, slaughter and blood collection

The quails were placed in transport crates with an available area of 70 cm^2 per animal (Petherick & Phillips, 2009). Mean temperature and humidity at transport were 22.2°C and 73.9%, respectively. The crates were randomly distributed in each of the two vehicles used for transportation. Transport started at 7 a.m. and finished at 7:56 a.m., with a total distance of 30 km. Slaughter was carried out in a provincially inspected slaughterhouse.

Slaughter started at 8:40 am. The quails were stunned with an electric current (265 V, 60 MA), using head-only stunning for 4 seconds (Tserveni-Gousi et al., 1999). Subsequently, the quails were bled by severing both carotid arteries and jugular veins. Blood samples were collected at bleeding from six quails per experimental unit, totalling 180 quails. On the day of blood collection, the material was sent to the university's own analysis laboratory, where the tubes were centrifuged at 3,000 rpm for 10 minutes to obtain the blood serum and plasma. Subsequently, the samples were frozen at -18ºC for 15 days until the analyses were conducted. After thawing, the concentrations of uric acid, glucose, total protein (Doles® commercial spectrophotometer), albumin and lactate (Bioclin® kits spectrophotometer) and creatine kinase (Doles® commercial spectrophotometer) were determined. The globulin concentration was calculated by the mathematical difference between the total protein and the albumin serum concentration.

Carcass and meat

The 300 carcasses were chilled for 15 minutes. Immediately after dripping (5 minutes), the carcasses were weighed to obtain the cold carcass weight.

Meat colour (L*, lightness; a*, redness; b*, yellowness) was evaluated on the medial surface (bone side) of each breast fillet, using a Hunter Miniscan EZ colorimeter. Measurements were made on the bone side to avoid colour changes on the surface associated with scalding (Fletcher et al., 2000).

For carcass and meat evaluation, the carcasses were thawed under refrigeration (1oC) for 48 hours (Ramos & Gomide, 2007). Subsequently, they were weighed with the head, neck and feet to obtain the weight after thawing. After this, the carcasses without head, neck and feet were weighed to obtain the cold carcass weight. Meat quality analyses were performed on the left *Pectoralis major* muscle, as described by Narinc et al. (2013). The pH and conductivity were measured at three muscle points (cranial, medial and caudal) by insertion of the glass electrode into the sample. Water-holding capacity was estimated using the filter paper method (Matos et al., 2015). Cooking loss was determined as the difference between sample weight before and after cooking (Ramos & Gomide, 2007).

Statistical analysis

Analysis of variance (ANOVA) was performed using the generalized linear models (GLM) procedure of the RStudio software to test the effects of floor pens, transport, pre-transport fasting times and their interactions with blood variables as well as carcass and meat characteristics. The general model used was as follows:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \alpha_i \beta_j + \alpha_{i\gamma k} + \beta_j \gamma_k + \alpha_i \beta_j \gamma_k + \varepsilon_{ijk},$$

in which y_{ijk} is the measured dependent variable; μ is the overall mean; α_i is the effect of pre-transport fasting times; β_j is the effect of floor pens; γ_k is the effect of transport; $\alpha_{i\gamma k}$, $\beta_{j\gamma k}$ and $\alpha_i\beta_j\gamma_k$ are the effects of interactions; ε_{ijk} is the random error associated with each observation.

The effects of floor pens and transport were not significant for blood variables as well as carcass and meat characteristics ($P \geq 0.05$); therefore, floor pens and transport were excluded as factors. When the model was significant ($P < 0.05$), orthogonal polynomial contrasts were used to test the linear effects of treatment on blood variables as well as carcass and meat characteristics. When the model was significant ($P < 0.05$), means were also compared using Dunnett's test ($P < 0.05$).

The Shapiro-Wilk test was used to check the normality of the data, and non-normally distributed data were log-transformed. For the analysis, each quail was considered an experimental unit. The data were analysed using the mixed model procedure. The statistical model was composed of the main effect (pre-transport fasting times) and the random effects: two vehicles and the possible interactions of the two factors. Body weight measured 2 days before slaughter (before treatment) was also included in the statistical model as a covariate ($P < 0.05$). Data were subjected to a linear regression test if the F-test was significant ($P < 0.05$). The slope coefficient was tested by the t-test ($P < 0.05$). In addition to the linear regression analysis, if the f-Test was significant, Dunnett's test ($P < 0.05$) was also performed to create confidence intervals for the differences between the mean of the control group (no fasting) and the means of the other treatments.

Results and Discussion

The blood glucose concentrations in quails increased linearly with increasing fasting time (Table 1). Uric acid, total proteins, albumin, lactate, creatine kinase, globulin and albumin/globulin were not significantly different among the groups.

Table 1
Blood biochemistry profile of Japanese quails at disposal age subjected to different pre-transport fasting intervals

Variable	Pre-transport fasting (hours)						SEM	P-value
	Zero	1 h 30	3 h	4 h 30	5 h 30	7 h		
Uric acid (mg dL^{-1})	16.21	13.87	14.62	17.54	14.19	15.25	0.48	0.405
Glu1 (mg dL^{-1})	202.28	195.56	226.75	219.85	234.03	252.31	6.38	0.017
TP (g dL^{-1})	5.62	5.01	6.46	7.24	5.62	5.50	1.05	0.645
Alb (g dL^{-1})	1.92	0.74	1.92	0.74	1.92	0.74	0.02	0.645
Lact (mg dL^{-1})	37.30	32.05	48.70	44.18	43.49	42.90	1.76	0.262
CK (UL^{-1})	758.58	446.68	467.74	1174.90	724.44	1122.02	1.17	0.347
Glob (g dL^{-1})	3.55	3.55	4.47	5.50	4.07	3.72	1.07	0.429
Alb/glob (g g^{-1})	1.55	1.51	1.41	1.35	1.38	1.48	1.02	0.450

Glu - glucose; TP - total proteins; Alb - albumin; Lact - lactate; CK - creatine kinase; Glob - globulin; Alb/glob - albumin globulin ratio. 1Yglucose = 195.95 + 0.12 (treatment), R2 = 0.84 ($P < 0.05$).

According to Reed (2009), when the carbohydrate stores are depleted due to fasting or starvation, glycogenolysis and gluconeogenesis occur. These processes are responsible for providing glucose to the central nervous system and red blood cells, using glucogenic amino acids, glycerol and lactate. Increases in blood glucose concentrations are associated with the fight-or-flight response, triggered by acute stress reactions (Broom & Fraser, 2010). In the case of our study, the potential stressor was fasting. Although the glucose concentration increased, there were no increases in the concentrations of plasma proteins, albumin and globulin. Animals under stress may show alterations in the levels of total blood proteins due to increased gluconeogenesis and decreased incorporation of proteins into tissues (Barnett et al., 1982).

Similar to what was observed in this study, A. A. Silva et al. (2022) also found an increase in blood glucose in meat quails with increasing pre-transport fasting (3, 6, 9 and 12 hours). However, in a study with broiler chickens, Savenije et al. (2002), who evaluated the effects of transportation and pre-slaughter fasting on broiler chickens (up to 6.5 hours), did not report any changes in blood glucose levels. The authors stated that although fasting and transportation were stressful stimuli, the amount of available energy (available glucose) was not compromised by the short intervals evaluated in their experiment. Saki et al. (2011) observed an increase in blood glucose in chickens up to 4 hours of fasting, remaining unchanged until 24 hours of fasting. This research demonstrates how different species respond differently to stressful factors, in this case, hunger.

Plasma CK and lactate levels may increase in response to stress (Awerman & Romero, 2010), which was not found in this study. The CK concentration is an indicator of possible muscle damage in animal tissues (Xing et al., 2014), whereas the lactate concentration is associated with fatigue (Broom & Fraser, 2010). Alterations in both parameters may also result in meat quality issues (Xing et al., 2014). Delezie et al. (2007) also reported no changes in the CK concentrations when evaluating pre-slaughter events of acute stress in broilers.

Hot carcass weight and yield and cold carcass weight decreased with increasing fasting times (Table 2). Quails fasted for 7 hours had lower hot and cold carcass weights compared to those of non-fasted animals. Moisture retention and cold carcass yield did not significantly differ among groups.

Table 2
Carcass characteristics of Japanese quails at disposal age submitted to different pre-transport fasting intervals

Variable	Pre-transport fasting (hours)						SEM	P-value
	Zero	1 h 30	3 h	4 h 30	5 h 30	7 h		
HCW (g)[1]	112.92a	109.79a	108.94a	113.55a	111.09a	105.07b	0.79	0.013
MR (%)	8.73	9.41	8.61	9.75	9.21	9.27	0.15	0.204
HCY (%)[2]	65.32	63.09	62.78	67.07	63.03	62.01	0.72	0.014
CCY (%)	71.56	69.66	68.70	74.36	70.98	69.77	0.93	0.596
CCW (g)[3]	108.33a	107.07a	108.23a	108.63a	107.11a	102.33b	0.61	0.017
CCWW (g)[4]	93.17a	91.52a	92.63a	92.34a	92.10a	87.27b	0.55	0.012

HCW - Hot carcass weight; MR - moisture retention; HCY - hot carcass yield; CCY - cold carcass yield; CCW - cold carcass weight; CCWW - cold carcass weight without head, neck and feet
Means followed by different letters within one row differ from the control (zero fasting) by Dunnett's Test ($P < 0.05$)
[1]YHCW = 112.42 - 0.01 (pre-transport fasting), $R^2 = 0.28$; ($P < 0.05$). [2]HCY = 65.13 - 0.36 (pre-transport fasting), $R^2 = 0.12$, ($P < 0.05$); [3]YCCW = 109.04 - 0.01 (pre-transport fasting), $R^2 = 0.45$;($P < 0.05$). [4]YCCWW = 93.51 - 0.01 (pre-transport fasting), $R^2 = 0.49$ ($P < 0.05$).

Garcia et al. (2008) also reported that the longer the broilers were fasted before slaughter, the lower the hot carcass weight, with no differences in carcass yield among treatments (4 to 17 hours of fasting). In a study evaluating different pre-slaughter fasting intervals (3 to 18 hours) in broilers, Castro et al. (2008) reported a decrease in hot and cold carcass yields with increasing fasting length. Moreover, hot carcass yield was higher in broilers fasted for 3 and 6 hours, and reductions were observed after 9 hours of fasting. Saki et al. (2011) did not observe an effect on the carcass characteristics of chickens that were fasted for 4 to 24 hours, concluding that a 4-hour fast can be used before slaughtering these birds.

In this study, despite the linear decrease in the carcass yields of quails fasted for up to 5 hours and 30 minutes, there was no significant change in carcass weights. As these weights did not change, the decrease in carcass yield was a result of the emptying of the gastrointestinal tract. Therefore, the gastrointestinal tract was emptied more efficiently after 5 hours and 30 minutes, with no loss of carcasses and fewer chances of contamination with gastrointestinal contents.

Quails fasted for 7 hours had lower hot and cold carcass weights compared with non-fasted animals. This result is similar to that reported by Warriss et al. (1999), who stated that in the first 4 to 6 hours of fasting, the birds' weight loss is mainly caused by the emptying of the gastrointestinal tract, with no negative influence on the carcass. However, the authors reported a loss of moisture and nutrients in body tissues after 6 hours of fasting, which may affect carcass characteristics. In this study, despite the changes in carcass weights and yields, especially in quails fasted for 7 hours, no

differences in the hydration status were observed since there was no change in plasma protein concentration (Thrall et al., 2015). Moreover, quails had access to water during fasting. Possibly, the mobilisation of body reserves was higher, which is supported by the blood glucose concentration and carcass weight data. Although the carcass weight progressively decreased over time, it reached its minimum after 7 hours of fasting; therefore, the carcass weights of quails fasted for 7 hours were significantly lower than those of the control animals (fasting time zero).

A. A. Silva et al. (2022) found no differences in the carcass weights of European quails fasted for 3 to 12 hours. In relation to carcass yield, an increase was observed up to 3 hours of fasting, remaining unchanged until 12 hours. In this experiment, although the quails showed a linear decrease in body weight, there were no changes in carcass weight, the main commercial product. As carcass weight did not change, the increase in carcass yield and its stabilisation after the third hour of fasting resulted from the emptying of the gastrointestinal tract.

The different pre-transport fasting times did not influence the meat quality traits (pH, L*, a*, b *, electric conductivity, water-holding capacity and cooking loss) of quails (Table 3).

Table 3
Meat quality traits of Japanese quails at disposal age submitted to different pre-transport fasting intervals

Variable	Pre-transport fasting (hours)						SEM	P-value
	Zero	1 h 30	3 h	4 h 30	5 h 30	7 h		
pH	6.16	6.24	6.17	6.18	6.20	6.18	0.02	0.744
L*	35.19	36.05	35.96	35.79	36.13	35.69	0.28	0.941
a*	7.69	7.42	7.09	7.75	7.75	7.74	0.16	0.782
b*	10.16	10.44	10.19	10.19	10.61	10.34	0.12	0.871
ECC (mV)	58.45	56.96	57.85	52.86	57.63	54.88	0.78	0.288
WHC (%)	21.84	21.75	27.42	25.03	26.32	26.01	0.78	0.251
CL (%)	21.23	19.97	24.42	18.03	21.12	22.04	0.87	0.424

L* - lightness; a* - redness; b * - yellowness; ECC - electric conductivity in cold carcass; WHC - water-holding capacity; CL - cooking loss.

Gewehr et al. (2023) and Schneider and Gewehr (2023), evaluating fasting times for broiler chickens (0 to 16 hours), and Oliveira et al. (2015), investigating fasting in fasting studies for free-range chickens (0 to 12 hours), also observed no effect of fasting on pH, colour, water retention capacity and cooking losses in breast meat samples. In general, due to the interdependence between meat quality characteristics and

pH (Sterten et al., 2009), maintaining the pH values results in unchanged levels of other parameters.

The main effects of stress on meat quality are related to colour, water-holding capacity and pH. Despite the similarity in the ultimate pH, the values observed are high (approximately 6.1) compared with those reported by Remignon et al. (1998) for quail meat (< 5.8), regardless of the fasting time. The lightness of the meat was also lower compared with that reported in studies using quail meat (L* ≅ 45) (Remignon et al., 1998; Genchev et al., 2008). The results of this study suggest a possible classification of the meat studied as a DFD (dark, firm and dry) meat; however, in the scientific literature, this classification only exists for broiler meat (Jiang et al., 2017; Sheard et al., 2012). Unlike what was observed in this research, A. A. Silva et al. (2022) found an increase in pH and water-holding capacity and a decrease in electrical conductivity, cooking loss and lightness of European quail meat as the fasting increased from 0 to 12 hours. The authors also commented that the results found for pH (5.9 to 6.1) and lightness (39.2 to 44.4) could configure the meat as DFD. Based on these results, studies must be carried out to create a classification for quail meat as it may be different from broiler meat due to its particularities.

Conclusions

Japanese quails subjected to pre-transport fasting exhibited higher glucose concentrations and lower carcass weights with increased fasting time, but no changes in meat quality were observed. Considering the studied pre-transport fasting interval, a duration of 5 hours and 30 minutes is recommended for the proper emptying of the digestive tract and the maintenance of the carcass weight.

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