


Rate of feed passage in Japanese quail

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*The rate of passage (ROP) in the gastrointestinal tract (GIT) influences the exposure time of food to the digestion and absorption processes. Consequently, ROP affects the efficiency of nutrient utilization and energy from the diet. This study aimed to determine the physiological parameters that characterize the digestive response, such as first appearance time (FAT), ROP, mean retention time (MRT) and transit time (TT) in adult Japanese quail (*Coturnix coturnix japonica*), and to evaluate the effects of sex, apparent metabolizable energy corrected for nitrogen balance (AME_n) content in the diet and different types of markers on these parameters. In the first trial, we investigated the effects of sex and AME_n level (high- and low-energy diet) on the FAT parameter. Thirty-two male and 32 female Japanese quail were randomly allocated to 8 battery cages and assigned to 4 treatments in a 2×2 factorial design with 4 replicates of 4 birds for each treatment. To determine the FAT, ferric oxide (1%) was added to the diet, and the excreta of the quail was monitored until the first appearance of the marker. The results indicated significant differences ($P < 0.05$) in the FAT between males (100 min) and females (56 min), regardless of the AME_n content. In the second trial, thirty-two 32-week-old female Japanese quail in the laying phase were assigned to four treatments in a 2×2 factorial design, in which the main independent variables were type of marker (Cr or Ti) and AME_n level (high- and low-energy diets). In order to determine ROP ($ET_{1\%}$), MRT and TT ($ET_{100\%}$), the markers (0.5%: Cr_2O_3 and 0.5%: TiO_2) were added to the diets, and the excreta were collected for 750 min. The excretion times for 1% ($ET_{1\%}$), 25% ($ET_{25\%}$), 50% ($ET_{50\%}$), 75% ($ET_{75\%}$) and 100% ($ET_{100\%}$) were estimated using cumulative excretion curves. No effect was detected for the AME_n level ($P > 0.05$); however, the effect of different marker types was significant ($P < 0.05$). This difference increased with time and $ET_{100\%}$ was estimated to occur at 59 min. The ROP was estimated to be 68 min. The TT was estimated to be 540 min using Cr and 599 min using Ti, with an average MRT value of 0930 h. Taken together, our findings support the hypothesis that Japanese quail digestion through the GIT can be dynamic and differ based on sex or marker type.*

Keywords: coturnix quail, mean retention time, markers, metabolizable energy, passage rate

Implications

This study shows that the rate of passage in Japanese quail differs from that of other commercial poultry species. In addition, the time required for feed to pass through the intestinal tract was not affected by metabolizable energy levels, despite the effect of marker type being significant. There is a lack of information on the dynamics of feed passage in Japanese quail; our results can be used in models to predict nutrient absorption and develop digestion simulation models to improve diet formulations for specific nutritional strategies.

Introduction

Egg production has been increasing in South America, particularly in Brazil (FAO, 2019). Commercial laying hens and Japanese quail (*Coturnix coturnix japonica*) are primarily responsible for this growth, with the latter expanding to comprise approximately 10% of all laying hens in Brazil, which was around 152 million in 2017 (IBGE, 2017). Although both commercial laying hens and Japanese quail have been selectively bred for egg production, some characteristics accentuate the differences between these birds, of which efficiency of utilization may be most important. Taking the apparent metabolizable energy corrected for nitrogen balance (AME_n) as an example, hens require 10.03 MJ/kg of egg mass (Sakomura, *et al.*, 2005), whereas Japanese quail require 17.51 MJ/kg (Jordão Filho *et al.*, 2011).

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Nutrient utilization depends on the interactions between digestive physiology, microbiota and the physical-chemical properties of food (Kaupp and Ivey, 1923; Kolakshyapati *et al.*, 2019). Thus, measures to improve the efficiency of nutrient content utilization in the diet are necessary to more accurately and efficiently develop diets for these birds. In this context, there is a need to understand the digestive response of Japanese quail to food, and first appearance time (FAT), rate of passage (ROP) mean retention time (MRT) and transit time (TT) are some simple physiological parameters that are useful for understanding the processes of digestion and absorption.

Some studies of this nature have been published but using laying hens. Kaupp and Ivey (1923) showed that MRT varies within species, according to the following physiological stages: growing (MRT: 0350 h), egg-laying (MRT: 0800 h) and brooding (MRT: 1100 h). Currently, MRT values of 0536 h for broilers and 0559 h for laying hens fed diets based on corn and soybean meal are accepted (Shires *et al.*, 1987; Rochell *et al.*, 2012). No reports have been published concerning the ROP, FAT, MRT and TT of Japanese quail, except for a study by Savory and Gentle (1976), who used excreta collection at spaced intervals, beginning 3 h after supplying the marked diet. However, these authors were unable to detect differences between sexes and ages, nor did they detect a response to different dilutions of AME_n using fiber sources in the diet. Therefore, questions remain about the variables that influence the ROP, FAT, MRT and TT in Japanese quail.

Due to its simplicity, the most common parameter used to characterize the responses of gastrointestinal tract (GIT) dynamics has historically been FAT. However, this parameter is sensitive and, depending on the objective, the FAT variable may be limited. Thus, determining ROP, MRT and TT with the use of indicators may be more informative. Although studies of Japanese quail are scarce, a recently published study of commercial laying hens showed the importance of evaluating the marker, fecal or radiographic, in order to understand the dynamics of the GIT (Kolakshyapati *et al.*, 2019). Given this information, the present study aimed to characterize FAT, ROP, MRT and TT in adult Japanese quail, evaluating the effects of sex and AME_n level in the diet on FAT and analyzing the effects of marker type and AME_n level on ROP, MRT and TT.

The preliminary results were published in an abstract form according to Nóbrega *et al.* (2019).

Material and methods

Two trials were performed in order to determine the FAT, ROP, MRT, excretion time (ET) and TT of food in Japanese quail fed low or high AME_n diets.

Trial 1: Determining the first appearance time of markers in excreta

Experimental design. Sixty-four (32 male and 32 female) 32-week-old quail were randomly assigned to four treatments

in a 2 × 2 factorial design to test the effects of sex (male and female) and low AME_n level in the diet (low and high AME_n), with four replicates of four birds for each treatment.

Experimental diets. One diet was formulated to contain a high AME_n level (15.06 MJ/kg) corresponding to 1.2 times the recommendation proposed by Silva and Costa (2009). Another diet was formulated to contain a low AME_n level (8.79 MJ/kg) containing 0.72 times the same recommendation (Table 1). Apart from AME_n level, both diets were formulated to meet or exceed the nutrient recommendations of Silva and Costa (2009).

Bird husbandry. A total of 64 Japanese quail were purchased from VICAMI® and were separated into 8 battery cages (100 × 100 × 50 cm) with 4 birds per cage, in which the birds had free access to food and water provided by trough feeders and nipple drinkers. **Prior to the study, the quail were selected based on average BWs of 134 g (± 10 g) for males and 154 g (± 10 g) for females,** with an average egg production of 86% homogenously distributed among the treatments. Battery cages were placed in a conventional room, in which the average temperature was 25 ± 3°C and the average air relative humidity was 54 ± 11%. The lighting program was set to 17L : 7D.

Determination of first appearance time. Ferric oxide was added to the diets in order to determine the first appearance of markers in the excreta, and marker inclusion was 1%. The quail were previously adapted to a standard diet (SD) for approximately 3600 h before the start of the trial. When the trial started at 0500 h, the quail were subjected to a 0200 h fasting period, and marked diets were provided at 0700 h. Then, trays were placed below the cages to collect excreta, and the excreta were monitored until the first appearance of the markers. The FAT was determined as the time between when the marked diet was supplied and the first appearance of the marker in the excreta.

Trial 2: Determining the rate of passage, mean retention time, excretion time and transit time

Experimental design. Thirty-two 32-week-old female quail were randomly assigned to four treatments in a 2 × 2 factorial design, in which the evaluated factors were two AME_n levels in the diet (low and high AME_n) and two indicators (chromium oxide and titanium oxide), with four replicates of four birds per treatment. Each marker made up 0.5% of its respective diet.

Experimental diets. The same diets as the first trial were also used in this trial. These consisted of a low AME_n diet (8.79 MJ/kg) and a high AME_n diet (15.06 MJ/kg).

Experimental procedures and evaluated variables. The quail were adapted to the SD for approximately 3600 h before the start of the trial. When the experiment began at 0500 h, the quail were subjected to a 0200 h fasting period, and marked

Table 1 Composition and nutritional content of experimental diets to Japanese quail

Composition (%)	Low energy	High energy
Corn (8.8%)	25.51	44.07
Soybean meal (45%)	29.56	24.08
Wheat bran	8.74	
Rice husk	19.36	
Inert ¹	2.47	0.5
Soy oil	–	12.79
Corn gluten (60%)	3.78	7.10
Limestone	6.83	7.27
Dicalcium phosphate	1.17	1.29
Salt	0.31	0.35
Choline chloride (60%)	0.10	0.10
Vitamin and mineral supplements ²	0.40	0.40
D,L-Methionine (99%)	0.36	0.33
L-Lysine HCl (78%)	0.28	0.42
L-Arginine (100%)	0.10	0.23
L-Threonine (98.5%)	0.02	0.03
L-Tryptophan (98.5%)	0.02	0.05
Chromium oxide	0.50	0.50
Titanium dioxide	0.50	0.50
Calculated nutritional contents		
Metabolizable energy (MJ/kg)	8.79 (8.86) ³	15.06 (14.37)
CP (%)	21.00 (20.55)	20.25 (20.56)
Digestible methionine + cysteine (%)	0.91 (0.92)	0.91 (0.92)
Digestible lysine (%)	1.11 (1.09)	1.11 (1.10)
Digestible threonine (%)	0.67 (0.65)	0.67 (0.66)
Digestible tryptophan (%)	0.23 (0.23)	0.23 (0.23)
Digestible valine (%)	0.83 (0.81)	0.83 (0.81)
Digestible isoleucine (%)	0.77 (0.75)	0.76 (0.74)
Digestible arginine (%)	1.29 (1.26)	1.29 (1.28)
Crude fiber (%)	10.59	1.94
Ca (%)	3.20	3.20
Available P (%)	0.33	0.33
Na (%)	0.16	0.16
K (%)	0.72	0.59

¹Inert – Washed sand.

²Content (per kg of the diet) – vitamin A, 7000 IU; vitamin D₃, 2000 IU; vitamin E, 8 IU; vitamin K₃, 2 mg; vitamin B₁, 1 mg; vitamin B₂, 3.5 mg; vitamin B₆, 2 mg; vitamin B₁₂, 5 mcg/kg; niacin, 25 mg; Cl, 0.26 g; pantothenate acid, 10 mg; Cu, 8 mg/kg; Fe, 50 g; Mn, 70 g; Zn, 50 g; I, 1.2 mg and Se 0.2 mg.

³Values in parentheses represent analyzed values.

diets were provided at 0700 h for the following 2 h. At 0900 h, these diets were replaced by the SD until the end of the trial. One hour after the quail started to feed on the marked diets (0800 h), all the excreta on the trays were collected at the following intervals: 30, 60, 90, 120, 150, 180, 240, 300, 360, 450, 540, 630 and 750 min. The excreta collected at each time point were properly identified, weighed and conserved in plastic bags at a temperature of –20°C before analysis.

We used a homogeneous pool of excreta per treatment due to the reduced sample volume prior to analysis. Dry matter content was determined according to the AOAC (2004), while Cr and Ti concentrations were quantified based on the methodologies outlined in Olukosi *et al.* (2012) and Myers *et al.* (2004), respectively.

The DM excreted (DME, g) at each time interval was obtained by multiplying the quantity of excreta (g) and the DM content calculated for each pool. To determine the excretion of Cr and Ti at each time point, the Cr and Ti concentrations were multiplied by the DME, resulting in markers excreted (M_{Ex}, g/g), Cr excreted (E_{Cr}, g) or Ti excreted (E_{Ti}, g) values. Then, the concentrations of Cr and Ti were relativized as percentages of the total amount excreted in the experimental period, which resulted in an excreted fraction of the total for each marker (f_{M_{Ex}}).

The MRT (min) was calculated using the following equation, described in Coombe and Kay (1965): $MRT = \frac{\sum(M_{Ex} \times t)}{\sum M_{Ex}}$, where M_{Ex} is the amount of excreted marker and *t* is the time in minutes.

Modeling the excretion and estimation of physiological parameters. The ROP, ET and TT were obtained by relating fM_{EX} as a function of time, as described in Ferrando *et al.* (1987). The relation between fM_{EX} and time was adjusted using a Gompertz function, according to the following equation: $fM_{EX} = cME \times e^{-e^{-k \times (t - T_{max})}}$, where cME is cumulative marker excretion, k is the inflection point of the curve and T_{max} is the time to achieve an inflection point, which also represents the time at which the excretion rate is at its peak.

The physiological parameters proposed by Ferrando *et al.* (1987) were determined by the inverse of the Gompertz function, subtracting the desired fM_{EX} to determine the corresponding ET, using the following formula: $ET = -(-kT_{max} + \ln(-\ln(fM_{EX}/cME)))/k$, where ET is the excretion time in minutes, and fM_{EX} is the input that determines the corresponding ET. The values used in this study were 0.01 (ET_{1%}), 0.25 (ET_{25%}), 0.5 (ET_{50%}), 0.75 (ET_{75%}) and 1 (ET_{100%}) of fM_{EX} . On the basis of this model, the ROP was considered to be ET_{1%} and TT was equal to ET_{100%}.

Statistical analysis

The FAT, M_{EX} and MRT variables were analyzed to verify the normality of errors and homogeneity of variances using Cramer-von Mises and Brown and Forsythe's tests, respectively. Once these presuppositions were satisfied, the data were submitted to ANOVA according to the following statistical model: $Y_{ijk} = \mu + \tau_i + \psi_j + (\tau\psi)_{ij} + \epsilon_{ijk}$, where Y_{ijk} is the observed response of the k th repetition of the i th level of the main factor and the j th of the secondary factor; μ is the effect of the general mean; τ_i is the effect of i th level of the main factor; ψ_j is the effect of j th level of the secondary factor; $(\tau\psi)_{ij}$ is the interaction effect between τ_i and ψ_j ; and ϵ_{ijk} is the experimental error associated with the Y_{ijk} observation.

It is worth noting that for the first trial, the main and secondary factors were the effects of sex and AME_n, respectively, while in the second trial, the main and secondary factors were AME_n level and marker type, respectively. In the second trial, a regression analysis was applied to interpret the excretion of Japanese quail as the function of time using the Gompertz function (Gompertz, 1825).

Results

Effects of sex and apparent metabolizable energy corrected for nitrogen balance content on the first appearance time of the marked excreta

The results shown in Table 2 indicate that AME_n level did not affect FAT in Japanese quail ($P > 0.05$), with the average FAT being 78 min. However, the values of FAT were significantly different between the sexes ($P < 0.05$). Male FAT values were approximately 1.79 greater than female values on average, with a mean of 56 min. There was no interaction between sex and AME_n ($P > 0.05$); therefore, FAT differed between males and females only, regardless of the AME_n content of the diet.

Effects of marker and apparent metabolizable energy corrected for nitrogen balance content on marker excretion and mean retention time

The results of the ANOVA shown in Table 3 indicate that the excretion of Japanese quail differed only in relation to the marker used ($P < 0.05$), and the lowest value was obtained using Cr. The AME_n content of the diet and the interaction between marker and AME_n content did not influence ($P > 0.05$) excretion. The mean MRT was estimated to be 428 min. The difference ($P < 0.05$) between markers was 10 min, with Ti taking longer than Cr.

Table 2 Rate of passage measured by the first appearance time of the marker in the excreta in male and female Japanese quail fed diets with low and high apparent metabolizable energy corrected for nitrogen balance content

Variable	Treatment				Average	SEM	Sex		Energy		P-values		
	T1	T2	T3	T4			Female	Male	Low	High	Sex	Energy	Sex × energy
FAT	59.75	95.00	52.00	105.25	78.00	6.19	55.88	100.12	77.38	78.62	<0.01	NS	NS

T1 = low-energy diet – female quail; T2 = low-energy diet – male quail; T3 = high-energy diet – female quail; T4 = high-energy diet – male quail; FAT = first appearance time (minutes); NS = not significant.

Table 3 Evaluation of marker excretion and mean retention time for diets with low and high energy in Japanese quail

Variables	Treatment				Average	SEM	Energy		Marker		P-values		
	T1	T2	T3	T4			Low	High	Cr	Ti	Energy	Marker	Energy × marker
M_{EX}	0.0075	0.0021	0.0074	0.0011	0.0045	0.0005	0.0048	0.0043	0.0016	0.0075	NS	<0.05	NS
MRT	428	424	438	422	428	2.00	426	430	423	433	NS	<0.05	NS

T1 = low-energy diet – Ti; T2 = low-energy diet – Cr; T3 = high-energy diet – Ti; T4 = high-energy diet – Cr; M_{EX} = marker excretion (g/g); MRT = mean retention time (minutes); NS = not significant.

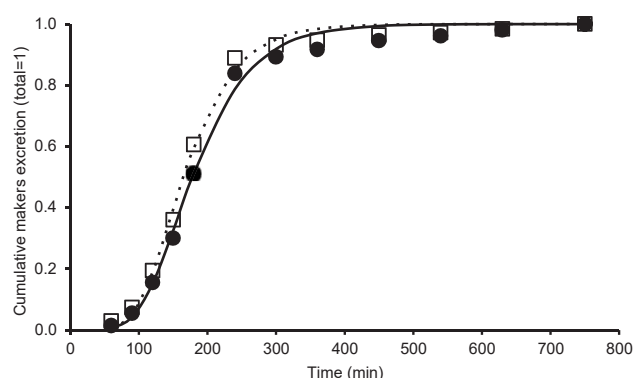


Figure 1 Cumulative Cr (● observed and — predicted) and Ti (□ observed and predicted) excretion to Japanese quail at 32 week old.

Modeling excretion and estimation of physiological parameters

The excretion of Japanese quail could be estimated by using adjusted models for each marker, Cr and Ti, as presented in Figure 1. The adjusted models were as follows:

$$\text{Cr} : fM_{\text{EX}} = 1 \times e^{-0.0193 \times (t-146.2)}$$

$$\text{Ti} : fM_{\text{EX}} = 1 \times e^{-0.0172 \times (t-156.7)}$$

Because cumulative marker excretion was estimated in Figure 1 for both markers, the obtained data allowed estimates of the total marker excretion to be calculated; thus, the corresponding t could also be calculated using these adjusted models. The k parameter, which represents the time of fM_{EX} excretion per minute, indicates that the ET for Cr was 11% faster than for Ti. The T_{max} parameter indicates the t at which the fM_{EX} parameter was maximized. At this point, the ET difference between the markers was approximately 7%. Therefore, the differences between the markers were less than 11 min until T_{max} or were inexpressible from a practical point of view.

Solving the adjusted model to obtain t when fM_{EX} was equal to 1%, 50% and 100% was helpful in obtaining ROP and TT, which can be used to interpret the dynamism of intestinal transit. Based on these models for Cr and Ti, the calculated values of t were 67 and 68 min, 165 and 178 min for ROP, and 540 and 599 min for T , respectively.

The difference in TT between the two markers was approximately 60 min, which is a considerable value in terms of the dynamics of intestinal transit (Table 4).

Discussion

This study evaluated the effects of sex, AME_n and marker type on the intestinal transit of adult Japanese quail. Moreover, this study revealed surprising results compared to other commercial poultry, such as broiler chickens and laying hens. In the first trial, the FAT differed significantly between males and females ($P < 0.05$), which was unexpected because it was expected that the null hypothesis (H_0) between the sexes would be rejected, thus justifying the use of males as an animal model for females in digestibility studies, in order to avoid collecting eggs with excreta in the trays. This null hypothesis was based on Savory and Gentle (1976), who observed more advanced anatomical development of the GIT in females compared to males, but the authors did not detect a significant difference in FAT between the sexes.

One possible explanation for this finding may be found in Savory *et al.* (1987). Their study evaluated the effect of physiological stage on voluntary consumption and, consequently, on the passage of food through the GIT. The lower frequency of feed intake by Leghorn roosters was presented as the main cause of differences between roosters and broilers in relation to the retention time of food in the GIT (Savory *et al.*, 1987). We believe that this argument may explain this finding. As male quail were in the maintenance stage while females were in the laying phase, their daily feed intakes were different (19 g/bird d for male *v.* 24 g/bird d for laying phase) and, consequently, there was a lower frequency of feed intake for males. A lower frequency of feed intake induces higher retention of food in the crop and gizzards of birds (Sibbald, 1979; Savory *et al.*, 1987).

In addition, other authors agree that the frequency of feed intake is a critical factor that influences the passage of food through the intestinal tract (Mateos *et al.*, 1982; Wetherbee *et al.*, 1990). Although there was an effect of sex on FAT, which supports the impossibility of reciprocal use of results obtained from males and females, FAT is one of the simplest variables among the physiological parameters used to evaluate digestive responses due to its methodological simplicity and susceptibility to the frequency of feed intake.

Table 4 Excretion time for 1%, 25%, 50%, 75% and 100% of Cr and Ti of the Japanese quail based on Gompertz model

Marker	ET ¹ _{1%}	ET _{25%}	ET ² _{50%}	ET _{75%}	ET ³ _{100%}
Cr	67	129	165	211	540
Ti	68	138	178	229	599
Difference ⁴	1	8	13	18	59

ET = excretion time (minutes).

¹Equivalent to rate of passage.

²Equivalent to mean retention time.

³Equivalent to transit time.

⁴Ti–Cr.

The other surprising finding obtained in this study was the similarity in FAT values between the high and low AME_n diets. It was expected that the high AME_n content diet would result in an increased FAT. However, based on Borella and Lippmann's (1980) findings, lipids (sesame oil) inhibit gastric emptying, slowing the passage of food from 64 to 28 mm/min in the GIT. Moreover, this effect of lipids was also observed in two other studies: in the first, FAT was delayed by 17 min in laying hens (Mateos and Sell, 1981) and, in the second study, Mateos *et al.* (1982) concluded that there was a linear effect of lipids in laying hens using the ¹⁴⁴Ce marker, as there was a 1.2 min increase per individual unit of lipid in the diet added to the 156 min intercept.

In this study, Japanese quail fed with a high AME_n content diet (with 12.79% soybean oil) did not differ from the quail fed a low-energy diet (without soybean oil and high fiber content). In a similar study, Tuckey *et al.* (1958) modified the diet by adding lipid sources and changing ingredients (wheat, soybean meal and animal fat) in order to maintain similar protein contents in the diets. The aforementioned study did not report any delays in FAT for the diet with 12% lipid inclusion in relation to the control diet, which did not contain any lipid sources.

In our study, fiber was not found to cause a delay in the passage of food to increase FAT. Two studies have reported on two characteristics that may lead to fiber delaying the passage of food in low-energy diets. The first characteristic is viscosity, because no neural action is mediated by fiber viscosity upon gastric emptying (Hunt and Macdonald, 1954). The second is that the increase in viscosity resulting from the solubility of non-starch polysaccharides (NSPs) is associated with low digestibility of diets (Almirall and Esteve-Garcia, 1994). However, these characteristics may not be applicable to this study because the rice husk used herein does not increase viscosity in the GIT, and this ingredient is not an NSP.

Although it was assumed that there would be a decrease in digestibility of the low AME_n diet due to the similarity between the calculated AME_n (8.79 MJ/kg) and analyzed (8.86 MJ/kg) values, the same may not be assumed for the high-energy diet since the difference between calculated and analyzed values was 0.69 MJ/kg (15.06 v. 14.37 MJ/kg), as demonstrated in Table 1. Correa-Castiblanco (2017) also verified that energy utilization is higher in Japanese quail fed on low-energy diets. The difference between the calculated and analyzed AME_n values in low- and high-energy diets used in the study of Correa-Castiblanco (2017) was +0.45 MJ/kg (6.28 to 6.73 MJ/kg) and -0.81 MJ/kg (13.60 to 12.79 MJ/kg), respectively. These findings support the hypothesis about the limit of lipid use for Japanese quail.

Because of the methodological simplicity of FAT, its inference power is limited; therefore, in the second trial, a methodology that supports more concise interpretations and inferences in relation to food dynamics in the GIT of females in the laying phase was performed. No effects of AME_n content on M_{EX} and MRT were detected, as previously determined in the first trial evaluating the FAT parameter. On

the other hand, there was a marker effect; although this effect created a difference of only 10 min (as presented in Table 3), this difference was 4.7 times higher than Ti considering the estimated excretion.

The interaction of both factors was evaluated using the Gompertz function, and until now, the models used did not allow the direct interpretation of their parameters (Almirall and Esteve-Garcia, 1994; Rochell *et al.*, 2012). It was possible to estimate the maximum recovery of the administered dose (1% or 100%) using the cME parameter, which is the asymptotic response of the model. The other parameters allowed differentiation of the markers under the same physiological conditions and the same recovery value when the cME was equal to 1. The *k* parameter represents the iM_{EX} excretion rate per minute, and it differed by 12% according to marker type, as confirmed by the ANOVA result (Table 3).

The third parameter, *T*_{max}, indicates the *t* at which iM_{EX} peaked and differed by 7% (11 min). Analyzing the *k* and *T*_{max} parameters point-to-point, the differences did not result in any real effects from a practical point of view; however, the cumulative effect of the adjusted model started with a difference between the Cr and Ti markers of 1 min for the estimated ROP, and the markers differed by 1 h in the estimated TT. Therefore, there is an express difference when the interpretation is completed with the aid of mathematical interpretation. Divergences between Ti and Cr markers have also been reported in other studies (Scott and Boldaji, 1997; Palander *et al.*, 2010; Alvarenga *et al.*, 2019). Interactions between marker type and dietary treatment have also been reported in other studies (Palander *et al.*, 2010; Wang *et al.*, 2017), but the individual effects of each type of marker remain mostly unexplainable.

The mean values of the models, adjusted for Cr and Ti, were considered to characterize the dynamics of food passage. The use of the Gompertz function allowed the interpretation of the physiological parameters related to the transit of food in the GIT. The FAT calculated in the first trial and the ROP determined in the second, both determined using females, differed by 12 min or approximately 21%. In this sense, the ROP of female Japanese quail is considered to be approximately 67 min and 30 s, thus eliminating any subjectivity regarding the visualization of the first appearance of excreta in the tray.

The MRT was calculated in 428 min and estimated at 172 min using the inverted Gompertz model to estimate the time required for 50% indicator excretion or ET_{50%}. The difference between MRT and ET_{50%} is related to the mathematical procedure used, whereas MRT assumes a linear relationship between marker excretion and time. The ET_{50%} showed that the trajectory of food in the Japanese quail GIT throughout the experimental period was of a non-linear nature.

The TT to excrete approximately 100% of the indicator was estimated to be 570 min (0930 h). The ROP, ET_{50%} and TT supported the understanding that different accelerations may exist in the passage of food in the GIT of Japanese

quail. It took 67 min to excrete the first tenth (0.01) of the marker, and it took 167 min to excrete the last tenth of the marker, in addition to the 404 min taken to excrete 0.99 of the ingested marker. Therefore, to excrete the final tenth of the indicator, approximately the same time was required as that to excrete the first 50% of the ingested marker.

These results suggest that the digestion rate in the GIT was not constant, and when associated with previous findings, such as better use of the low-energy diet, it is expected that the Japanese quail ceca have a significant effect on digestion delay in the GIT, measured herein by the recovery of the markers in the excreta. Since the first half (ET_{50%}) of the ingested indicator was excreted in 172 min or 0252 h, and the other half was excreted in 398 min or 0638 h, it took a total of 0930 h TT for complete marker elimination in the excreta. Although there was a 0930 h methodological difference, only the study by Kaupp and Ivey (1923) using brooding birds approached the value determined in the present study.

To our knowledge, no study has yet determined the ROP, ET_{50%} and TT for Japanese quail. However, birds can undergo striking anatomical changes due to cross-breeding and selection processes (Didio, 1986), supporting the hypothesis that ROP and TT differ among some species. When comparing the findings of the present study with findings for other species, Rochell *et al.* (2012) reported that the time necessary to reach 1% marker excretion was close to 0127 h, while 50% excretion took 0446 h in broiler chickens. These values are 60% and 49% higher than the present outcomes obtained for Japanese quail, respectively, which indicates a faster gastrointestinal transit in broiler chickens than in quail.

However, the MRT determined herein using a linear procedure (0708 h) was higher than the 0536 h observed in broiler chickens (Ferrando *et al.*, 1987) and the 0600 h determined in laying birds (Shires *et al.*, 1987), indicating that the digesta are retained longer in quail than in these species before excretion. Although these outcomes seem contradictory, some hypotheses could explain such results: (1) the relative capacity of the gizzard in broilers may be higher than that of quail, since the relative weight of this organ is lower in quail (Savory and Gentle, 1976; Shires *et al.*, 1987; Rezaei *et al.*, 2014), thus delaying gastrointestinal transit more in broilers than in quail.


In addition, Savory and Gentle (1976) and Borella and Lippmann (1980) claimed that the gizzard and crop are the principal components that influence the ROP (Savory *et al.*, 1987); (2) interspecies differences in the influence of gastric reflux, gastroduodenal reflux and/or cecum reflux caused by peristaltic movements in these regions (Rocha *et al.*, 2017); (3) considering that the relative weight and relative length of the total GIT and cecum are higher in quail than in broilers, a higher retention time in these regions is expected in quail (Vieira *et al.*, 2017).

Therefore, these outcomes reinforce the importance of the main aims of this study, which were to characterize digestive responses FAT, ROP, MRT, ET_{50%} and TT in adult Japanese

quail, and supported evidence of differences between birds of the genus *Coturnix* and *Gallus*.

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Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Ethics statement

All animal care procedures were approved by the Institutional Animal Care and Use Committee under protocol no. 6.725/15, prior to the beginning of the trials.

Software and data repository resources

None of the data were deposited in an official repository. The data can be obtained from the authors upon request.

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