http://dx.doi.org/10.1590/1678-4162-12052

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Arq. Bras. Med. Vet. Zootec., **v.73, n.2, p.495-507, 2021**

Incubation variables, performance, and morphometry of the duodenal mucosa of Japanese quails (*Coturnixcoturnix japonica*) submitted to different incubation temperatures and thermally challenged after hatching

[*Variáveis de incubação, desempenho e morfometria da mucosa duodenal de codornas japonesas (*Coturnix coturnix japonica*) submetidas a diferentes temperaturas de incubação e desafiadas termicamente após eclosão*]

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ABSTRACT

This study aimed to evaluate the effects of different temperatures on incubation variables, performance, and morphometry of the duodenal mucosa of Japanese quails (*Coturnix coturnix japonica*) submitted to chronic heat stress after hatching. We distributed 540 eggs in three incubators with a temperature of 37.8°C and 60% of humidity. From the 6th day of incubation until hatching, the temperatures were adjusted to (37.8°C, 38.5°C and 39.5°C). After hatching, quails were evaluated for the quality score, weighed, and distributed in a completely randomized design with three incubation temperatures (37.8, 38.5, and 39.5°C) and two ambient temperatures (stress and thermoneutral). At 10, 20, 30, and 40 days they were weighed to determine the live weight (g) and weight gain(g). To collect the duodenum and determine morphometric parameters, we euthanized four quails of each treatment. The data were analyzed, and the differences between the means determined by the Tukey test at 5%. The incubation temperature of 39.5°C provided lower hatching rate and the live weight at birth; however, from the 10th day of age, increased live weight, weight gain, and positively influenced the morphological parameters of the duodenal mucosa in situations of chronic stress.

Keywords: absorption area, stress, histology, incubation, temperature

RESUMO

Objetivou-se avaliar os efeitos de diferentes temperaturas de incubação sobre as variáveis de incubação, desempenho e morfometria da mucosa duodenal de codornas japonesas (Coturnix coturnix japonica) *submetidas ao estresse térmico crônico por calor após eclosão. Foram distribuídos 540 ovos em três incubadoras, com temperatura de 37,8°C e umidade 60%. A partir do sexto dia de incubação até a eclosão, as temperaturas foram ajustadas para 37,8°C, 38,5°C e 39,5°C. Após a eclosão, as codornas foram avaliadas quanto ao escore de qualidade, pesadas e distribuídas em um delineamento inteiramente ao acaso, com três temperaturas de incubação (37,8ºC, 38,5ºC e 39,5°C) e duas temperaturas ambientes (estresse e termoneutro). Aos 10, 20, 30 e 40 dias, foram pesadas para determinar o peso vivo (g) e o ganho de peso(g). Quatro codornas de cada tratamento foram eutanasiadas para coleta do duodeno, para determinar os parâmetros morfométricos. Os dados foram analisados e as diferenças entre as médias foram determinadas pelo teste de Tukey a 5%. A temperatura de incubação de 39,5°C proporcionou menor taxa de eclosão e menor peso vivo ao nascer, entretanto, a partir do 10° dia de idade, essa temperatura aumentou o peso vivo, o ganho de peso e influenciou positivamente os parâmetros morfológicos da mucosa duodenal em situações de estresse crônico.*

Palavras chave: área de absorção, estresse, histologia, incubação, temperatura

Recebido em 9 de junho de 2020
Aceito em 27 de outubro de 2020
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INTRODUCTION

Coturniculture is expanding widely, being an activity with low investment and rapid financial return. The characteristics of Japanese quails (Coturnixcoturnix japonica) include rapid growth, high productivity, low feed intake, early sexual maturity and a long period of production, due to these unique characteristics, coturniculture is gaining prominence in the poultry sector (Silva *et al*., 2018). Factors such as genetic improvement, nutrition, sanitary management, artificial incubation, and environmental temperature control are fundamental for the development of the sector. Among these factors, the ambient temperature of the breeding site is considered as the main stress factor, and consequently responsible for the success or failure of a poultry company (Sousa *et al*., 2014).

Birds are more susceptible to thermal variation than mammals due to anatomical and physiological characteristics such as the presence of feathers, absence of sweat glands, and high production of metabolic heat. Their thermoregulatory system is more conducive to retaining heat than dissipating it (Furlan *et al*., 2012). Heat stress occurs when the amount of heat produced by birds exceeds the dissipation capacity (Alagawany *et al*., 2017). In this situation, physiological and behavioral changes occur in quails, which severely affect feed intake and cause structural changes in the intestinal epithelium, decreasing digestibility and absorption of nutrients, which interferes with weight gain and growth rate. Also, changes in blood pH, electrolyte balance, and immunosuppression due to high circulating cortisol levels are observed, raising the mortality rate (El-Kholy *et al*., 2017; Santos *et al*., 2017).

The thermal comfort temperatures for quails are different from those observed for other birds. Hence, quails are more tolerant to heat stress compared to chickens; this fact is due to the higher surface/volume ratio, providing quails higher ability to dissipate endogenous heat. The thermal comfort zone for quails from the fourth week after hatching ranges from 25 to 26°C, presenting severe stress when the temperature reaches 33°C (Sousa *et al*., 2014; Santos *et al*., 2017).

Incubation temperature influences the entire process of embryo development, growth, and metabolism (Reyna and Burggren, 2017) and performance after hatching (Burggren and Elmonoufy, 2017). Thermal manipulation during incubation is suggested as an alternative to improve the physiological responses of birds in stressful situations, especially heat stress, throughout their lives. This event promotes non-gene changes during specific phases of embryonic development, which allows adaptation of birds to high environmental temperatures (Piestun *et al*., 2011). However, studies on thermal manipulation and thermotolerance acquisition in Japanese quails are scarce.

According to Loyau *et al*. (2016), embryos submitted to high and low temperatures during incubation improve their ability to adapt to hot and cold environments, respectively, throughout life. According to Piestun *et al*. (2009), to obtain the lasting effect of thermotolerance after hatching, thermal manipulation during incubation should be performed during the development period of the hypothalamic-pituitary-thyroid axis and hypothalamic-pituitary-adrenal axis. The thyroid hormones (T3 and T4) and heat shock proteins are responsible for the acquisition of thermotolerance in situations of heat stress in chickens, which reduce basal cell metabolism and increase tissue stability, respectively (Al-Zghoul, 2018).

Considering that changes in incubation temperature can beneficially influence the variables of performance, digestive system development, and trigger thermotolerance in birds throughout the productive life, this work aimed to evaluate the effects of different temperatures on the incubation variables, performance, and morphometry of the duodenal mucosa of Japanese quails (*Coturnix coturnix japonica*) submitted to heat stress after hatching.

MATERIAL AND METHODS

The Ethics Committee on The Use of Animals of the Federal Rural University of the Semi-Arid (CEUA/UFERSA) approved the present study under No. 37/2016. We carried out the incubation trial in the multidisciplinary laboratory of the Bioscience department of the Federal Rural University of the Semi-Arid (UFERSA). We used 540 eggs of Japanese quails acquired from a commercial hatchery of a 19-week-old lot (Granja Fujikura/Suzano-SP). We considered suitable for

hatching the eggs of the clean, whole, and pigmented shell, elliptical form, and discarded those that were pointed, with the presence of mold and broken.

We performed the incubation in a room with controlled temperature and humidity. A total of 180 eggs were randomly distributed in each incubator (BROOD Chocadeira MAIA 60® produced in Santa Catarina/SC), and each egg considered an experimental unit. The temperature set to 37.8°C and relative humidity to 60%. On the 6th day of incubation until the time of hatching, a protocol of three incubation temperatures was established for 24 h. The incubator temperatures adjusted to 37.8°C (standard), 38.5°C (intermediate), and 39.5°C (high) and humidity of 65%, the eggs were kept horizontally and automatically turned every two hours in the three treatments. On the 15th day of incubation, the incubators were programmed to stop turning the eggs. The birth of quails was followed every 3 hours, from the 16th day of incubation (384 hours) to the 21st day of incubation (505 hours). We opened all unhatched eggs to determine the period of embryonic mortality. We considered three phases of embryonic death. Phase I comprised the period from zero to seven days of development, phase II, the period from eight to eleven days, and phase III, the period from twelve to eighteen days. We broke the eggs to visualize the consistency, physical form, and development status of the quail embryo, according to Ainsworth *et al*. (2010).

After hatching, the quails were weighed and evaluated for the chick quality score from 0 to 100 points, according to the methodology of Tona *et al*. (2003). They were uniformly distributed in wooden and galvanized wire cages with 50cm long and 120cm wide, the floor covered with 3cm of wood-shavings bedding, in the aviary house in the poultry sector of UFERSA. They were forming a factorial design (3x2) with three incubation temperatures (37.8, 38.5, and 39.5°C) and two ambient temperatures (stress and thermoneutral), the temperature remained constant from 1° to 40° days of age at 40°C and ±60% humidity (RU), to subject Japanese quails to a situation of chronic heat stress. We used four infrared drying lamps, to maintain this temperature, and externally insulated the cages with plastic tarps, and made eight vents for air renewal.

Air temperature and relative humidity were measured using thermo-hygrometers at the back of the Japanese quails. To maintain a thermal comfort environment, we established the following temperatures of 35°C-23°C over the 40 days, with a reduction of 3°C each week (El-Tarabany 2016, Bayrakdar *et al*., 2017). Therefore, there were six treatments: T1 (37.8°C/stress), T2 (38.5°C/stress), T3 (39.5°C/stress), T4 (37.8°C/thermoneutral), T5 (38.5°C/thermoneutral) and T6 (39.5°C/ thermoneutral). The birds received water and feed *ad libitum*. The diets were formulated with corn bran and soybean meal, following the nutritional recommendations of the age (Rostagno *et al*., 2011). The management was carried out daily at 08:00 am, 02:00 pm, and 08:00 pm. On the 10th day, all birds were vaccinated (live attenuated vaccine) against Newcastle disease by eye at a dose of 0.06ml, according to the manufacturer (New Vacin, La Sota, Fort Dodge).

At 10, 20, 30, and 40 days all quails were weighed to determine the live weight(g) and weight gain(g). We euthanized four quails of each treatment, under dissociative anesthesia, associated with ketamine hydrochloride (40mg/Kg – IM), xylazine hydrochloride (1,5mg/KG – IM), and atropine sulfate (0,02 mg/KG – IM). After induction, cervical dislocation was performed with subsequent necropsy to collect the duodenum, which was weighed to determine the absolute weight(g) and relative weight (%) by the formula: (absolute weight/weight of the animal) x100.

For morphometric evaluation of the duodenum, we used four animals per treatment and collected fragments of approximately 2cm of the mean portion of the duodenum. The samples were washed with distilled water and fixed in 10% formaldehyde for 24 hours. Tissue samples were dehydrated in an increasing series of alcohols (70, 80, 90, and 100%) diaphanized in xylol and included in paraffin. After semi-serial microtomy of 5µm thickness we stained histological samples with hematoxylin and eosin. The slides were sealed with coverslips using Canadian Balm and observed under light microscopy. Photographs of fields containing vertically oriented villi were taken. The Axio Vision program performed the analysis of the images.

The villus height and crypt depth were measured after the conversion of pixels into micrometers, and the villus/crypt ratio was calculated. We measured the villus height from the basal region of the villus, coincident with the upper portion of the crypts at its apex. The crypts were measured from their base to the crypt/villus transition zone and, the absorption area was determined according to the methodology proposed by (Kisielinski *et al*., 2002). Twenty measurements were performed per bird and 80 in each treatment and 480 in each age, for each variable studied. The live weight at birth, hatchability index (%), mortality, chick quality score, live weight, absolute and relative weight, and morphometric analysis were submitted to variance analysis in the SAS System (Statistical Analysis System) version 2001. The Tukey test compared significant means at 5% probability. Weight gain data were submitted to descriptive analysis using the Microsoft Excel program.

RESULTS

According to (Table 1) for live weight at birth (g), there was a statistical difference between incubation temperatures (P<0.05), with the best weight observed at the standard temperature of 37.8°C. However, the highest hatching index was observed at an intermediate temperature of 38.5°C, and this did not reflect in better body score quality. The lowest hatching rate, which reflected in a higher percentage of embryonic mortality, was observed at a high temperature of 39.5°C, and the intermediate period between 8° and 11° of incubation presented a higher mortality rate (17.78%).

There was no interaction (P>0.05) for incubation temperature and ambient temperature for live weight at different ages (Table 2). The quails incubated at 39.5°C showed higher live weight (P<0.05) compared to other incubation temperatures, regardless of room temperature. The temperature of 38.5°C showed the lowest live weight of all ages. When we evaluated the ambient temperature factor, quails kept in the thermoneutrality situation presented (P<0.05) higher live weight than quails submitted to chronic heat stress.

The body weight gain was higher at ten days of age in quails submitted to chronic heat stress; however, in other ages, the high ambient temperature promoted a negative effect on weight gain. We observed the highest weight gain at 10, 20, and 30 days in quails from the incubation temperature of 39.5°C, but this effect was not noticed at 40 days when the highest weight gain came from the temperature 38.5°C (Figure 1).

The results for the absolute weight of the duodenum of Japanese quails can be found in (Table 3). There was interaction (P<0.05) between factors of incubation temperature and environment at 20 days of age. The birds incubated at 37.8°C and reared in a situation of conical stress by heat showed a higher absolute weight of the duodenum. There was no difference between incubation temperatures when the birds were reared in a situation of thermoneutrality. At 30 days of age, the highest absolute weight of the duodenum was observed in quails reared in a situation of thermoneutrality. At 10 and 40 days of age, there was no (P>0.05) interaction between the factors studied. For the relative weight of the duodenum (Table3), at ten days of age, the highest weight was observed in quails submitted to chronic heat stress. No differences were observed between the factors studied in the other ages.

Table 1. Live weight at birth, hatchability, quality and mortality score of Japanese quails (*Coturnix coturnix japonica*) subjected to thermal manipulation during incubation

Variables	Incubation temperature (°C)			P value
	37.8	38.5	39.5	
Live weight at birth in (g)	9.83±0.80a	8.02±0.84b	7.76±0.91b	0.0296
Hatchability (%)	81.11b	87.22a	70.55c	<.0001
Incubation Quality	95.48±5.16a	91.90±3.20b	93.08±3.30ab	<.0001
Maximum -Minimum	100 - 88	100 - 63	100 - 66	
Mortality	18.89b	12.78c	29.45a	<.0001
Precocious (%)	7.22a	3.88c	10.0b	<.0001
Intermediate (%)	6.12c	7.23b	17.78a	<.0001
Late (%)	5.55a	1.67b	1.67c	<.0001

Same letters on the line do not differ by Tukey's test (P<0.05).

Table 2. Average live weight (g) of Japanese quails (*Coturnix coturnix japonica)* at 10, 20, 30 and 40 days of age submitted to thermal manipulation during and after hatching

		10 days	20 days	30 days	40 days
Treatments	37.8/stress	35.94	67.08	103.38	130.74
	38.5/ stress	31.30	61.22	99.76	129.14
	39.5/ stress	36.18	68.38	106.80	133.06
	37.8/thermoneutral	30.63	70.42	121.03	143.43
	38.5/thermoneutral	29.24	66.00	117.00	148.09
	39.5/thermoneutral	35.44	75.61	126.73	158.41
Incubation temperature	Standard (37.8°C)	33.28 b	68.75 b	112.20 b	137.08 b
	Intermediate (38.5°C)	30.27 c	63.61 c	108.38 c	138.61 b
	High (39.5°C)	35.81 a	71.99 a	116.76 a	145.41 a
Room temperature	Stress	34.24 B	65.63 B	102.76 B	130.58 B
	Thermoneutral	35.44 A	75.61 A	126.73 A	158.41 A
Significance level	TI	<.0001	<.0001	0.0005	0.05
	TA	0.0014	<.0001	<.0001	<.0001
	TIXTA	NS	NS	NS	NS

A-B (room temperature); a-c (incubation temperature) indicates that the means in the same column are different by the Tukey test (P <0.05), NS not significant. TI= Incubation temperature; TA= Room temperature and TIxTA= Interaction between temperatures.

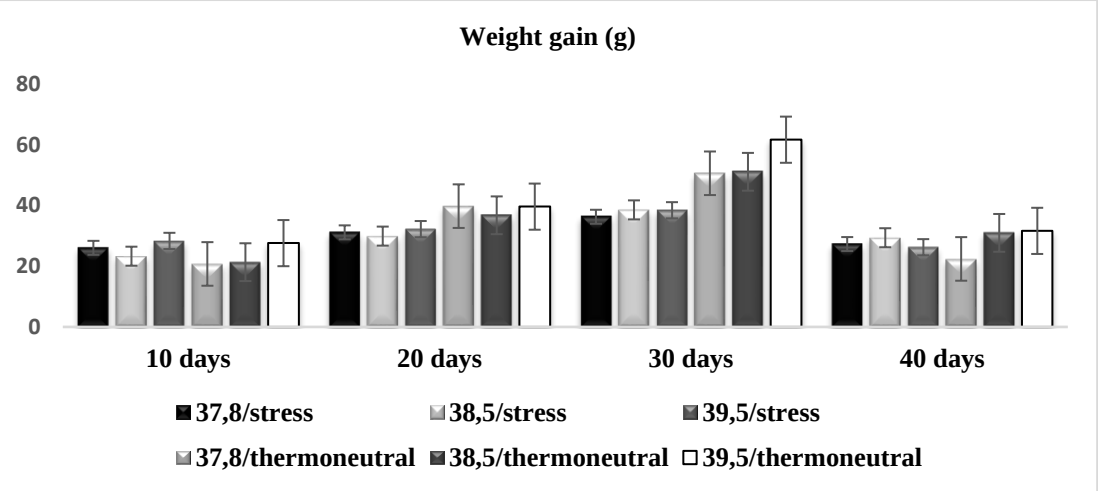

Figure 1. Weight gain (g) of Japanese quails (*Coturnix coturnix japonica*) at 10, 20, 30 and 40 days of age submitted to thermal manipulation by heat during and after hatching.

For the factors of incubation temperature and room temperature, there was significant interaction (P<0.05) for the height of duodenal villi at all ages (Table 4). Heat stress after hatching promoted a positive effect (P<0.05) on mucosal integrity, increasing the height of villi in all ages studied. The incubation temperature 39.5°C promoted a positive effect (P<0.05) at 10 and 30 days of age compared to the other temperatures, reflecting positively on the height of the duodenal villi of quails submitted to chronic heat stress after hatching at all ages evaluated. However, when the birds were reared at thermoneutrality temperatures, the incubation temperature of 39.5°C has no positive effect at 20 and 40 days of age. Quails incubated at the standard temperature 37.8°C maintain the integrity of the duodenal mucosa in a situation of heat stress after hatching at 20, 30, and 40 days old. When reared in a situation of thermoneutrality, they present (P<0.05) higher villi at 20 and 40 days compared to the other temperatures. Quails incubated at 38.5°C presented the lowest heights of duodenal villi at all ages evaluated and at the two ambient temperatures (P<0.05).

Table 3. Averages of absolute (g) and relative (%) weight of the duodenum of Japanese quails (*Coturnix coturnix japonica*) at 10, 20, 30 and 40 days of age submitted to thermal manipulation during and after hatching

Absolute weight (g)		10 days	20 days	30 days	40 days
Treatments	37.8/stress	0.67	1.20 aA	1.39	1.86
	38.5/ stress	0.66	0.72 cA	1.29	1.37
	39.5/ stress	0.71	0.94 bA	1.10	1.51
	37.8/thermoneutral	0.69	0.88 aA	1.48	1.44
	38.5/thermoneutral	0.64	0.94 aA	1.35	1.49
	39.5/thermoneutral	0.59	0.97 aA	1.54	1.31
Incubation temperature	Standard (37.8°C)	0.68	1.04 a	1.43	1.65
	Intermediate (38.5°C)	0.65	0.83 b	1.32	1.43
	High (39.5°C)	0.65	0.95 a	1.32	1.41
Room temperature	Stress	0.68	0.95	1.26 B	1.58
	Thermoneutral	0.64	0.93	1.45 A	1.41
Significance level	TI	NS	0.02	NS	NS
	TA	NS	NS	0.04	NS
	TIXTA	NS	0.02	NS	NS
Relative weight (%)		10 days	20 days	30 days	40 days
Treatments	37.8/stress	2.09	2.00	1.44	1.32
	38.5/ stress	2.10	1.34	1.25	1.14
	39.5/ stress	2.21	1.33	1.05	1.21
	37.8/thermoneutral	2.15	1.36	1.26	1.05
	38.5/thermoneutral	1.87	1.49	1.30	1.19
	39.5/thermoneutral	1.85	1.30	1.18	1.08
Incubation temperature	Standard (37.8°C)	2.12	1.68	1.35	1.18
	Intermediate (38.5°C)	1.98	1.41	1.27	1.16
	High (39.5°C)	2.03	1.31	1.11	1,14
Room temperature	Stress	2.13 A	1.55	1.24	1.22
	Thermoneutral	1.95 B	1.38	1.24	1.10
Significance level	TI	NS	NS	NS	NS
	TA	0.02	NS	NS	NS
	TIXTA	NS	NS	NS	NS

A-B (room temperature); a-c (incubation temperature) indicates that the means in the same column are different by the Tukey test (P <0.05), NS not significant. TI= Incubation temperature; TA= Room temperature and TIxTA= Interaction between temperatures.

Table 4. Average villus height (µm) of the duodenum of Japanese quails (*Coturnix coturnix japonica*) at 10, 20, 30 and 40 days of age submitted to thermal manipulation during and after hatching

		10 days	20 days	30 days	40 days
Treatments	37.8/stress	687.57 bA	711.09 aA	722.25 aA	778.57 aA
	38.5/ stress	746.69 bA	628.34 bA	656.31 bA	738.02 bA
	39.5/ stress	826.22 aA	700.18 aA	730.31 aA	793.07 aA
	37.8/thermoneutral	704.40 aB	675.57 aB	690.88 aB	720.05 aB
	38.5/thermoneutral	587.75 bB	638.20 aB	648.90 bB	634.45 cB
	39.5/thermoneutral	677.96 aB	567.64 bB	666.98 aB	645.38 bB
Incubation temperature	Standard (37.8°C)	695.98 b	693.33 a	706.56 a	749.31 a
	Intermediate (38.5°C)	667.22b	633.20 b	652.60 b	686.23 c
	High (39.5°C)	752.09 a	633.91b	698.64 a	719.22 b
Room temperature	Stress	753.49 A	679.87 A	702.95 A	769.88 A
	Thermoneutral	656.96 B	627.13 B	668.92 B	666.62 B
Significance level	TI	<.0001	<.0001	<.0001	<.0001
	TA	<.0001	<.0001	<.0001	<.0001
	TIXTA	<.0001	<.0001	<.0001	<.0001

A-B (room temperature); a-c (incubation temperature) indicates that the means in the same column are different by the Tukey test (P <0.05), NS not significant. TI= Incubation temperature; TA= Room temperature and TIxTA= Interaction between temperatures.

There was significant interaction (P<0.05) between the incubation temperature and room temperature factors for duodenal crypt depth at 20, 30, and 40 days (Table 5). At ten days, the standard incubation temperature of 37.8°C showed a higher crypt depth concerning the different incubation temperatures. At 20 and 30 days, quails incubated at 38.5°C presented higher crypt depths when reared in a situation of chronic heat stress and a situation of thermoneutrality. However, at 40 days, this effect was not observed, where the crypt depth was high (P<0.05) in quails incubated at temperatures of 37.8°C and 39.5°C.

For the villus/crypt ratio, there was a significant interaction (P<0.05) between the incubation temperature and room temperature factors at 10, 30, and 40 days, but there was no interaction (P>0.05) between the factors at 20 days (Table 6).

Table 5. Crypt depth averages (µm) of the duodenum of Japanese quails (*Coturnix coturnix japonica*) at 10, 20, 30 and 40 days of age submitted to thermal manipulation during and after hatching

		10 days	20 days	30 days	40 days
Treatments	37.8/stress	47.65	40.36 bA	46.48 aA	39.98 aA
	38.5/ stress	45.52	45.58 aA	40.46 aA	37.61 bA
	39.5/ stress	45.98	43.70 bA	35.94 bA	37.64 bA
	37.8/thermoneutral	46.53	41.90 bA	38.40 bB	40.35 aA
	38.5/thermoneutral	46.41	45.20 aA	40.25 aB	36.04 bA
	39.5/thermoneutral	44.80	38.52 bA	40.94 aB	38.76 aA
Incubation temperature	Standard (37.8°C)	47.09 a	41.13 b	42.44 a	40.16 a
	Intermediate (38.5°C)	45.96 b	45.39 a	40.35 a	36.82 b
	High (39.5°C)	45.39 b	41.11 b	38.44 b	38.2 a
Room temperature	Stress	46.38	43.21	40.96 A	38.41
	Thermoneutral	46.91	41.87	39.86 B	38.38
Significance level	TI	0.02	<.0001	<.0001	<.0001
	TA	NS	NS	0.0004	NS
	TIXTA	NS	<.0001	<.0001	<.0001

A-B (room temperature); a-c (incubation temperature) indicates that the means in the same column are different by the Tukey test (P <0.05), NS not significant. TI= Incubation temperature; TA= Room temperature and TIxTA= Interaction between temperatures.

Table 6. Average villus/crypt ratio (µm/µm) of the duodenum of Japanese quails (*Coturnix coturnix japonica*) at 10, 20, 30 and 40 days of age submitted to thermal manipulation during and after hatching

		10 days	20 days	30 days	40 days
Treatments	37.8/stress	14.62 bA	17.86	15.66 bA	19.51 bA
	38.5/ stress	16.59 bA	14.86	16.40 bA	19.72 aA
	39.5/ stress	18.25 aA	16.11	20.44 aA	21.14 aA
	37.8/thermoneutral	15.39 aB	17.62	18.09 aB	17.93 aB
	38.5/thermoneutral	13.09 bB	14.41	16.17 bB	17.69 aB
	39.5/thermoneutral	15.35 aB	14.95	16.40 bB	16.71 bB
Incubation temperature	Standard (37.8°C)	15.00 b	17.74 a	16.87 b	18.72
	Intermediate (38.5°C)	14.84 b	14.63 b	16.28 b	18.70
	High (39.5°C)	16.80 a	15.53 b	18.42 a	18.92
Room temperature	Stress	16.48 A	16.27	17.50 A	20.12 A
	Thermoneutral	14.61 B	15.66	16.88 B	17.44 B
Significance level	TI	<.0001	<.0001	<.0001	NS
	TA	<.0001	NS	<.0001	<.0001
	TIXTA	<.0001	NS	<.0001	<.0001

A-B (room temperature); a-c (incubation temperature) indicates that the means in the same column are different by the Tukey test (P <0.05), NS not significant. TI= Incubation temperature; TA= Room temperature and TIxTA= Interaction between temperatures.

For the incubation temperature factor, we observed the best means in quails from the incubation temperature of 39.5°C at 10 and 30 days. However, at 20 days, the best villus/crypt ratio (P<0.05) was observed in quails from the standard incubation temperature of 37.8°C. In the ambient temperature factor, quails submitted to chronic heat stress presented better (P<0.05)

averages at 10, 30, and 40 days of age compared to quails kept in thermoneutrality. These facts contributed to the better (P<0.05) villus/crypt ratio, to be observed in quails incubated at a temperature of 39.5°C. After hatching, they were kept in a situation of thermal stress by heat, at 10, 30, and 40 days.

In all ages evaluated, there was significant interaction (P<0.05) between the incubation temperature and room temperature factors, where quails from incubation temperature 39.5°C presented better (P<0.05) absorption area in situations of chronic heat stress after hatching, at all ages (Table 7).

Table 7. Averages of the absorption area of the duodenum of Japanese quails (*Coturnix coturnix japonica*) at 10, 20, 30 and 40 days of age submitted to thermal manipulation during and after hatching

		10 days	20 days	30 days	40 days
Treatments	37.8/stress	14.64 bA	15.82 aA	15.84 bA	18.41 aA
	38.5/ stress	14.69 bA	13.39 bA	15.57 bA	17.68 bA
	39.5/ stress	15.82 aA	14.64 aA	18.98 aA	18.70 aA
	37.8/thermoneutral	13.79 aB	14.66 aA	17.50 aA	16.69 aB
	38.5/thermoneutral	11.84 bB	14.88 aA	15.86 bA	15.51 bB
	39.5/thermoneutral	13.67 aB	13.50 bA	16.96 aA	15.16 bB
Incubation temperature	Standard (37.8°C)	14.21 a	14.97 a	16.67 b	17.55 a
	Intermediate (38.5°C)	13.26 b	14.13 a	15.71 b	16.59 b
	High (39.5°C)	14.74 a	13.98 b	17.97 a	16.93 ab
Room temperature	Stress	15.05 A	14.37	16.79	18.26 A
	Thermoneutral	13.01 B	14.34	16.77	15.78 B
Significance level	TI	<.0001	<.0001	<.0001	<.0001
	TA	<.0001	NS	NS	<.0001
	TIXTA	<.0001	<.0001	<.0001	<.0001

A-B (room temperature); a-c (incubation temperature) indicates that the means in the same column are different by the Tukey test (P <0.05), NS not significant. TI= Incubation temperature; TA= Room temperature and TIxTA= Interaction between temperatures.

DISCUSSION

In the present study, we observed the highest hatching rate in eggs incubated at a temperature of 38.5°C (87.22%) followed by the standard temperature 37.8°C (81.11%) and high temperature 39.5°C (70.55%). Thus, the highest percentage of embryonic mortality was observed at high temperatures, especially in the intermediate phase at 8° and 11th days of incubation. Burggren *et al*. (2008) observed similar results in which the most critical phase for embryonic development is the intermediate phase. Studies of incubation temperatures for Japanese quails observed that embryos are resistant to high incubation temperature (up to 40°C) in the initial phase of embryogenesis; however, in the final stages of incubation, temperatures of 39 and 41°C increased embryonic mortality (Romão *et al*. 2009).

The quality chick score can identify possible problems during incubation. According to Flores *et al*. (2017), thermal manipulation during incubation does not interfere in the quality of broilers after hatching. These data corroborated by Nyuiadzi *et al*. (2017), who worked with three incubation temperatures with values lower than recommended and did not observe any difference in the quality of quails. In the present study, incubation temperatures 37.8°C and 39.5°C presented the best score (95.48±5.16a and 93.08±3.30ab) respectively. In the present study, incubation with a temperature of 39.5°C reduced live weight at birth. According to Abuoghaba (2016), the increase in incubation temperature in broilers decreases the live weight at birth, this result reflects in lower live weight throughout the life of the bird, since increased temperature promotes morphological changes in the digestive system that interfere in the development of the bird. Different results from those observed in this study were described by Romão *et al*. (2009), where the best live weight at birth was observed in quail from temperatures between 38 to 38 to 40°C.

As expected in the present study, we observed that heat stress after hatching decreases the live weight and weight gain of Japanese quails significantly. Birds kept under high ambient temperatures reduce feed intake to minimize the caloric

increase resulting from the digestion process. Besides, birds need a considerable amount of energy to dissipate heat and maintain homeothermy. Therefore, decreased energy is available for physiological processes, such as the development of fast-growing tissues as muscles and bones. The result of this process is the decrease in live weight and weight gain, as reported here and by other authors (Abdellhady *et al*., 2017; El-Kholy *et al*., 2017, 2018; Erisir *et al*., 2018).

Sousa *et al*. (2014) evaluating the limits of thermal comfort for quails, found that most quails kept in comfort treatments had higher voluntary feed intake in the final stage of rearing. Given this premise, we can assure that the higher the ambient temperature, the lower the voluntary food intake, because the caloric increase from the diet, at high temperatures, is not satisfactory, and the bird needs to eliminate this excess of heat. In the present study, at ten days of age, the weight gain was higher in the situation of heat stress. After 20 days, the weight gain declined compared to quails maintained in situations of thermoneutrality. The thermoregulatory system of birds is anatomically formed after hatching; however, it is not entirely functional, with the need for heating. According to Santos *et al*. (2017), Japanese quails subjected to high ambient temperatures significantly reduce eating behavior and increase the behavior of drinking water, become more agitated and began to spread more wings to increase the dissipation of body heat to the environment.

Anato-physiological characteristics such as the presence of feathers, absence of sweat glands, and high metabolic rate favor birds to have limited capacity to cope with high ambient temperatures (Furlan *et al*., 2012). Thermal manipulation during incubation emerges as a strategy to alleviate this problem and improve the adaptive capacity of birds to heat stress situations (Piestun *et al*., 2011). Quails incubated in the present study at a temperature of 39.5°C showed higher live weight and weight gain after hatching, results similar to these were observed in quails by (Alkan *et al*., 2013) and in broilers by (Al-Zghoul *et al*., 2013; Piestun *et al*., 2011; Zaboli *et al*., 2016. According to Al- Zghoul (2018), the increase in temperature in the final incubation phase alters the dynamics of the expression of heat shock proteins and thermal shock factors, which are involved in the thermotolerance of birds when subjected to environmental situations of heat stress.

The small intestine acts as an essential organ for the processes of digestion and absorption of food, being one of the organs most susceptible to possible damage of thermal stress. Thus, the data of absolute and relative weight of the duodenum, morphological variables such as villus height, crypt depth, villus/crypt ratio, and absorption area can be used as a parameter to determine the welfare of birds, their growth capacity, production and thermotolerance in the face of environmental challenges.

According to Lopes *et al*. (2015), birds kept at high ambient temperatures reduce the size of the viscera to try to offset the heat load that will be dissipated into the environment. Xiaofang *et al*. (2018) observed similar results, where the weight of the duodenum and ileum of broilers submitted to heat stress for seven days decreases significantly. These results may be associated with low food intake, low digestibility, and higher energy expenditure in an attempt to lose heat to maintain homeostasis during heat stress. In the present study, thermal stress reduced the absolute weight of the duodenum at 30 days. Regarding relative weight at ten days of age, thermal stress increased the weight of the duodenum. These data corroborate Bonfim *et al*. (2016). They reported that quails reared at room temperature at 32°C up to 42 days of age did not affect the relative weight of the small intestine compared to quails of the air-conditioned room treatment.

According to these authors, quails are more efficient at dissipating metabolic heat to the environment than broilers. A study with broilers observed that the relative weight of the intestine was higher in birds subjected to thermal stress, the authors attribute to this fact the supplementation of the diet with zinc and Vitamin E (Lopes *et al*. (2015). However, Xiaofang *et al*. (2018) suggest that heat stress can reduce the weight of the digestive organs, which contributed to shorter intestinal villi, even though the length of the intestine was significantly longer. However, in the present study, duodenum morphometry data show that thermal heat stress increased the height of villi at all ages studied. Similar results were observed by Bayrakdar *et al*. (2017) in Japanese quails submitted to a temperature of 33°C for 6 hours per day, from 7 to 42 days of age. Although,

the beneficial effect of heat stress on duodenal morphometry did not reflect on the live weight and weight gain parameters. The high energy expenditure necessary to maintain the process of cell renewal (proliferation, differentiation, and extrusion) that occurs in the intestinal mucosa can explain this fact. The digestion and absorption processes use about 20% of their energy to maintain the intestinal mucosa, diverting from the growth of the bird (McBride and Kelly 1990). Therefore, the more extensive the repairs of the intestinal mucosa, the lower the availability of net energy for bird growth.

Wu *et al*. (2018) report that low feed intake at high ambient temperature promotes deleterious effects on the intestinal mucosa since the presence of food in the intestinal mucosa acts as a trophic effect for its development. Besides, thermal stress promotes changes in the epithelial surface, which allows the binding of pathogens in the epithelium resulting in reduced villus height and increased crypt depth, impairing the integrity of epithelial tissue of the intestine (Burkholder *et al*., 2008). According to Xiaofang *et al*. (2018), to maintain the structure and function of the digestive system, the process of cell renewal must be fast, for this, it is necessary to have the presence of nuclear antigen of cell proliferation (PCNA). However, thermal stress by heat considerably reduces this antigen expression, both along the villus and in the crypt region.

The height of villi and the depth of the crypt are considered good indicators of bowel development in birds reared in situations of thermoneutrality. We observed the presence of basal cells responsible for the villus cell renewal process in the region of the intestinal crypt, thus, the higher the depth of the crypt, the higher the height of the villus. According to Barri *et al*. (2011), after hatching, intense hyperplasia occurs in the crypt region between the 4th and 14th day, allowing the development of the intestinal epithelium, enterocytes take about four days to migrate to the tip of the villi. In the present study, we observed the best villus/crypt ratio at 10, 30, and 40 days of age in quails submitted to heat stress. Similar results were observed by Fernandes *et al*. (2017) in broilers submitted to thermal oscillation.

Sandikci *et al*. (2004), Mehaisen *et al*. (2017) observed in Japanese quails submitted to heat stress results different from those observed in the present study. According to these authors, exposure of quails to high ambient temperatures causes structural changes in the intestinal epithelium affecting the processes of digestion and absorption of nutrients. Corroborating Porto *et al*. (2015), Fernandes *et al*. (2017), Wu *et al*. (2018) observed that the increase in room temperature reduced the parameters of intestinal morphometry in broilers. According to Porto *et al*. (2015), during thermal heat stress, there is a reduction in the energy available to the gastrointestinal tract, which slows the development of the intestinal mucosa, resulting in shorter villi, greater depth of crypts and lower villus/crypt ratio.

The climatic warming that has been occurring over the years is a concern for the coturniculture sector since heat stress promotes several changes in the behavior and physiological mechanisms of quails, culminating with low performance of these birds and economic losses for the sector (Nyoni *et al*. 2018). Thus, it is essential to find ways to minimize the adverse effects of heat stress. The increase in incubation temperature can induce physiological changes that act as epigenetic thermal adaptation, improving the thermotolerance of birds throughout life (Piestun *et al*. 2009). In the present study, quails from incubation with a temperature of 39.5°C presented the best means for villus height, villus/crypt ratio and absorption area, when quails were submitted to chronic heat stress at all ages evaluated. A study also observed a positive effect on intestinal morphometry in chickens inoculated with Salmonella Enteritidis and submitted to high incubation temperature (Moreira Filho *et al*., 2015). According to Barri *et al*. (2011), thermal manipulation during incubation is a positive factor for increased intestinal villi that can be observed from the sixth day after hatching.

For Piestun *et al*. (2009), acquisition of thermotolerance in situations of heat stress in birds occurs when thermal manipulation is performed during the embryonic development of the hypothalamic-pituitary-thyroid axis and hypothalamic-pituitary-adrenal axis. According to Al-Zghoul (2018), thermal manipulation with a temperature of 39.5°C promotes the reduction of circulating plasma levels of T3 in broilers submitted to chronic heat stress at 35 days of age, this reduction in metabolic rate is related to the acquisition of thermotolerance. For Al-Zghoul *et*

al. (2013), thermal manipulation during incubation favors thermal conditioning in birds throughout their life by increasing heat shock proteins expression (Hsp70), when subjecting birds to thermal challenges. Thus, the increase in incubation temperature during the development of the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-thyroid axes is an alternative to deal with deleterious effect situations of thermal stress after hatching.

CONCLUSION

The incubation temperature of 39.5°C reduces the hatching rate and the live weight at birth but does not interfere with the chick quality score. Besides, it improves the integrity and absorption area of the duodenal mucosa of quails subjected to heat stress after hatching. These improvements may be associated with the acquisition of thermotolerance of Japanese quails after hatching that may have been triggered by increased incubation temperature.

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