

Effects of Thermal Manipulation of Japanese Quail Embryo on Post-hatch Carcass Traits, Weight of Internal Organs, and Breast Meat Quality

Key words

Coturnix japonica;
meat-type quail;
breast traits;
thermal biology

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Abstract: Embryonic thermal manipulation was known as an effective protocol for improving post-hatch growth performance and thermotolerance acquisition among avian species. Previously, we evaluated the impact of embryonic thermal manipulation of Japanese quail on embryonic development, hatchability, and post-hatch performance. We conducted the current study to further elucidate the effects of thermal manipulations of Japanese quail embryos on internal organ weights, carcass traits, and meat quality parameters at post-hatch day 35. Quail eggs of control group were incubated at 37.7 °C and relative humidity (RH) 55%. Three thermally manipulated groups of quail eggs were incubated intermittently at 41°C and 65% RH intermittently (3 hours/day): the early embryonic group (TM₁) was thermally challenged at embryonic day (ED6) to ED8, the late embryonic group (TM₂) was thermally challenged at ED12-14, and early/late embryonic group (TM₃) was thermally challenged in both time windows. Quail meat quality parameters, carcass traits, and internal organ weights were evaluated at post-hatch day 35. The results revealed that early embryonic thermal manipulation (TM₁ group) is an effective protocol for decreasing fat pad accumulation. The pH value of breast meat in all TM treatments revealed significant ($P < 0.05$) decreases by 5% in comparison with that of control without any negative effects on breast meat composition or sensory criteria. Early embryonic thermal manipulation would be recommended as an enhanced protocol that can be used to reach the favored lucrative effects of the thermal treatment in meat-type quail.

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Introduction

Several countries depend on Japanese quail (*Coturnix japonica*) as a source of meat and eggs. Japanese quail is a migratory bird that is characterized by short generation intervals besides its distinctive physical, physiological, and behavioral features, that's why, it is a very important animal model in scientific research and lab experiments (1, 2).

Embryonic thermal manipulation (TM) was identified as a beneficial technique that improves muscle development and growth in avian species, in which the embryos are challenged with high or low incubation temperatures during a critical time window in their embryonic life (3, 4). Adaptation for thermotolerance and breast muscle yield were enhanced

without any significant alterations in the characteristics of breast meat quality (5). The intermittent embryonic TM of Japanese quail at ED 6 - 8 improved final body weight at post-hatch day 35 which was manifested by significant breast muscle hypertrophy (6, 7). The reduction of the abdominal fat pad relative weight is one of the goals of broiler management. Embryonic TM had been identified as an effective technique to decrease abdominal fat accumulation (4). To date, there are no studies on quail that have indicated whether TM has a beneficial effect on meat quality, internal organ weights, and carcass traits or not. Therefore, the current study aimed to elucidate the effects of embryonic TM on quail breast meat quality, carcass traits, and internal organs weights at post-hatch day 35.

Materials and methods

Egg incubation and hatching management

The study was conducted at the Faculty of Veterinary Medicine, Cairo University, Egypt (FVMCU). All experimental procedures and management conditions used in this study were approved by the Institutional Animal Care and Use Committee (IACUC; Reference No. Vet CU16072020190).

One thousand eggs were obtained from a quail maternal flock of 13 weeks old from the experimental farm, Faculty of Agriculture, Cairo University, Egypt. After egg selection, individually weighted and disinfected, a final number of 816 quail eggs were randomly assigned into four groups (204 eggs per group), incubated in four homologous incubators (PTO- Italy) with continuous turning system, and fully digital programmable temperature and humidity. Incubation conditions for the control group (Ctrl) were 37.7°C and 55% relative humidity (RH) from embryonic day 0 (ED0) to ED17 (8). Three thermally treated egg groups: incubation temperature was increased to 41°C and 65% RH - intermittently (3 hours/day) during early embryogenesis (ED6-8) in the TM1 group and during late embryogenesis (ED12-14) in the TM2 group. The last group (TM3) was thermally treated in both time windows. Immediately after the thermal manipulation techniques were terminated, incubation conditions were restored to the standard conditions (37.7°C and 55% RH). During the incubation period, the eggs in all incubators were automatically turned through 270° every hour. The eggs were transferred to hatching trays on the 15th day of incubation and RH% was increased to 60-65%.

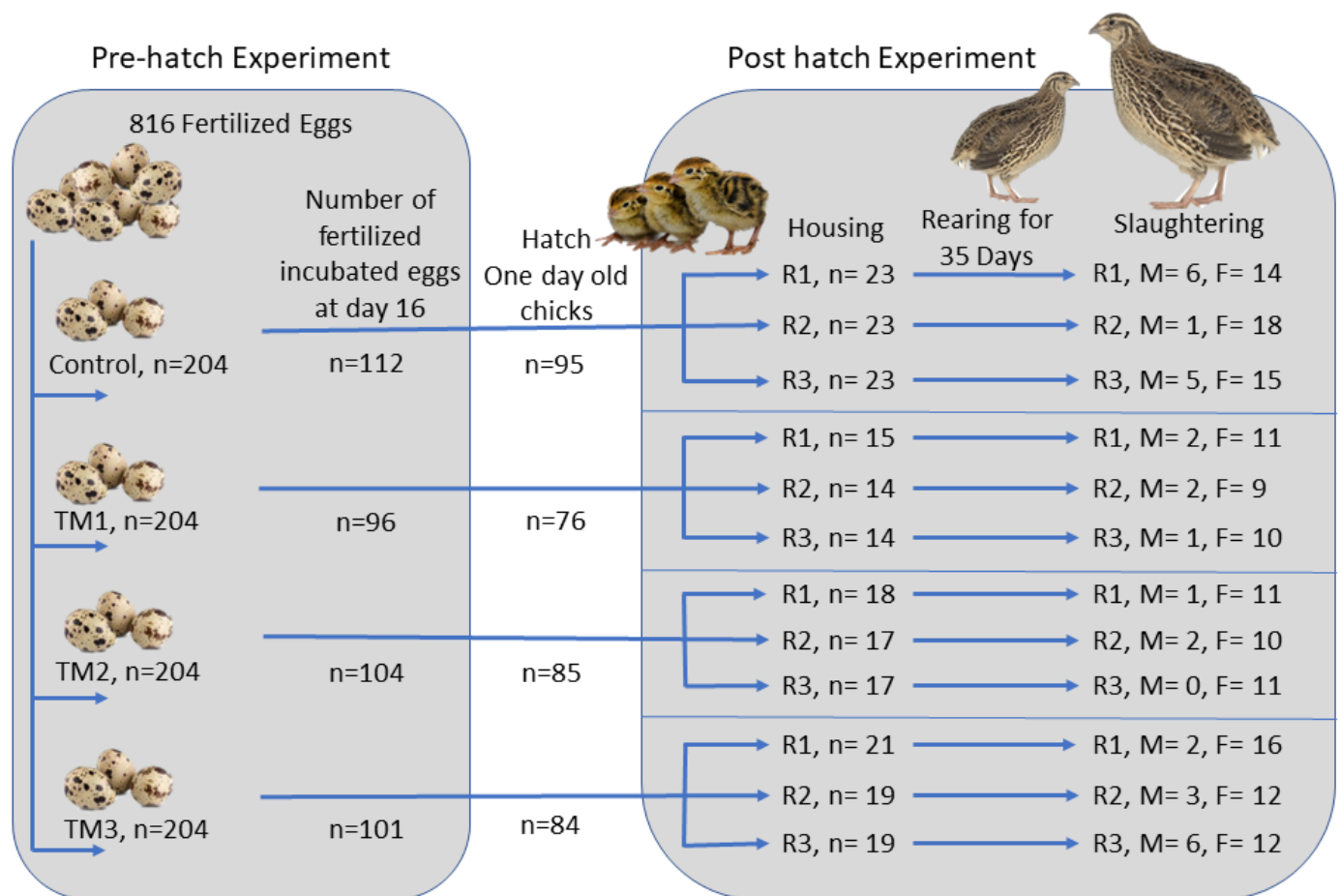


Figure 1: Experimental design for the post-hatch experiment. 12 pens were created from the total hatched chicks (R1,R2,R3; three replicate pens per group, M; Male, F; Female).

Housing and rearing

The chicks were randomly housed on a deep litter system in 12 separate pens (three replicate pens per group) under the same managemental conditions with ad libitum feeding and free access to water. The experimental design is shown in figure 1. The starting room temperature was 35°C in the first week of age and then decreased by 1.5 °C per week until the 5th week of age. Quail chicks were exposed to 23 h light and 1h darkness. During the first 20 days of age, quail chicks were fed with crumbled starter feed (Leader-Egypt TM) containing 3.000 kcal/kg metabolic energy and 23% crude protein. The grower feed (Leader-Egypt TM) containing 2.900 kcal/kg metabolic energy and 21% crude protein was introduced from day 20 till the end of the experiment.

Slaughtering and carcass traits

At 35 days old, after 4 hours of feed deprivation, three birds per replicate were weighed and slaughtered following the halal slaughter procedures according to ethical approval by the Institutional Animal Care and Use Committee (IACUC; Reference No. Vet CU16072020190). Weights of the carcass, abdominal fat, breast, breast muscle, and internal organs (heart, liver, spleen, gizzard, and intestine) were determined for each bird and expressed as a percentage of live body weight. The lengths of the intestines and caeca were measured and recorded. All measurements were taken immediately after slaughtering. Then the carcasses were chilled at 4 °C for 24 hours then examined.

Breast meat quality Parameters

Nine birds from each group (3 birds/replicate) were slaughtered and examined for breast meat quality parameters (proximate compositional analysis and Physico-chemical characteristics). Boneless breasts without skin on both sides of the sternum were separated.

Proximate composition analysis. The proximate chemical analysis was conducted for raw quail meat. Moisture, protein, fat, and ash contents of quail meat were determined for each group after the processing according to the method of the Association of Official Analytical Chemists (9). For determination of moisture contents (g/100g or %), 3 g of sample were dried at 100°C until a constant weight was obtained. Protein content (g% sample) was determined using the Kjeldahl method of analysis. For the conversion of nitrogen into crude protein, a factor of 6.25 was used. Fat (g/100g or %) was determined by 6-cycle extraction with petroleum ether in a Soxhlet apparatus and the weight loss was calculated. Ash was determined by ignition at 500 °C for 5 hours (g% sample).

Physico-chemical characteristics

Shear force. The quail meat was cooked in a convection oven (Heraeus, D-63450 Hanau, Germany). The oven was

adjusted at 150 °C for an internal temperature of 75 °C (average cooking time 20 min). The cooking temperature was monitored by a needle thermocouple probe attached to a previously calibrated hand-held thermometer (Hanna HI 9850911; Pasadena, Texas, USA). The cooked meat was cooled to room temperature. Six core samples (each of 1.27 cm diameter) were collected parallel to the surface using a hand-held coring device. Each core sample was sheared once with a Warner-Bratzler shear force (WBSF) device attached to an Instron Universal Testing Machine (Model 2519 105; Instron Corp., Canton, Massachusetts, USA) with a 55-kg tension/compression load cell and a crosshead speed of 200 mm/min. The average shear force value was calculated and recorded for each sample (10).

Color evaluation. The surface color of freshly cut raw quail meat was measured using a Chroma meter (Konica Minolta, model CR 410, Japan) calibrated with a white plate and light trap supplied by the manufacturer. The L (lightness), a (redness), and b (yellowness) values were obtained using Commission International de l'Eclairage (CIE) standard illuminant D65 light source. The color was expressed using CIE (11).

Cooking loss (CL%). The cooking was performed in a convection oven (Heraeus, D-63450 Hanau, Germany) adjusted at 150 °C for an internal temperature of 75 °C (average cooking time 20 min). Cooking loss was calculated as outlined by Neel et al. (12). Due to the small size of quail carcass, the whole dressed, eviscerated, and cleaned bird carcass (approximately 70.9 g) was cooked. To obtain more accurate results, the readings were taken from different locations of the cooked quail carcass so the reported results will be representative of the whole cooked quail sample. The meat samples were blotted with blotting paper and weighed accurately just before cooking. After cooking, the samples were cooled, wiped with blotting paper, and weighed immediately. The cooking loss as a percentage was determined as the difference in the weights of the sample before and after cooking.

$$CL\% = \frac{(W1-W2)}{W1} \times 100$$

pH value analysis. A meat/muscle homogenate was prepared from a 5 g sample and 20 mL of distilled water. Measurement of pH using a digital pH meter (Lovibond Senso Direct) previously calibrated with 7.0 and 4.0 buffers using a probe-type electrode (Senso Direct Type 330). Three pH readings for each replicate were recorded and the average was calculated according to Abdel-Naem et al. (13).

Sensory analysis

Sensory analysis was carried out by panelists (25 experienced panelists were selected based on their previous

experience in consuming quail meat) from the members of the Food Hygiene and Control Department, Faculty of Veterinary medicine, Cairo University, Egypt. Different cooked quail samples were randomly coded, and the panelists were asked to score the samples using a five-point hedonic scale for tenderness (five denotes extremely tender and one denotes very tough), flavor (five denotes extremely strong flavor and one denotes extremely bland flavor) and juiciness (five denotes very juicy and one denotes very dry) according to the guidelines provided by the American Meat Science Association (14).

Statistical analyses

To compare carcass's traits, weights of internal organs, and breast meat quality parameters among treatment groups, a one-way analysis of variance test (ANOVA) was used. Levene's test was used to evaluate the homogeneity of variances (homoscedasticity), and the Shapiro-Wilk test was utilized for normality analysis of the variables. The data that were not normally distributed or violated the homogeneity of variance assumption were analyzed with the Kruskal-Wallis test (Meat tenderness, flavor, and juiciness). Tukey's Studentized Range (HSD) test was used for post-hoc analysis. All P values less than 0.05 were considered statistically significant. Analyses were performed with SAS® version 9.4

(15). All data were presented as the mean ± standard error of the mean (SEM).

Results and Discussion

Carcass traits and internal organ weights

Analysis of the results revealed that the means final body weights of TM1 were numerically higher but there were no significant differences in final body weights among TM1, TM3, and control. Table 1 shows the effects of embryonic thermal manipulation on carcass traits and internal organs weight of 35-day-old Japanese quails. Thermal manipulation at both early and late embryogenesis (TM3) resulted in a significant reduction in the relative weights of carcass, breast, and breast muscle when compared to the control ($P < 0.05$). Abdominal fat weights in TM1 groups were significantly lower ($t(28) = 2.79$, $P = .0441$) than those in the control group.

Embryonic TM had no significant effect ($P > 0.05$) on the weights of the internal organs (liver, spleen, heart, and intestine) as well as the length of the intestine and caeca. Regardless of treatments, the relative weight of the heart is significantly larger ($t(28) = 3.03$, $P = 0.0052$) in males (0.92 ± 0.02 g) than in females (0.84 ± 0.01 g).

Table 1: Influence of embryonic thermal manipulation on carcass traits and weights of internal organs of 35-day-old meat-type Japanese quails¹

Parameters ³	Treatments ²				Comparisons	
	Ctrl	TM1	TM2	TM3	F-statistics (df ₁ , df ₂)	P value
Live Body weight (g)	197.89 ± 6.52 ^a	205.61 ± 4.56 ^a	175.80 ± 6.06 ^b	192.33 ± 7.63 ^{ab}	5.09 (3, 171)	.0021
Carcass weight % (Without blood, feather, Head, and shank)	85.28 ± 0.63 ^a	83.99 ± 0.42 ^a	83.36 ± 0.82 ^{ab}	81.08 ± 0.44 ^b	6.88 (3, 28)	.0013
Carcass weight % (Without viscera)	72.00 ± 0.35 ^a	71.52 ± 0.36 ^{ab}	70.71 ± 0.32 ^{ab}	69.48 ± 0.87 ^b	4.15 (3, 28)	0.0149
Breast weight %	35.90 ± 0.54 ^a	35.81 ± 0.20 ^{ab}	34.87 ± 0.42 ^{ab}	34.12 ± 0.83 ^b	3.64 (3, 28)	0.0248
Breast muscle weight %	20.54 ± 0.22 ^{ab}	21.80 ± 0.42 ^a	20.01 ± 0.61 ^b	19.56 ± 0.45 ^b	6.15 (3, 28)	0.0024
Abdominal fat weight %	1.81 ± 0.18 ^a	1.10 ± 0.09 ^b	1.21 ± 0.16 ^{ab}	1.22 ± 0.25 ^{ab}	3.09 (3, 28)	0.0429
Heart weight %	0.87 ± 0.03	0.89 ± 0.02	0.86 ± 0.03	0.85 ± 0.02	0.51 (3, 28)	0.6776
Spleen weight %	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.56 (3, 28)	0.6450
Liver weight %	2.02 ± 0.12	2.44 ± 0.12	2.30 ± 0.18	2.05 ± 0.18	1.34 (3, 28)	0.2821
Intestine weight %	4.12 ± 0.30	4.30 ± 0.10	4.55 ± 0.25	4.30 ± 0.21	0.51 (3, 28)	0.6783
Gizzard weight %	2.09 ± 0.08 ^{ab}	2.26 ± 0.11 ^a	2.30 ± 0.06 ^a	1.84 ± 0.05 ^b	6.89 (3, 28)	0.0013
Intestine length (cm)	60.33 ± 2.22	62.78 ± 2.36	58.67 ± 2.30	58.67 ± 2.22	0.46 (3, 28)	0.7138
Length of caeca (cm)	7.67 ± 0.36	8.33 ± 0.29	7.89 ± 0.27	8.06 ± 0.26	0.89 (3, 28)	0.4604

¹All data are presented as the mean ± standard error of the mean (SEM). Within the same row, means followed by different superscript letters are significantly different at $P < 0.05$. ²Treatments (Ctrl: control, TM1: early embryogenesis, TM2: late embryogenesis, TM3: early/late embryogenesis). ³The relative weights of carcass, breast, muscles, abdominal fat, and internal organs were expressed as a percentage of live body weight.

Earlier studies revealed varied and sometimes contradictory results, Collin et al. (16) reported that no effects of thermal manipulation on gross weight were detected during the entire growth period, however, breast yield was higher in late-term TM chickens than in controls at 43 days old. Al-Zghoul and El-Bahr (17) reported that chicken embryo TM induced a significant increase in broiler carcass weight, breast muscle weight, and breast weight/carcass weight percentage if compared with control at post-hatch days 28 and 35. We suggest that the difference in body weight between the TM chicks and the control disappeared after day 35 of age due to the phenomenon of compensatory growth in broilers (18, 19). It is also worth considering the method of measuring and recording temperatures when comparing the results of thermal treatments. It has previously been shown that there can be a significant difference between incubator air temperature and eggshell temperature (EST) (20). Previous studies in quail and chicken revealed that TM chicks had a larger relative breast muscle weight (4, 5, 7, and 19). These results are attributable to a higher percentage of larger diameter (hypertrophy) muscle fibers than the control group (7, 19).

Our results indicated that the relative weight of the abdominal fat pad was markedly decreased in the early-term TM1 group. We suggest that early TM in quail embryos may interfere with adipocyte development and reduce the fat pad in the long term as suggested by Hammond et al. (21) in broiler chicken embryo TM. The reduction of the abdominal fat pad relative weight is one of the aims of broiler

management, and TM had been identified as an effective way to decrease abdominal fat accumulation (4, 19).

Concerning liver weight, our results disagree with Al-Zghoul and El-Bahr (17) who reported that embryonic TM caused a significant increase in liver weight of broiler chickens when compared to control chicks on post-hatch day 35. Massimino et al. (22) documented that embryonic TM in mule ducks resulted in the heavier liver (foie grass production). These significant increases in the liver weight in both species were obtained after long thermal treatment which extends to more than 16 hours/day, so the duration of thermal manipulation is one of the major aspects that should be taken into consideration. The increased liver weight in the TM chicken and duck may be attributed to the increase in glycogen or fat accumulation in the liver (liver steatosis). Our findings agree with Al-Zghoul and El-Bahr (17) who illustrated that broiler embryo TM caused no changes in the spleen, intestine weights as well as intestine and cecum lengths in all TM groups compared to controls.

Breast meat quality

The Physico-chemical characteristics of breast meat are illustrated in Table 2.

The pH values of all TM treatments revealed significant ($P < 0.05$) decreases in comparison with that of the control. The pH value is considered one of the shelf life-determining factors and lower pH values “more acidic” means longer product shelf life (23).

Table 2: Influence of embryonic thermal manipulation on breast meat quality parameters of Japanese quails at 35-day-old¹

Parameters ³	Treatments ²				Comparisons	
	Ctrl	TM1	TM2	TM3	Test statistics	P value
Moisture %	72.30 ± 0.13	72.26 ± 0.16	72.94 ± 0.43	72.39 ± 0.10	$F_{3,8} = 1.88$	0.2118
Fat %	2.37 ± 0.22	2.73 ± 0.04	2.51 ± 0.25	2.98 ± 0.21	$F_{3,8} = 1.86$	0.2141
Protein %	23.30 ± 0.53	23.38 ± 0.18	22.88 ± 1.04	22.99 ± 0.45	$F_{3,8} = 0.14$	0.9311
Ash %	1.46 ± 0.03	1.57 ± 0.05	1.50 ± 0.05	1.53 ± 0.06	$F_{3,8} = 1.07$	0.4155
pH	6.17 ± 0.09 ^a	5.80 ± 0.06 ^b	5.77 ± 0.03 ^b	5.90 ± 0.00 ^b	$F_{3,8} = 10.76$	0.0035
Cooking loss %	23.12 ± 0.49	22.81 ± 0.17	22.65 ± 0.51	23.66 ± 0.46	$F_{3,8} = 0.26$	0.8521
Shear force (N)	1.68 ± 0.07	1.76 ± 0.03	1.77 ± 0.04	1.80 ± 0.07	$F_{3,8} = 0.74$	0.5551
L (Lightness)	41.32 ± 1.04	42.67 ± 0.42	42.45 ± 0.53	43.96 ± 0.36	$F_{3,8} = 2.85$	0.1052
a (Redness)	13.33 ± 0.70	12.94 ± 0.08	13.66 ± 0.59	12.14 ± 0.30	$F_{3,8} = 1.81$	0.2225
B (Yellowness)	3.32 ± 0.86	3.46 ± 0.19	4.49 ± 0.19	4.27 ± 0.32	$F_{3,8} = 2.08$	0.1820
Tenderness	4.00 ± 0.00	4.00 ± 0.21	4.00 ± 0.11	4.00 ± 0.08	$\chi^2(3) < 0.001$	> 0.999
Flavor	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	5.00 ± 0.00	$\chi^2(3) < 0.001$	> 0.999
Juiciness	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	$\chi^2(3) < 0.001$	> 0.999

¹All data are presented as the mean ± standard error of the mean (SEM). Within the same row, means followed by different superscript letters are significantly different at $P < 0.05$. ²Treatments (Ctrl: control, TM1: early embryogenesis, TM2: late embryogenesis, TM3: early/late embryogenesis).

Meat composition (fat%, protein %, ash %, moisture %) and sensory quality (cooking loss %, juiciness, flavor, tenderness) showed no significant differences among treatments. Our results are in agreement with Loyau et al. (5) and Collin et al. (16) who revealed that the embryonic TM favored breast muscle growth without significant alterations in breast meat characteristics. The cooking loss, moisture %, and fat % represent important parameters that reflect the quality of processed meat and are important for the juiciness and mouth feel of cooked meat. The current study revealed no significant difference between the control and TM groups in the sensory examination of breast meat, which could explain why TM did not alter the sensory criteria.

Importantly for the poultry industry, embryonic TM modifies the physiology and body composition of broilers without negatively affecting the processing quality of breast meat at slaughter age. For instance, TM chickens were 1.4% lighter and had 8% less relative abdominal fat pad than controls while consistently having larger myofiber and 4.6% heavier relative breast muscle at 35 days compared to the controls (24). Loyau et al. (5) also reported that breast muscle yield was enhanced by TM, especially in females, without significant change in breast meat characteristics (pH, color, drip loss). These studies highlight the possibility that embryonic TM can promote breast muscle yield without lowering the quality of the muscle.

Conclusions

The quail early embryonic TM is an effective protocol in decreasing fat pad accumulation and pH value of breast meat without any negative effects on breast meat composition or sensory criteria. Intermittent early embryonic TM could be used to reach the favored lucrative effects of the thermal treatment in quail broilers.

Acknowledgments

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The authors declare no conflicts of interest.

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Učinki toplotne manipulacije zarodkov japonskih prepelic na lastnosti trupa po izvalitvi, težu notranjih organov in kakovost prsnega mesa

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Izvleček: Toplotna manipulacija zarodka različnih vrst ptic je znana kot učinkovit protokol za izboljšanje rasti po izvalitvi in pridobivanje odpornosti na povišano temperaturo. Predhodno smo ocenili vpliv embrionalne toplotne manipulacije zarodkov japonskih prepelic na embrionalni razvoj, valilnost in uspešnost po izvalitvi. V tej študiji smo natančneje pojasnili učinke toplotne manipulacije zarodkov japonskih prepelic na težo notranjih organov, lastnosti trupa in parametre kakovosti mesa na 35. dan po izvalitvi. Jajca prepelic kontrolne skupine so bila inkubirana pri 37,7 °C in 55-odstotni relativni vlažnosti. Tri preiskovane skupine prepeličjih jajc so bile občasno izpostavljene temperaturi 41 °C in relativni vlagi 65 % (3 ure/dan): zgodnja embrionalna skupina (TM1) je bila izpostavljena toploti 6.– 8. embrionalni dan (ED), pozna embrionalna skupina (TM2) 12.–14. ED, zgodnja/pozna embrionalna skupina (TM3) pa v obeh časovnih intervalih. Parametre kakovosti mesa prepelic, lastnosti trupa in težo notranjih organov smo ocenili na 35. dan po izvalitvi. Rezultati so pokazali, da je toplotna manipulacija v zgodnjem embrionalnem obdobju (skupina TM1) učinkovit protokol za zmanjšanje kopičenja maščobnih blazinic. Vrednost pH prsnega mesa pri vseh toplotno manipuliranih skupinah se je znatno ($P < 0,05$) znižala za 5 % v primerjavi s kontrolno skupino, brez negativnih učinkov na sestavo prsnega mesa ali senzorične kriterije. Zgodnjo embrionalno toplotno manipulacijo bi bilo priporočljivo uporabiti kot izboljššan protokol, s katerim bi bilo mogoče doseči prednostne donosne učinke pri mesnih prepelicah.

Ključne besede: *Coturnix japonica*; mesna prepelica; lastnosti prsnega mesa; toplotna biologija