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# *Effect of Thermal Embryonic Manipulation on the Quality of Male and Female Broiler Meat Submitted to Thermal Stress Pre-Slaughter*

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## ABSTRACT

Adverse environmental conditions during rearing may negatively affect productivity and meat quality of the modern fast-growing broiler strains. Temperature manipulation during sensitive embryonic development periods may affect broilers' physiological responses to environmental conditions during rearing on commercial farms. The objective of this trial was to evaluate the effect of temperature manipulation during incubation and breeder age on the meat quality of male and female broilers submitted to heat stress during the pre-slaughter period. In this experiment, 1280 broiler chicks were distributed according to a completely randomized experimental design in a 2x2x2 factorial arrangement. Treatments consisted of two breeder ages (30 and 60 weeks), two temperature programs applied in the last four days of incubation (standard or high temperature), and sex (male and female). Birds were submitted to 32 °C for 48 hours before slaughter, on day 46.Meat quality parameters (pH, temperature, color, and weight loss) were evaluated. There was significant effect ($p<0.05$) of sex on meat redness (a*) and fat percentage, with females presenting higher values than males in both measurements. Males incubated at the higher temperature presented higher ($p<0.05$) meat weight loss by pressure than females. The thermal manipulation applied during the final stage of incubation did not affect the meat yield or meat quality of broilers submitted to heat stress.

## INTRODUCTION

Extreme environmental conditions may pose serious challenges for modern broiler strains genetically selected for fast growth (Yahav *et al*., 2004). Heat stress is one of the factors that causes performance losses in the poultry industry. When environmental temperature exceeds the comfortable temperature range, broilers reduce their feed intake and spend energy to dissipate heat in order to maintain homeothermia, resulting in poor performance (McKee & Sams, 1997) and meat quality defects. Heat stress increases the need of energy production via anaerobic glycolytic processes, with consequent production of lactic acid (Berri *et al.,* 2005). The combination of low meat pH due to high lactic acid content with high meat temperature causes muscle protein denaturation, thereby, reducing meat protein stability and water retention capacity (Berri *et al*., 2005; Collin *et al*., 2007).

A meat quality defect, consisting of the presence of several degrees of white striping on the breasts of broilers, which follow the direction of the muscle fibers, has been recently reported. The incidence of the defect increases with age (Petracci & Cavani, 2011). Although its cause has not been elucidated yet, it is possible that the fast development of broilers, in association with inadequate environmental conditions may be contributing factors. This supports the need to search for

alternatives that effectively reduce the impacts of high environmental temperatures on broiler performance (Collin *et al*., 2007).

As a consequence, if the higher growth rate of modern broilers, the proportion between pre- and post egg-hatching life periods was reduced to less than 1:1.5. Therefore, the development of their thermoregulatory system, which controls body temperature during the incubation phase, needs to be better understood, as well as to how to induce acclimation to adverse environmental conditions during the post egg-hatching period.

A protocol of single-stage incubation that includes periodic stimuli, which consists of increasing temperatures during specific sensitive periods of embryonic development, has been applied and it is referred as circadian incubation. It is believed that slight incubation environment variations induce variations in gene expression; as a result, different phenotypes are expressed according to environmental agents (Boerjan, 2010). The most sensitive period in the development of the embryonic thermoregulatory system is during the last days of incubation. During this period, some factors, such as incubation temperature, may induce changes in the perinatal epigenetic programming of body functions (Tzschentke & Plageman, 2006).

Many studies have focused on intermittent temperature manipulation during incubation, ranging from 3 to 12 hours per day and deviating 1-2°C from standard incubation temperature, with very variable results (Moraes *et al*., 2004; Yalçin & Siegel, 2003; Collin *et al*., 2005; Yalçin *et al.,* 2005; Collin *et al*., 2007; Yalçin *et al*., 2008; Piestun *et al*., 2008, Ferreira *et al*., 2015). For instance, Tzschentke & Halle (2009) increased the incubation temperature from 38.2°C to 38.4°C for two hours daily between days 18 and 21 of incubation and observed greater body weight in male broilers at slaughter age. Today, it is difficult to reach consensus relative to protocols for the manipulation of the incubation environment of broiler embryos at industrial level to try to improve live performance in the field (Boerjan, 2010).

Another issue is whether the development of the broilers' metabolic functions can be improved by short-time temperature stimuli. The development of all tissues is affected by incubation temperature, suggesting that embryonic development can be manipulated and may allow pre-conditioning broiler embryo metabolism to post-hatching environmental conditions (Aksit*et al*., 2010; Molenaar *et al*., 2010). Some authors show, for instance, that broiler muscle development can be stimulated by temperature changes at different incubation stages, such as Maltby *et al*. (2004) during initial phase of incubation, Halevy*et al*. (2006) between day 16 and 18,or during the first days post-hatching (Halevy *et al*., 2001). According to Collin *et al.* (2007), incubation temperature manipulations may allow broilers to cope with environmental temperature changes without spending energy, and therefore, to obtain good post-hatching performance. However, temperature stimuli must be applied during the last days of incubation. Currently, new investigations are being conducted to define the maximum intensity and length of temperature stimuli for different types of eggs (Boerjan, 2010).

In addition of varying with genetic selection and embryonic development stage, heat production depends on embryo size, which is determined by broiler breeder age. Hamidu *et al.* (2007) found that the metabolism of embryos from smaller eggs laid by young breeder hens is slower because they receive oxygen lower supply as a result of their thicker shells relative to eggs laid by older breeders. Oxygen is the fuel used for the beta-oxidation of fatty acids in the egg yolk, affecting the embryos' metabolic rate or heat production (Molenaar *et al.*, 2010).

Therefore, the aim of this study was to evaluate the effects of incubation temperature manipulation and breeder age of breeders on the meat quality of male and female broilers submitted to heat stress during the pre-slaughter period.

## MATERIALS AND METHODS

In total 1,280 eggs were distributed in the hatchery according to a completely randomized design in a 2 x 2 x 2 factorial arrangement, consisting of two breeder ages (30 and 60 weeks), two temperatures applied during the last four days of incubation (37.2 - 37.4°C and 38.2 - 38.4°C for 4 hours/d), and sex (males and females), resulting in eight treatments with four replicated of 40 eggs each. Eggs were incubated in single-stage incubator (James Way, model Platinum) at 96.5-100.4° F, 40% relative humidity (RH), and 10,000 ppm $CO_2$ until day 10 and 5,000 ppm $CO_2$ from day 11 to day 21 of incubation.

After hatch, birds were reared in the experimental poultry house of the Federal University of Parana, Palotina, PR, Brazil. The environmentally-controlled broiler house was equipped with evaporative cooling pads and 15-m length and 12-m width vents, divided into 32 pens measuring 3.75 m2. Water and a commercial feed were supplied *ad libitum*. A five-phase

feeding program was applied: pre-starter (23.30% CP, 3,000 kcal ME/kg, 1.25% Lys), starter I (23.00% CP, 3,100 kcal ME/kg, 1.250% Lys), starter II (22.12% CP, 3,150 kcal ME/kg, 1.2% Lys), grower (21.25% CP, 3,200 kcal ME/kg, 1.16% Lys), and finisher (20.25% CP, 3,280kcal ME/kg, 1.111% Lys).

From day 1 to 44, house temperature was maintained in the thermal neutral zone, according to the strain's management guide (Cobb, 2008). On day 44, house temperature was increased up to 32ºC and fan speed was decreased (1.5m/sec) for 48 hours.

On day 46, eight birds per treatment were individually weighed and sacrificed, and their carcasses were used to evaluate meat quality in the Laboratory of Poultry Experimentation of UFPR. Birds were bled, and their carcasses were scalded, plucked, and eviscerated. Carcass yield was calculated as the ratio between hot eviscerated carcass and live body weight. Prime cut yields (whole breast, thigh and legs, with skin and bones) were calculated as their absolute weight relative to eviscerated carcass weight. Abdominal fat around the cloaca, bursa of Fabricius, gizzard, proventriculus, and adjacent abdominal muscles was removed and weighed. Abdominal fat yield was calculated as its weight relative to eviscerated carcass weight.

All breasts were assessed for the presence of white striping. Breasts without visible white striping were considered negative, whereas those presenting clearly visible whitish stripes parallel to muscle fibers on the muscle surface were classified as positive. Negative and positive percentages were calculated relative to the total number of breasts/ treatment.

The right *Pectoralis major* muscle (breast fillet) of each bird was identified and maintained at room temperature for 15 min *post-mortem* for pH and temperature measurements. Measurements were performed within the first hour *post mortem* (initial pH and temperature) and 24 hours after slaughter under refrigeration at 0 ± 2ºC (ultimate pH and temperature).

Color was read on the ventral side of the breast fillet 24 hours *post-mortem*, at three different points using a colorimeter (Minolta CR10). Meat luminosity (L*), redness (a*), and yellowness (b*) values are expressed according to the CIELAB color system.

Water loss by pressure was evaluated in 2 g of the right breast muscle. The samples were weighed on a semi-analytical scale, placed between two filter sheets (Whatman n.1) and pressed between two acrylic plates under a 10-kg weight for 5 minutes, and then weighed.

The left *Pectoralis major* muscle was weighed and roasted on a pre-heated electric oven at 180ºC until reaching 72ºC internal temperature of (5 minutes on each side). After cooking, samples were cooled at 4 ± 2ºC for 12 hours and weighed again to determine cooking water loss.

The left *Pectoralis minor* muscle was weighed and frozen for 24 hours; after defrosting, it was weighed again to determine defrosting water loss.

Data were submitted to statistical analysis using the Statistical Analysis System (SAS) at 5% significance level. Data relative to the incidence of white striping did not present a normal distribution, and therefore were transformed by RANK procedure of SAS, and then submitted to analysis of variance by the GLM procedure of SAS.

## RESULTS

Incubation temperature, breeder age, or broiler sex did not influence (p>0.05) had no effect on carcass, breast, or leg yields. However, sex affected abdominal fat percentage (Table 1), with females presenting higher levels (p<0.05) than males, independently of breeder age or of incubation temperature.

**Table 1 –** Carcass and prime parts yields of male and female broilers derived from 30- and 60-week-old breeders and submitted to different temperatures during incubation.

| | Yield, % | | | |
|---|---|---|---|---|
| Incubation temperature | Carcass | Breast | Leg | Fat |
| Normal | 78.16±0.17 | 36.69±0.49 | 27.49±0.48 | 2.26±0.11 |
| Circadian | 77.90±0.21 | 37.11±0.31 | 26.40±0.23 | 2.26±0.15 |
| p-value | 0.5661 | 0.6348 | 0.0530 | 0.9969 |
| Broiler breeder age | | | | |
| 30 | 77.94±0.17 | 37.16±0.27 | 26.81±0.22 | 2.26±0.14 |
| 60 | 78.13±0.21 | 36.64±0.52 | 27.08±0.50 | 2.26±0.13 |
| p-value | 0.7572 | 0.9001 | 0.6135 | 0.9784 |
| Sex | | | | |
| Male | 77.93±0.21 | 37.02±0.35 | 26.90±0.24 | 1.85$^{b}$±0.10 |
| Female | 78.14±0.16 | 36.78±0.47 | 26.99±0.49 | 2.67$^{a}$±0.12 |
| p-value | 0.2063 | 0.9644 | 0.2871 | <0.0001 |
| Interactions | | | | |
| Incubation x Age | 0.1550 | 0.1937 | 0.9384 | 0.2748 |
| Incubation x Sex | 0.3615 | 0.5126 | 0.6866 | 0.7749 |
| Age x Sex | 0.7970 | 0.5310 | 0.3900 | 0.3097 |
| Incubation x Age x Sex | 0.2668 | 0.4663 | 0.4929 | 0.3042 |
| CV, % | 1.28 | 4.55 | 4.90 | 28.48 |

Relative to the measured meat quality parameters, initial and ultimate pH and temperature values were not affected (p>0.05) by the evaluated factors, as shown in Table 2. Cooking, defrosting, and pressing weight loss of the breasts are presented in Table 3, according to incubation temperature, breeder age, and sex. Pressing weight loss was affected by the interaction (p<0.05) between incubation temperature and broiler breeder age. The breakdown of this interaction (Table 4) shows that the meat of the progeny of 30-week-old breeders, when submitted to higher temperature stimuli from 16 to 19 days of incubation, presented greater pressing weight loss than the progeny of older breeders.

**Table 2 –** Breast meat initial and ultimate pH and temperature values of male and female broilers derived from 30- and 60-week-old breeders and submitted to different temperatures during incubation.

| | At slaughter | | 24 hours *postmortem* | |
|---|---|---|---|---|
| Incubation temperature | pH | T°C | pH | T°C |
| Normal | 6.25±0.07 | 34.44±0.46 | 5.74±0.05 | 10.09±0.37 |
| Circadian | 6.29±0.04 | 34.58±0.33 | 5.75±0.04 | 9.37±0.32 |
| p-value | 0.771 | 0.6928 | 0.8132 | 0.3840 |
| Broiler breeder age | | | | |
| 30 weeks | 6.28±0.04 | 34.84±0.38 | 5.75±0.04 | 9.30±0.38 |
| 60 weeks | 6.27±0.07 | 34.18±0.42 | 5.74±0.05 | 10.16±0.30 |
| p-value | 0.3027 | 0.4777 | 0.6423 | 0.2519 |
| Sex | | | | |
| Male | 6.34±0.04 | 34.43±0.33 | 5.78±0.04 | 9.61±0.38 |
| Female | 6.21±0.06 | 34.60±0.46 | 5.71±0.05 | 9.85±0.32 |
| p-value | 0.1947 | 0.2906 | 0.3427 | 0.7070 |
| Interactions | | | | |
| Incubation x Age | 0.7296 | 0.5727 | 0.7572 | 0.4108 |
| Incubation x Sex | 0.7896 | 0.6636 | 0.6742 | 0.4108 |
| Age x Sex | 0.9405 | 0.6161 | 0.8327 | 0.4901 |
| Incubation x Age x Sex | 0.2780 | 0.0840 | 0.9556 | 0.3710 |
| CV,% | 3.25 | 5.33 | 3.11 | 21.14 |

**Table 3 –** Cooking, defrosting, and pressing weight loss of breast meat samples of of male and female broilers derived from 30- and 60-week-old breeders and submitted to different temperatures during incubation.

| | Loss % | | |
|---|---|---|---|
| Incubation temperature | Cooking | Defrosting | Pressing |
| Normal | 23.25± 0.68 | 17.53[a]± 1.71 | 8.76± 0.56 |
| Circadian | 22.05± 1.30 | 15.88[b]± 3.16 | 12.00± 1.99 |
| p-value | 0.6710 | 0.0591 | 0.5675 |
| Broiler breeder age | | | |
| 30 weeks | 21.91± 1.29 | 15.94± 1.86 | 10.95± 1.59 |
| 60 weeks | 23.38± 0.68 | 17.48± 3.01 | 9.78± 1.34 |
| p-value | 0.4912 | 0.6738 | 0.2167 |
| Sex | | | |
| Male | 23.20± 0.74 | 13.38± 1.86 | 11.24± 1.59 |
| Female | 22.09± 1.26 | 19.96± 3.16 | 9.50± 0.67 |
| p-value | 0.7310 | 0.0887 | 0.2113 |
| Interactions | | | |
| Incubation x Age | 0.6007 | 0.5072 | 0.0345 |
| Incubation x Sex | 0.8893 | 0.7087 | 0.6658 |
| Age x Sex | 0.4675 | 0.4528 | 0.7201 |
| Incubation x Age x Sex | 0.1577 | 0.2994 | 0.1896 |
| CV, % | 18.26 | 58.81 | 34.71 |

**Table 4 –** Statistical breakdown of the incubation temperature x broiler breeder age interaction for pressing weight loss due of broiler breast meat.

| | Broiler breeder age | | p value |
|---|---|---|---|
| Incubation temperature | 30 weeks | 60 weeks | |
| Normal | 8.39[Aa] | 9.13[Aa] | 0.5357 |
| Circadian | 10.75[Aa] | 7.81[Ba] | 0.0503 |
| p-value | 0.0818 | 0.1935 | |

Means followed by equal lowercase letters in the same column do not significantly differ (p>0.05). Means followed by equal uppercase letters in the same row do not significantly differ (p>0.05).

Table 5 shows that there were no effects (p>0.05) of incubation temperature or breeder age, on meat luminosity (L*), redness (a*), or yellowness (b*) values. However, higher redness values (p<0.05) were observed in the breast meat of females than in males. The presence white striping on the breast was not significantly influenced (p>0.05) by the evaluated factors.

**Table 5 –** Meat color measurements (L*, a* e b* values) and the incidence of white striping on the breast of of male and female broilers derived from 30- and 60-week-old breeders and submitted to different temperatures during incubation.

| | Color | | | White striping, % | |
|---|---|---|---|---|---|
| Incubation temperature | L | a | b | No | Yes |
| Normal | 57.50±0.54 | 1.91±0.22 | 12.97±0.36 | 31.25 | 68.75 |
| Circadian | 57.80±0.60 | 1.86±0.18 | 12.86±0.41 | 28.13 | 71.88 |
| p-value | 0.7195 | 0.8482 | 0.8534 | 0.7892 | |
| Broiler breeder age | | | | | |
| 30 weeks | 57.96±0.57 | 1.71±0.18 | 12.78±0.38 | 34.38 | 65.63 |
| 60 weeks | 57.34±0.57 | 2.06±0.22 | 13.05±0.38 | 25.00 | 75.00 |
| p-value | 0.4653 | 0.2181 | 0.6227 | 0.4237 | |
| Sex | | | | | |
| Male | 58.02±0.54 | 1.52$^{b}$±0.17 | 12.84±0.40 | 31.25 | 68.75 |
| Female | 57.28±0.60 | 2.25$^{a}$±0.21 | 12.99±0.37 | 28.13 | 71.88 |
| p-value | 0.3803 | 0.0130 | 0.7967 | 0.7892 | |
| Interactions | | | | | |
| Incubation x Age | 0.3145 | 0.8036 | 0.3464 | 0.7892 | |
| Incubation x Sex | 0.8466 | 0.5085 | 0.4746 | 0.4237 | |
| Age x Sex | 0.6171 | 0.8244 | 0.1613 | 0.0653 | |
| Incubation x Age x Sex | 0.6927 | 0.4844 | 0.7770 | 0.4237 | |
| CV, % | 5.80 | 60.05 | 17.34 | 45.81 | |

## DISCUSSION

The present trial was to evaluate the effect of increasing incubation temperature (38.2 - 38.4ºC for 4 hours/d on days 16 to 19 of incubation) on the meat yield and quality of broiler submitted to heat stress during the last two days of rearing. Our hypothesis was that circadian incubation could induce embryo training or imprinting by early acclimation of the thermoregulatory system, allowing the broilers to cope better with heat stress. However, increasing incubation temperature at the end of incubation did not affect carcass yield prime parts yields of broilers subsequently submitted to heat stress. This result is not in agreement with those reported in literature. Collin *et al*. (2007) and Piestun *et al*. (2008) obtained heavier breast muscles when broiler embryos were incubated at high intermittent temperature (3h, 39.5ºC, 16 to 18 days of incubation).

Females presented greater fat deposition than males. According to Mendes & Komiyama (2011), females have specific physiological processes for reproductive activity, leading to a greater accumulation of fat that increases with advanced age, thereby bringing losses in meat carcass yield.

Halle & Tzschentke (2010) observed that incubation at high temperatures caused long-term changes in body functions and improved acclimation to heat.

Variations in meat color are associated with differences in muscle myoglobin content, morphology, and pH (Mendes & Komiyama, 2011). As observed in Table 2, females presented meat higher redness values (a*), which is directly related to myoglobin content in the muscle, indicating higher *post-mortem* glycolysis rate, which results in muscle color change (McKee & Sams, 1997). Abreu *et al*. (2014) also observed that the meat of female quails is redder than that of males. The reason for this difference between female and male broilers remains to be determined in future experiments.

It is known that male broilers are more vulnerable to environmental changes than females (Bogdanova & Nager, 2008). However, in the present trial, the meat quality of neither male or female broilers was affected by the high incubation temperature applied during the sensitive period of embryonic thermoregulatory system development. On the other hand, Ferreira *et al.* (2015) showed that high incubation temperatures increased chicken meat toughness (shear force) and reduced meat redness values, but did not change meat cooking loss, pH, or luminosity.

Appearance is an important meat quality characteristic related to the acceptance or rejection of products by consumers. One of the factors that affects chicken meat appearance is water retention capacity (Garcia *et al*., 2012), particularly during storage (Berri *et al*., 2005; Tona *et al*., 2008). It is known the effect of breeder age influences the live performance of their progeny (Peebles *et al.,* 1999). When the embryos were submitted to high temperatures between 16 and 19 days of incubation, the meat of broilers derived from 30-wk-old breeders presented higher pressure weight

loss compared with those from 60-wk-old breeders. This effect may be explained by the higher nutritional content and more balanced nutrient profile of the yolk of the eggs laid by old breeders, promoting uscle development and enhancing the functional properties of broiler meat.

Yalçin *et al.* (2005) investigated the relationship between broiler breeder age breeders and the effectiveness of pre- and postnatal conditioning induced thermal tolerance. According to those researchers, although pre- and postnatal conditioning may help broilers cope with heat stress during grow-out, breeder age has a strong influence on the thermoregulation ability of broilers. Thermal conditioning of embryos and additional conditioning the chicks thereafter may eliminate or at least reduce the adverse effects of heat stress on broilers from younger parents. The possible interference of breeder nutrition with these mechanisms remains to be explored.

Although the numerical increase in the incidence of white striping on the breasts of the progeny of older breeders was not statistically significant, this trend warrants further investigation. According to Yalçin *et al.* (2005), breeder age plays an important role in the response of the embryo temperature manipulation during incubation; this may influence the performance during the post egg-hatching period. Nevertheless, no references on the influence of breeder age on the incidence of that myopathy in the progeny were found in literature.

The efforts to increase the body weight of broilers should not be limited to management and care during the post-hatching period. Incubation factors that affect broiler performance should also be determined. Circadian incubation has been evaluated as an alternative to induce phenotypes adapted to different rearing environments, and that therefore could present adequate live performance and meat quality under adverse environmental conditions.

Thermal manipulation during incubation failed to improve the thermal tolerance of broilers submitted to heat stress during the pre-slaughter period. This may be due to a lowe degree of thermal stimulation and/or short duration of thermal stimulation. However, it is also possible that thermal stimulation was not applied during the optimal sensitive period (Collin *et al.*, 2007; Tona *et al.,* 2008) in the present study. This warrants further research on the optimal conditions for thermal manipulation.

Another hypothesis is that an additional stimulation is required to obtain long-term thermal tolerance. In a recent study, Ferreira *et al* (2015) reported that high incubation temperature during the embryonic phase plus *in-ovo* vitamin C injection prevented the thermal stress effects of high rearing temperatures.

In addition, optimal embryo requirements regarding incubation temperature, relative humidity, and $CO_2$ concentration to obtain maximum hatchability, hatchling quality, and subsequent thermal tolerance are still not completely known. Therefore, studies on the thermal manipulation of chick embryos must consider the influence of all these parameters on longer-lasting thermoregulatory responses and on the acquisition of thermal tolerance.

The results showed that incubation temperature manipulation during the final incubation phase (16 to 19 days/4 hours daily) did not influence the meat yield or meat quality of male and female broilers submitted to heat stress before slaughter.

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