

Thermal stimulation of Ross®-lineage embryos on a commercial scale

Estimulação térmica dos embriões da linhagem Ross® em escala comercial

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ABSTRACT

Artificial incubation is an essential process to obtain healthy birds with good performance; nevertheless, it requires sustained improvement. During this process, incubation temperature is considered a critical factor, which has been studied. The objective of this study was to evaluate the development of Ross® embryos after hot and cold thermal stimulation. To this end, temperatures 1.39°C above the standard temperature and a temperature fixed at 36.00°C that varied 1.00 to 0.30°C below the standard temperature were applied during the final embryonic development period (days 14 to 18) for three hours, on a commercial scale. Results revealed that hot and cold thermal stimulations did not cause embryo mortality; the hatching and chick quality index were maintained and even increased. Therefore, we believe that thermal stimulation has the potential to improve hatchery index, and thus grange performance; however, adjustments are needed, varying according to each individual hatchery, before it can used as a protocol.

Key words: commercial incubation, embryonic development, hatching, thermal conditioning.

RESUMO

A incubação artificial é um processo fundamental para obtenção de aves saudáveis e com bom rendimento, contudo, necessita de constantes melhorias. Nesse processo, a temperatura de incubação é considerada um fator crítico e vem sendo estudada. O objetivo deste trabalho foi avaliar o desenvolvimento de embriões da linhagem Ross® frente a estimulações térmicas por calor e por frio. Para tanto, temperaturas de 1,39°C acima da temperatura padrão e de 36,00°C fixos, abaixo da temperatura padrão (variando de 1,00 a 0,30°C), foram aplicadas no último período de desenvolvimento embrionário (14^o ao 18^o dia) por três

horas de duração, em escala comercial. Com esse experimento, percebeu-se que as estimulações térmicas por calor e por frio não causaram mortalidade embrionária, mantiveram e até aumentaram os índices de eclosão, bem como a qualidade do pintinho. Dessa forma, acredita-se que as estimulações térmicas têm potencial de melhorar os índices do incubatório, e conseqüentemente o desempenho na granja, contudo necessita de ajustes, que dependem de cada incubatório para ser utilizada como protocolo.

Palavras-chave: condicionamento térmico, desenvolvimento embrionário, eclosão, incubação comercial.

INTRODUCTION

Cellular differentiation during incubation results in embryonic development of organs and physiological regulation systems, such as thermoregulatory system, with the developmental phases overlapping in a continuous process. Organs involved in thermoregulation (hypothalamus, thyroid, and pituitary gland) develop during growth phase, but their final maturation occurs in the last days of incubation and shortly after hatching (FLORES et al., 2013). Thus, incubation climate may influence embryonic development, and also affect post-hatching performance. In this context, slight variations in environment during embryonic development, in particular temperature variations, are believed to

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induce epigenetic adaptations (GILBERTS & EPEL, 2009) that are important for bird adaptability.

Different temperatures during incubation may have different effects on broiler weight after hatching, and may affect the final slaughter weight (TONA et al., 2004; WILLEMSSEN et al., 2008); these variations may also increase tolerance to environmental temperature challenges (MORAES et al., 2003; COLLIN et al., 2007), alter post-natal growth (COLLIN et al., 2005; HALEVY et al., 2006), and resulted in improved performance on site at 38 days of age (SHINDER et al., 2009). These long-term adaptations occur after application of periodic thermal manipulation during the last phase of maturation, when embryos are most responsive to “training” (TZSCHENTKE & HALLE, 2009). Thus, the objective of this study was to evaluate the development of Ross® embryos on a commercial scale, by assessing hatchery productivity index and chick quality after subjecting them to two thermal stimulations (hot and cold) during the final stage of embryonic development (days 14 and 18) for a period of three hours each.

MATERIALS AND METHODS

Eggs from the same batch of Ross® breeder hens between 61 and 63 weeks of age, were subjected to temperature variations during embryonic development in a modular single-stage incubator (SmartPro 77; Pas Reform Hatchery Technology, 2014, Zeddum, The Netherlands), which was used to combine different treatments. The tests were applied on a commercial scale, with the machines loaded at maximum capacity (76.800 eggs) using the hatchery incubation program as a baseline. T1 was 1.39°C above the 36.55°C programmed value for the 14th day of embryonic development (ED), and this was increased by 1.39°C for each subsequent programmed value until the 18th day (ED), for three hours per day; and T2 was a cold stimulus fixed at 36.00°C, varying 1.00°C to 0.30°C below the programmed temperature from the 14th to the 18th day of ED, for three hours per day. There were “control” incubations in the same single-stage machines (SS Cont), and some hatchings were also monitored in multiple-stage machines (CASP CM 125R, Amparo, Brazil) (MS Cont), both without thermal stimulation. The incubation environment (temperature, humidity, gas exchange, and ventilation) was monitored in real time using Smart Center software (Pas Reform Hatchery Technologies, 2014, Zeddum, The Netherlands).

Egg shell surface temperatures were recorded before and after the stimulations in 42

eggs distributed over three points in the incubator on pre-established and identified trays (as shown in Figure 1), using an infrared thermometer (ITR 4520, Braun Termoscan®, Kronberg, Germany) with an accuracy of $\pm 0.20^\circ\text{C}$. Thus, all trays in the upper, middle, and lower parts of the trolleys and the incubator were sampled. After hatching, an embryo diagnosis was performed to categorize losses in non-hatched eggs. Six trays with a capacity of 150 eggs each that had been monitored for temperature, were analyzed for each treatment.

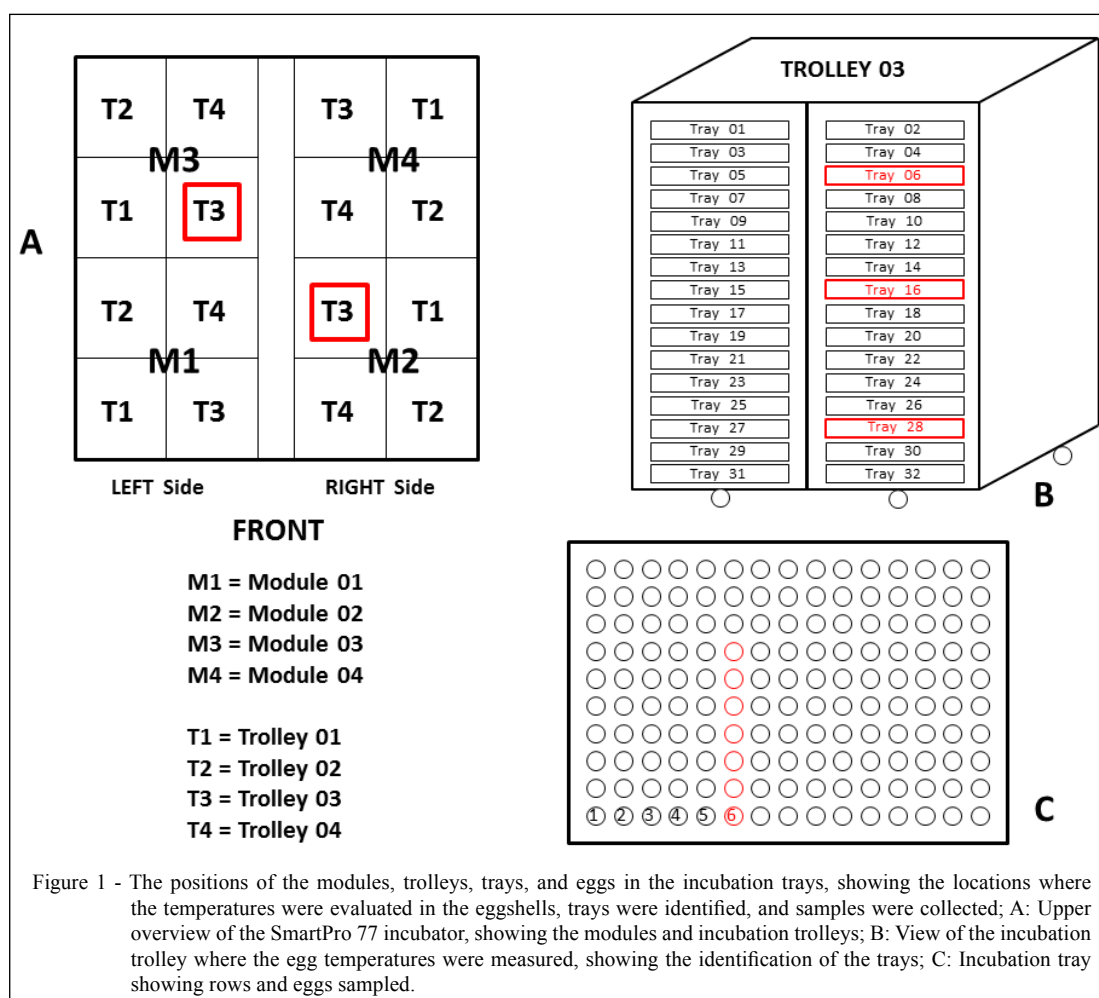
From these same trays, a random sample of 25 chicks (male and female) was evaluated for general quality using the Pasgar score (VAN DE VEN, 2011). Five items were verified: vitality (reflexes), navel, legs, beak, and abdomen. In addition, the cloacal temperatures of 15 males were measured using an infrared thermometer (ITR 4520). They were later sacrificed by cervical dislocation, in accordance with animal welfare standards. Next, the animals and their organs (yolk sac, heart, gastrointestinal tract [GIT], duodenal loop with pancreas, proventriculus, and ventriculus) were weighed using a scale (WeighMax® W- 3805 -100G, China) with a precision of $\pm 0.01\text{g}$.

In addition, at the end of incubation, the hatchability of the fertile eggs (total chicks hatched/total fertile eggs $\times 100 = \%$), the total hatching rate (number of chicks/number of incubated eggs $\times 100 = \%$), and the fertility (total fertile eggs/total incubated eggs $\times 100 = \%$) were calculated. This study was performed in a random and observational manner in a factorial scheme (1 \times 2 \times 2 – one age range of breeder hens \times two thermal treatments and two controls). The data were subjected to analysis of variance (ANOVA) through PROC GLM; later, averages were compared using Tukey tests with a 95% confidence level using SAS version 9.0 (2010).

RESULTS AND DISCUSSION

The surface temperatures were higher among the chicks that had received thermal treatments than for those in the control groups (no stimulation) (Table 1). The weight of the heart was lower in the T1 group (hot stimulus: 1.39°C) than in other groups. This observation was consistent with the literature, in which overheated embryos showed reduced heart size and altered cardiac muscle development (LEKSRISOMPONG et al., 2007) with an eggshell temperature of 38.90°C.

The masses of the GIT and the duodenal loops with the pancreas were greater ($P < 0.05$) in the single-stage incubated groups, both control and



stimulation, compared to the MS control group (no stimulation - multiple stage). Previous studies also reported reduced GIT tissue mass associated with exposure to elevated temperatures (WINELAND et al., 2006ab; LEKSRIOMPONG et al., 2007). The weights of the proventriculus and ventriculus were lower in the group subjected to thermal stimulation with heat (1.39°C) and in the MS control group (no stimulation - multiple stage). It is important to note that the MS group showed lower weights for GIT and duodenal loop with pancreas (Table 1), suggesting undesired and random temperature variations in the MS group.

Total weight of chicks and free weight of yolks did not differ significantly ($P>0.05$) between groups (Table 1). Tendencies towards lower average total weight were observed in T1 (heat stimulation: 1.39°C), followed by the SS control group (no stimulus - single stage) and the T2 group (cold

stimulation: 36.00°C). WINELAND et al. (2006ab) and LEKSRIOMPONG et al. (2007) reported that lower average weights of chicks and their organs after heat stimulation. Other researchers (YALÇIN et al., 2008) increased the incubation temperature to 38.5°C for six hours per day from the 10th to the 18th day, and reported accelerated growth compared to the control group with greater chick weight. This effect was not observed by YALÇIN & SIEGEL (2003), who assessed the effects of higher temperature (39.00°C). Note that the difference is only 0.50°C, which essentially corresponds to 1.00°F, the unit normally used in incubation (FLORES et al., 2013). This emphasizes the narrowness of temperature variation, and that its effects are dependent on the period of ED, as well as the frequency and intensity of the thermal stimulation. Stimulation used in this study was at most 38.33°C for heat and 36.00°C for cold, in alternation; that is, the embryos received both

Table 1 - Average surface temperatures, weights, embryo diagnoses, and hatchery productivity in the thermal stimulation and control groups.

Analyses	Variable	Treat.	Average	Variable	Treat.	Average
Surface Temperature and Weight	Surface temperature (°C)	T1	37.99 ^a ±0.22	Weight of heart (g)	T2	0.41 ^a ±0.01
		T2	37.84 ^a ±0.22		SS Cont.	0.40 ^a ±0.01
		MS Cont.	37.47 ^{ab} ±0.38		MS Cont.	0.30 ^b ±0.02
		SS Cont.	37.13 ^b ±0.22		T1	0.30 ^b ±0.01
	Total weight (g)	MS Cont.	48.63 ^a ±1.76	Weight of GIT (g)	T4	2.12 ^a ±0.09
		T1	47.40 ^a ±1.02		SS Cont.	2.06 ^a ±0.09
		SS Cont.	47.01 ^a ±1.02		T1	1.99 ^a ±0.09
		T2	44.24 ^a ±1.02		MS Cont.	1.51 ^b ±0.15
	Free weight of yolk (g)	SS Cont.	41.82 ^a ±0.89	Weight of duodenal loop and pancreas (g)	T2	0.54 ^a ±0.02
		MS Cont.	40.86 ^a ±1.54		SS Cont.	0.54 ^a ±0.02
		T1	40.81 ^b ±0.89		T1	0.50 ^a ±0.02
		T2	39.14 ^b ±0.89		MS Cont.	0.31 ^b ±0.04
Weight of yolk (g)	MS Cont.	7.77 ^a ±0.57	Weight of PVV (g)	T2	3.03 ^a ±0.08	
	T1	6.58 ^{ab} ±0.33		SS Cont.	2.93 ^a ±0.08	
	SS Cont.	5.37 ^b ±0.33		T1	2.47 ^b ±0.08	
	T2	5.05 ^b ±0.33		MS Cont.	2.45 ^b ±0.14	
Infertility (%)	T1	12.3	Pecked dead (%)	T1	0.4	
	SS Cont.	15.6		SS Cont.	0.1	
	T2	14.2		T2	0	
Mort. 1-4 days (%)	T1	5.6	Rotten (%)	T1	0.5	
	SS Cont.	5.2		SS Cont.	2.1	
	T2	9.4		T2	0.7	
Mort. 5-14 days (%)	T1	1.3	Cracked (%)	T1	0	
	SS Cont.	0.6		SS Cont.	0.4	
	T2	1.8		T2	1	
Mort. 5-14 days (%)	T1	1.3	Non-pecked live (%)	T1	0.1	
	SS Cont.	0.6		SS Cont.	0.3	
	T2	1.8		T2	0	
Mort. 15-21 days (%)	T1	2.9	Fungus (%)	T1	0.1	
	SS Cont.	3.9		SS Cont.	0	
	T2	2.6		T2	0	
Pecked live (%)	T1	0.6	Total eggs not born/not evaluated	T1	214/900	
	SS Cont.	0.4		SS Cont.	258/900	
	T2	0.6		T2	272/900	
General hatching (%)	T1	72.95	Fertility (%)	T2	87.7	
	SS Cont.	67.54		SS Cont.	84.39	
	MS Cont.	66.56		MS Cont.	-	
	T2	67.27		T2	85.79	
Incubation Index	Waste (%)	T1	1.42	Pasgar score (%)	T1	8.8
		SS Cont.	1.74		SS Cont.	8.8
		MS Cont.	2.74		MS Cont.	-
		T2	2.29		T2	9.08
Hatchability (%)	T1	83.18				
	SS Cont.	80.03				
	T2	78.41				

T1 = Hot stimulus 1.39°C above programmed temperature for ED days 14 to 18/3 hours per day; T2 = Cold stimulus fixed at 36°C varying from an additional 1.0 to 0.3°C below programmed value for ED days 14 to 18/3 hours per day; S Cont. = Single stage; MS Cont. = Multiple stage; GIT = gastrointestinal tract; PVV = proventriculus and ventriculus; Treatments with different letters have statistical significance ($P < 0.05$) by Tukey test.

higher and lower temperature shocks. We were able to observe that the resulting alterations in embryonic development in this study did not compromise chick hatching rates or their quality.

The average yolk weight in the MS group (no stimulus - multiple stage) was significantly different from those of the other groups ($P < 0.05$). According to the literature, increased residual yolk is a sign of overheating (LEKSRIOMPONG et al., 2007), which reaffirms the possibility that non-scheduled variations at inadequate times may negatively affect normal development. In the industry, temperature variability in different regions of the machine is often observed (FRENCH, 2002), since there are eggs of various origins and in different phases of development, in addition to differences in metabolism, oxygen consumption, and size due to breeder hens of different ages (LOURENS et al., 2006; HAMIDU et al., 2007). This study evaluated the development of embryos from Ross® breeder hens aged between 61 and 63 weeks. Thermal stimulation of other eggs from different age ranges of breeder hens is currently being evaluated as a continuation of the current study.

The residual yolk weights were 15.97%, 13.87%, 11.41%, and 11.42% for the control EM (no stimulation – multiple stage), T1 (hot stimulus: 1.39°C), SS control (no stimulation – single stage), and T2 (cold stimulus – fixed at 36.00°C) groups, respectively. Values lower than 10% of the chick's weight are recommended for good physical development (MEIJERHOF, 2005).

The chick overall quality can be used to identify possible incubation problems. Pasgar scores of at least nine points have been suggested to indicate non-problematic incubation (PASREFORM, 2010ab). The T1 (hot stimulus: 1.39°C) and the SS control (no stimulation – single stage) treatments scored 8.8 points, while the T2 group (cold stimulus – fixed at 36.00°C) scored 9.1 points.

Incorrect incubation temperatures may reduce hatchability, chick quality, and particularly affect post-hatching performance (HULET et al., 2007). In this study, the hatching rate of the T1 eggs (hot stimulus – 1.39°C) was greater than expected (Table 1), while the rates in the other groups did not differ significantly from the standard hatchery percentages. Previous studies have reported that thermal manipulation during the last development stage improves hatching by 1.5% and growth of males by 2.9%, with better food conversion (TZCHENTKE & HALLE 2009).

Evaluation of the non-hatched eggs (Table 1) revealed no indications of late embryonic

mortality that could be attributed to the high incubation temperatures, nor were significant percentages of non-pecked eggs found, which could be attributed to low temperatures. Average embryo temperatures were monitored and it did not exceed the standard limit. In addition, after the stimulation, the eggs would return to normal temperature within three hours.

LOURENS et al. (2005) established a target temperature of approximately 37.78°C for broiler embryos from the first day of incubation until transfer. Technical recommendation for single-stage incubations is 38.33°C in the last three days of incubation before transfer (maximum 38.61°C) (PAS REFORM, 2010ab) - that is, an increased embryonic temperature is recommended at the end of the cycle.

The exposure of eggs to high temperatures (38.50°C) for 4-6 hours between the 10th and 16th days of incubation may improve the capacity to adapt to heat stress in the fifth week (AKSIT et al., 2010). YALÇIN et al. (2010) reported better adaptation to high temperatures in broilers between the third and sixth post-hatching weeks, minimizing the negative effects caused by heat stress on slaughter weight and breast yield after exposure to temperatures of 39.60°C for 6h from the tenth to the eighteenth days of incubation. This study assessed the effects of temperatures 1.39°C above the scheduled values for each day of ED and with a temperature below these values (36.00°C), both for a period of three hours, from the 14th to the 18th days of ED. Results of this study showed no increase in embryonic mortality and improved hatching and quality index, suggesting the potential for improved farm performance.

However, depending on how the eggs are incubated, broilers reared in sheds may respond positively or negatively to temperature variations (LEKSRIOMPONG et al., 2009). For this reason, field data should be evaluated together with incubation data, and these batches should be monitored until slaughter. Considering that incubation conditions affect embryonic development, changes in broiler performance and health are to be expected (HULET et al., 2007; OVIEDO-RONDÓN et al., 2009). These effects may be observed in the batch averages or in small groups that experienced harmful microenvironments in the incubator. The current challenge is in reaching a consensus and defining protocols at industrial level that may improve productivity in the poultry sector (BOERJAN, 2010). In the meantime, the effects of sub-optimal incubation may cause problems in viability and poor health.

CONCLUSION

Thermal stimulation administered for three hours from ED days 14 to 18, with temperatures 1.39°C above the standard temperature and at 36.00°C (below the standard), did not cause negative effects. Thus, thermal stimulation may be a potential tool for use in hatchery protocols; however, optimal temperatures should be determined based on the type of incubator.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

This study was performed in accordance with the ethical principles of animal testing adopted by the Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL), and with the current legislation (Law 11,794 from 10/08/2008 and Decree 6,899 from 07/15/2009) under protocol number 3503-1, approved by the UNICAMP Ethics and Animal Welfare Committee.

ACKNOWLEDGEMENTS

The Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for granting doctoral fellowship - Process n° 162678/2011-8

REFERENCES

- AKSIT, M. et al. Brooding temperatures for chicks acclimated to heat during incubation: effects on post-hatch intestinal development and body weight under heat stress. **British Poultry Science**, v.51, p. 444-452, 2010. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/20680880>>. Accessed: Sept. 25, 2014. doi: 10.1080/00071668.2010.495746.
- BOERJAN, M. Circadian incubation for robustness. In: EUROPEAN POULTRY CONGRESS, 13., 2010. Tours, France. **Proceedings...** Tours: European Poultry Congress, 2010. 263p.
- COLLIN, A. et al. Effects of thermal manipulation during early and late embryogenesis on thermotolerance and breast muscle characteristics in broiler chickens. **Poultry Science**, v.86, p.795-800, 2007. Available from: <<http://ps.oxfordjournals.org/content/86/5/795.full.pdf+html>>. Accessed: Sept. 17, 2015. doi: 10.1093/ps/86.5.795.
- COLLIN, A. et al. The effect of duration of thermal manipulation during broiler chick embryogenesis on body weight and body temperature of post-hatched chicks. **Animal Research**, v.54, p.105-111, 2005. Available from: <hal.archives-ouvertes.fr/hal-00890025>. Accessed: Sept. 17, 2015. HAL Id: hal-00890025.
- FLORES, F. et al. Thermal variation during incubation of eggs and its effects on ingredients immunologic embryo. **Enciclopédia Biosfera**, v.9, p.2594-2612, 2013. Available from: <<http://www.conhecer.org.br/enciclop/2013b/CIENCIAS%20AGRARIAS/variacao%20termica.pdf>>. Accessed: Aug. 09, 2015.
- FRENCH, N.A. Managing the incubation environment in commercial hatcheries to meet the requirements of the embryo. **Avian and Poultry Biology Reviews**, v.13, p.179-185, 2002. Available from: <<http://www.ingentaconnect.com/content/stl/apbr/2002/0000013/00000003/art00008?token=0049133281b275c277b42573a6754487425447b496e75592f653b672c57582a72752d70fa>>. Accessed: Mar. 19, 2015. doi: 10.3184/147020602783698511.
- GILBERT, S.F; EPEL, D. **Ecological developmental biology: integrating epigenetics, medicine and evolution**. Massachusetts: Sinauer Associates, 2009. 1949p.
- HALEVY, O. et al. In ovo exposure to monochromatic green light promotes skeletal muscle cell proliferation and affects myofiber growth in posthatch chicks. **American Journal of Regulatory Integrative and Comparative Physiology**, v.290, p.1062-1070, 2006. Available from: <<http://ajpregu.physiology.org/content/290/4/R1062.long>>. Accessed: Apr. 15, 2015. doi: 10.1152/ajpregu.00378.2005.
- HAMIDU, J.A. et al. The effect of broiler breeder genetic strain and parent flock age on eggshell conductance and embryonic metabolism. **Poultry Science**, v.86, p.2420-2432, 2007. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/17954594>>. Accessed: Apr. 15, 2015. doi: 10.3382/ps.2007-00265.
- HULET, R. et al. Influence of egg shell embryonic incubation temperature and broiler breeder flock age on posthatch growth performance and carcass characteristics. **Poultry Science**, v.86, p.408-412, 2007. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/17954594>>. Accessed: Apr. 15, 2015. doi: 10.3382/ps.2007-00265.
- LEKSRIOMPONG, N. et al. Broiler incubation-1. Effect of elevated temperature during late incubation on body weight and organs of chicks. **Poultry Science**, v.86, n.12, p.2685-2691, 2007. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/18029817>>. Accessed: Mar. 21, 2015. doi: 10.3382/ps.2007-00170.
- LEKSRIOMPONG, N. et al. Broiler incubation-2. Interaction of incubation and brooding temperatures on broiler chick feed consumption and growth. **Poultry Science**, v.88, p.1321-1329, 2009. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/18029817>>. Accessed: Mar. 21, 2015. doi: 10.3382/ps.2007-00170.
- LOURENS, A. et al. Effect of eggs shell temperature during incubation on embryo development, hatchability and post-hatch development. **Poultry Science**, v.84, p.914-920, 2005. Available from: <<http://ps.oxfordjournals.org/content/84/6/914.abstract>>. Accessed: Jun. 04, 2015. doi: 10.1093/ps/84.6.914.
- LOURENS, A. et al. Effect of egg size on heat production and the transition of energy from egg to hatchling. **Poultry Science**, v.85, p.770-776, 2006. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/17878449>>. Accessed: Mar. 26, 2015. doi: 10.1093/ps/86.10.2194.
- MEIJERHOF, R.; VAN BEEK, G. Mathematical modelling of temperature and moisture loss of hatching eggs. **Journal of Theoretical Biology**, v.165, p.27-41, 1993.
- MORAES, V.M.B. et al. Effect of thermal conditioning during incubation embryo development on aspects of physiological responses of broilers to heat stress. **Journal of Thermal Biology**, v.28, p.133-140, 2003. Available from: <<http://base.repositorio.unesp.br/handle/11449/33643>>. Accessed: Mar. 14, 2015. doi: 10.1016/S0306-4565(02)00049-9.

- OVIEDO-RONDÓN, E.O. et al. Incubation conditions affect leg health in large, high-yield broilers. **Journal of Applied Poultry Research**, v.18, p.640-646, 2009.
- PAS REFORM HATCHERY Technologies. **Research & development**. Technical publications. Incubation Academy. Rio Claro, São Paulo, Brazil. 2010a. 8 p.
- PAS REFORM HATCHERY Technologies. **Incubation Guide**. Rio Claro, São Paulo Brazil, 2010b. Version 4.1. 107 p.
- SAS Institute Inc. **SAS**. Version 9.2. Cary, NC, 2010. 2200 p.
- SHINDER, D. et al. Effect of repetitive acute cold exposures during the last phase of broiler embryogenesis on cold resistance through the life span. **Poultry Science**, v.88, p. 636-646, 2009. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/19211536>>. Accessed: Aug. 17, 2015. doi: 10.3382/ps.2008-00213.
- TZSCHENTKE, B.; HALLE, I. Influence of temperature stimulation during the last 4 days of incubation on secondary sex ratio and later performance in male and female broiler chicks. **British Poultry Science**, v.50, p.634-640, 2009. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/19904643>>. Accessed: Feb. 03, 2015. doi: 10.1080/00071660903186570.
- TONA, K. et al. Comparison of embryo physiological parameters during incubation, chick quality, and growth performance of three lines of broiler breeders differing in genetic composition and growth rate. **Poultry Science**, v.83, p.507-513, 2004. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/15049506>>. Accessed: Aug. 10, 2013.
- VAN DE VEN, L.J. et al. Hatching system and time effects on broiler physiology and posthatch growth. **Poultry Science**, v.90, p.1267-1275, 2011. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/21597068>>. Accessed: Nov. 13, 2014. doi: 10.3382/ps.2010-00876.
- WILLEMSEN, H. et al. Critical assessment of chick quality measurements as an indicator of posthatch performance. **Poultry Science**, v.87, p.2358-2366, 2008. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/18931188>>. Accessed: Nov. 13, 2014. doi: 10.3382/ps.2008-00095.
- WINELAND, M.W. et al. Incubator temperature and oxygen concentration at the plateau stage in oxygen consumption affects intestinal maturation of broiler chicks. **International Journal of Poultry Science**, v.5, p.229-240, 2006a. Available from: <<http://scialert.net/abstract/?doi=ijps.2006.229.240>>. Accessed: May 27, 2015. doi: 10.3923/ijps.2006.229.240.
- WINELAND, M.J. et al. Incubator environment interacts with genetic line of broiler at the plateau stage to affect embryo plasma thyroxine and triiodothyronine concentrations. **International Journal of Poultry Science**, v.58, p.714-722, 2006b. Available from: <<http://scialert.net/abstract/?doi=ijps.2006.714.722>>. Accessed: Jun. 26, 2015. doi: 10.3923/ijps.2006.714.722.
- YALÇIN, S.; SIEGEL, P.B. Exposure to cold or heat during incubation on developmental stability of broiler embryos. **Poultry Science**, v.82, p.388-392, 2003. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/12967250>>. Accessed: Jun. 26, 2015. doi: 10.1093/ps/82.9.1388.
- YALÇIN, S. et al. Acclimation to heat during incubation. 2. Embryo composition and residual egg yolk sac fatty acid profiles in chicks. **Poultry Science**, v.87, p.1229-1236, 2008. Available from: <<http://www.ncbi.nlm.nih.gov/pubmed/18493015>>. Accessed: Dec. 01, 2014. doi: 10.3382/ps.2007-00436.