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EFFECT OF THERMAL STIMULATION IN EMBRYOS FROM COBB®GENETIC STRAIN UNDER COMMERCIAL SCALE

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ABSTRACT: Studies aimed at producing stronger birds and with better growth performance are being developed involving artificial incubation. To that extent, thermal stimulation by the heat of 1.39 ºC and 1ºC above the standard, and by cold of 36 °C fixed, were applied in the last week of the embryonic development (day 14 to day 18) to Cobb® strain birds of 33 to 53 weeks age. With the purpose of evaluating the behavior of the embryos facing these temperature stimuli to identify what is the best amplitude, frequency and the most appropriate period to perform temperature variation, obtaining more adapted birds to the field. It has been observed that heat or cold stimulation did not cause embryo mortality and did not affect the hatching and the quality of the chick for this strain negatively. The weight of the pullet, the residual weight of the yolk, and the peak of birth were not modified. The thermal stimulation during the last week of the birds' incubation is an available tool to improve hatchery production, quality and performance. However, several variables must be tested.

KEYWORDS: hatchability, incubation, chick quality, thermal variation.

INTRODUCTION

Embryo temperature during incubation is considered the most important physical factor in the success of commercial poultry incubation (WILLEMSEN et al., 2011), as it alters the embryo development supported by the cell gene expression (FLORES et al., 2013, SARDARY & MOHAMMAD, 2016). It is known that small variations in the cell ambient might induce changes in the expression of genes with the potential of pre-conditioning of birds metabolism after hatching (AKŞIT et al., 2010; PIESTUN et al., 2011). It might occur in a long range (SHINDER et al., 2009; WALSTRA et al., 2010; PIESTUN et al., 2013), changing the development of the whole broiler embryos including the muscle, skeleton and the liver (PIESTUN et al., 2015; LOYAU et al., 2013, 2014a). These so-called epigenetic effects are the origin of the management of circadian incubation process, contrasting with the used incubation processes in which the fluctuation of environment data is avoided. However, there are still variations from the maternal influence on the quality of the pullet development since the incubated eggs reflect the stress and immunity level of the female breeder (HERNIKSEN et al., 2013).

During the thermal manipulation, the embryo receives cold and hot stimuli in determined periods during the embryo development. However, thermal manipulation is only favorable when applied in a clear and controlled way, at the right moment, and during the proper time (TZSCHENTKE, 2009; MOHAMMAD & SARDARY, 2016). The metabolic rate in broiler embryos 14-18 days old decreased after thermal manipulation (12h day^{-1} at 39.5°C day^{-1}) (PIESTUN et al., 2013). TZSCHENTKE (2009) found that the thermal manipulation during the last phase of the embryo development induces in the brain change in the neurons related to thermoregulation and metabolism. WALSTRA et al. (2010) indicated the best adaptability of broilers thermally manipulated in the 8th week of life. In the other hand, WILLEMSEN et al. (2011) did not find change in the embryo development and in the hatching rate when the birds were exposed to environmental temperature 3ºC above and below the normal incubation temperature in an intermittent way, 4h day^{-1} during the 16^{th} and the 18^{th} day of embryo development

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Broilers were particularly sensible to ambient temperature fluctuations, and low temperatures might induce oxidative stress (LOYAU et al., 2014b). However, there is a lack of information on the exposition of embryos to incubation temperature lower than the pattern. SHINDER et al. (2011) found that embryos exposed to low incubation temperature tend to present sensibility to cold and show ascites after exposition to cold in a tardy way. YALÇIN et al. (2012) revealed that is an interaction between the age of the female breeders and the period of incubation under cold temperature on the pullet weight at birth and particularly on the relative heart weight.

Most data in current literature were obtained in the experimental and controlled scheme. Therefore, the aim of this study was to evaluate the performance indexes of embryos from Cobb® genetic strain to different thermal stimulation in the last phase of embryo development under commercial scale.

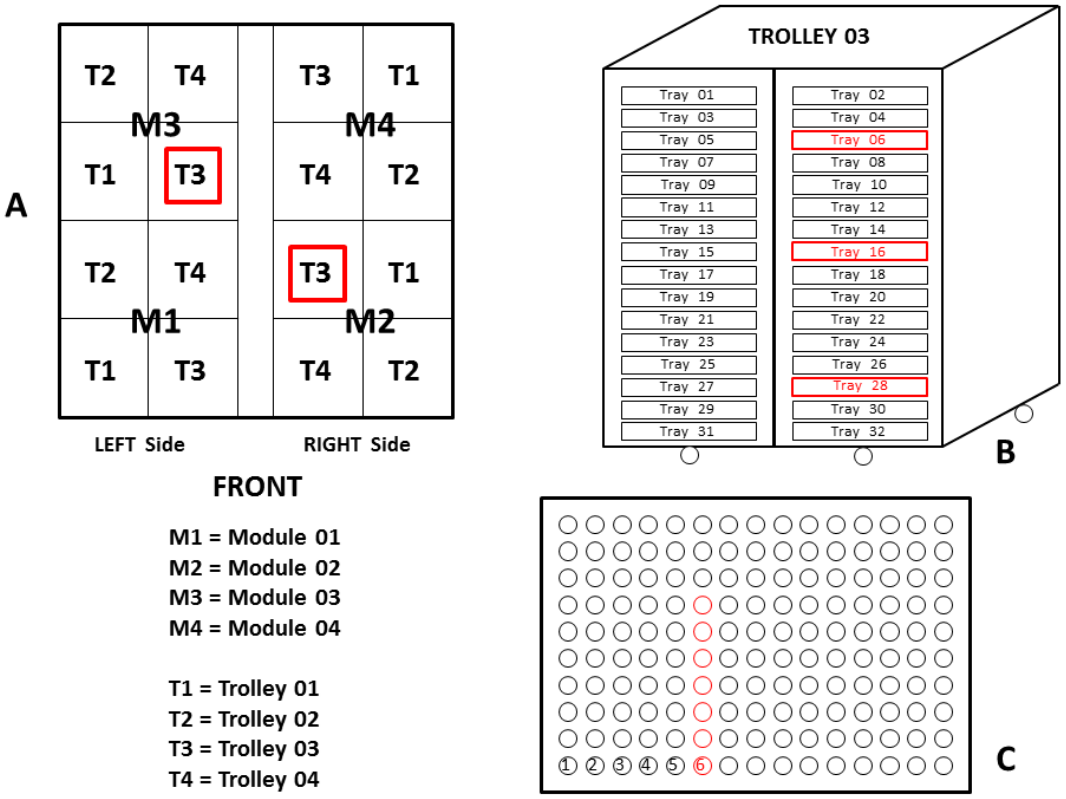
MATERIAL AND METHODS

Fertile eggs from four batches of Cobb® genetic strain from female breeders with age from 33 to 53 weeks were exposed to thermal stimulation combined with frequency, intensity, and duration. The study was randomly designed, observational and used a factorial scheme (2x3x2 – two ages of female breeders x three thermal manipulations and two controls) in a commercial incubator. Data were subjected to ANOVA using PROC GLM. The means were compared by the Tukey test adopting the confidence level of 95% using the software SAS® version 9.0 (2010).

The thermal simulations were done in the incubator SmartPro-77 of the single modular stage (PasReform Hatchery Technology, Zeddam, The Netherland) in a commercial hatchery. The machines had a total of 76,800 fertile eggs in all incubations. Control evaluation was done in a single stage machine (control SS – no thermal stimulation), and some batches were also observed in a multiple stage machine (CASP CM 125R, Amparo, Brasil) (control MS no thermal stimulation).

The thermal treatments were described using as the parameter the standard incubation program varying gradually according to the temperature established for that specific day of embryo development (E.D.). Two tests were done using hot stimuli called (T1 and T2), and one test with the cold stimulus was performed (T3). By definition, T1= increase of 1.39ºC above the set point temperature on the 14th day of the embryo development (E.D.) (36.55ºC), increasing 1.39ºC in each set point until the 18th day of E.D. with three hours of duration; T2= increase of 1.00ºC above the set point temperature at the 16th to the 18th day of E.D. with 2 hours of duration; and T3= cold stimulus fixed at 36.00ºC varying from 1.00ºC to 0.30ºC below each set point from the 14th to 18th day of E.D. with 3 hours of duration.

The incubations were monitored in real-time during the thermal stimulations using the software SmartCenter (PasReform Hatchery Technology, Zeddam, The Netherland). The eggshell temperature was registered on the equatorial line of 42 eggs from three places (Figure 1) in the incubation trays before and after thermal stimulation using an infrared thermometer (ITR 4520, Braun Termoscan®, Kronberg, Germany), with an accuracy of ± 0.2ºC. After hatching an embryo, the diagnosis was performed in the eggs that not hatched in trays where the embryo temperature was monitored.



SmartPro 77 incubator schematics

FIGURE 1. The positions of the modules, trolleys, trays, and eggs in the incubation trays, showing the locations where the temperatures were evaluated in the eggshells, trays were identified, and samples were collected; A: Upper overview of the SmartPro 77 incubator, showing the modules and incubation trolleys; B: View of the incubation trolley where the egg temperatures were measured, showing the identification of the trays; C: Incubation tray showing rows and eggs sampled (FLORES et al., 2016)

The behavior and quality of the chicks at birth were done in a sample of 25 birds in each batch (males and females) using the Pasgar® score methodology (VAN DE VEN, 2011). Five points were verified: vitality (reflex), belly button health, leg health, beak, and abdomen. At the final of the incubation the fertile hatching egg (FHE) was calculated using [eq. (1)].

$$FHE = \text{Total of chicks hatched / total of fertile eggs} \times 100\ (\%) \quad (1)$$

Fifteen male chicks were randomly separated into previously identified boxes. They were numbered following the sequence of the position of the boxes in the incubation tray (low, mean, and high). The surface temperature of the cloaca was registered using an infrared thermometer (Braun Termoscan® - ITR 4520). After the measurements and evaluations, the birds were sacrificed according to the welfare protocol approved by the UNICAMP ethics committee (protocol number 3503-1).

The total weight of the chick, yolk weight (yolk sac), heart weight, weight of the gastrointestinal tract (GIT), weight of the duodenal loop with the pancreas, weight pro-ventricular and ventricular (PVV, gizzard) were individually evaluated using a scale (WeighMax® W- 3805 - 100G, China) with a precision of 0.01g. The following variables were subtracted from the total weight and the egg yolk weight to obtain the free weight of the egg yolk and the percentage of the residual egg yolk. The performance was assessed in the birds that were reared afterward.

RESULTS AND DISCUSSION

The surface temperature of the groups was smaller in those without the thermal stimulation in multiple stages (38.02ºC), and in the single stage (37.53ºC). However, results differed ($p<0.05$) at T2 (an increase of 1.00ºC above the set point temperature at the 16th to the 18th day of E.D. with two hours of duration; 36.50ºC; Table 1). The temperature of the birds after hatching is influenced by the environment and when measured at birth must be between 40-40.5ºC in the chicks (MACARI et al., 2013a), and must remain during the process, and in the expedition room. In the current study, body temperature was monitored by the cloaca surface temperature, and, because of these procedure results were smaller than that indicated in the literature (Table 1 and 2).

Heart weight was less (0.27g) in the T1 group (an increase of 1.39ºC in each set point until the 18th day of E.D. with three hours of duration) in the relation to the group without thermal stimulation in multiple stages (0.37g). Embryos heated also show a reduction in the heart, as happened in the present study. The high eggshell temperature during incubation (38.9ºC) changed the development of the cardiac muscle (LEKSRISOMPONG et al., 2007) and might cause right ventricular hypertrophy and consequent increase in mortality caused by ascites (MOLENAAR et al., 2011).

The weight of the intestine tract (GIT), the duodenal loop with the pancreas, the pro- ventricle and ventricle (PVV) did not differ ($p>0.05$) between the treatments and the non-stimulated groups. In contrast research results indicate that high temperatures reduce the gastrointestinal tissue mass (LEKSRISOMPONG et al., 2007). The total weight of the chicks and the free weight of the egg yolk differed ($p<0.05$). The values were higher in the T2 group (increase of 1.00ºC above the set point temperature at the 16th to the 18th day of E.D. with two hours of duration; 36.50ºC) than T3 (cold stimulus fixed at 36.00ºC varying from 1.00ºC to 0.30ºC below each set point from the 14th to 18th day of E.D. with 3 hours of duration) and the group without stimulation in multiple stage (Table 1). A study points out that the mean weight of the chicks was reduced in 5%, that corresponds to a loss of 2-3g, but the relative size about the pro-ventricle and ventricle weight and the intestine were reduced in 13% and 16%, respectively (LEKSRISOMPONG et al., 2007). Another research indicates that the thermal stimulation induced an increase in the relative chick weight in the first week after hatching (MOHAMMAD & SARDARY, 2016). However, in the current study, the weight of the GIT, pro-ventricle and ventricle and the duodenal loop with the pancreas did not differ ($p>0.05$) between the thermally stimulated or the non-stimulated group.

The egg yolk weight differed in the groups ($p<0.05$) and it was higher (6.44g) for the group MS (no stimulation- multiple stages) and in the T2 (6.14g) when compared to the other groups. The increased residual egg yolk is also evidence of overheating (LEKSRISOMPONG et al., 2007), and it means that the embryo development did not happen as expected, as it means a residual weight inferior to 10% of the chick weight (Table 1).

TABLE 1. Average values about the combined heat treatment and the variables analyzed.

Variable	Treatment	Mean	N	Variable	Treatment	Mean	N
Surface temperature (ºC)	MS	38.02a ±0.48	5	Heart weight (g)	MS	0.37a±0.02	5
	SS	37.53ab±0.28	15		T3	0.36ab±0.01	15
	T3	37.47ab±0.28	15		T2	0.35ab±0.01	15
	T1	37.02bc±0.28	15		SS	0.31bc±0.01	15
	T2	36.50c±0.28	15		T1	0.27c±0.01	15
Total weight (g)	T2	47.27a±1.13	15	GIT weight (g)	T2	2.04a±0.06	15
	MS	45.92ab±1.96	5		MS	1.99a±0.10	5
	T1	40.83bc±1.13	15		T3	1.94a±0.06	15
	T3	40.46c±1.13	15		T1	1.84a±0.06	15
	SS	40.08c±1.13	15		SS	1.82a±0.06	15
Free egg yolk weight (g)	T2	41.13a±0.92	15	Weight of the duodenal loop+ pancreas (g)	T3	0.49a±0.02	15
	MS	39.49ab±1.60	5		SS	0.45a±0.02	15
	T1	36.31b±0.92	15		MS	0.45a±0.03	5
	SS	36.11b±0.92	15		T2	0.45a±0.02	15
	T3	35.98b±0.92	15		T1	0.43a±0.02	15
Egg yolk weight (g)	MS	6.44a±0.53	5	Weight of the PVV (g)	T2	2.70a±0.07	15
	T2	6.14a±0.31	15		T3	2.70a±0.07	15
	T1	4.50b±0.31	15		MS	2.69a±0.12	5
	T3	4.48b±0.31	15		SS	2.48a±0.07	15
	SS	3.97b±0.31	15		T1	2.45a±0.07	15

T1 = heat stimuli 1.39ºC)(14^{th} to 18^{th} E.D./3h); T2 = reduced heat stimuli 1ºC)(16^{th} to 18^{th} E.D./2 h); T3= cold fixed stimuli 36ºC (14^{th} to 18^{th} E.D./3h); control MS: no stimuli - multiple stages); control SS: no stimuli - single stage). Treatments with different letters are significant ($p<0.05$) by the Tukey test.

In the present study, group MS (no stimuli - multiple stages) showed higher weight egg yolk than the other groups, representing 14.01% of the chick weight, followed by treatment T2 with residual egg yolk of 12.9%. Since the multiple stage incubator has fertile eggs in different stages of embryo development, and eggs from females breeders of different ages, therefore the eggs have different pattern contributing to the presence of several microenvironments leading to hyper-heating in some areas. For the treatments T1, T3, and in the control group without stimulation in multiple stages machines the values were 11.02%; 11.07%, and 9.90%, respectively.

Young female breeders presented higher temperature than others ($p<0.05$). The heart weight, the GIT weight, the duodenal loop weight, and the PVV weight did not differ ($p>0.05$) within the range of average production (age of 48 and 53 weeks) *versus* the young females production (33, 34, 35, and 36 weeks). Related to the total weight, the egg yolk weight, and the free weight of egg yolk, the highest value was found in the female age in the average production ($p<0.05$) (Table 2), agreeing with current literature (HAMIDU et al., 2007). There was a difference between heat production, metabolism, and oxygen consumption between young female breeders (<35 weeks) and the old compared to those eggs from female breeders in the average period of production (35-50 weeks) (HAMIDU et al., 2007). As the female breeder age increase, the size of the eggs also increases leading to egg's lack of uniformity.

About the chick quality (Table 3), the mean Pasgar® score should be 9 (PAS REFORM, 2010). Treatments T1 and T3 presented 9.2, and the other groups remained close to 8. The physic changes found in the birds were helpful to determine the incubation profile and promote actions to mitigate the increase in embryo mortality or tardy hatching (HULET et al., 2007; LEKSRISOMPONG et al., 2007). None of these characteristics were found in the present study suggesting that the thermal stimulation applied were not prejudice to the birds. Hatching was not particularly affected by the thermal stimulation (Table 3). According to the genetic strain manual,

the hatching index for eggs from female breeders 33 to 36 weeks old is nearly 90% while it is in the range of 80-84% for older breeders (not considering the spiking effect of males). In the current study, it was verified that fertile eggs from female breeders 48 and 53 weeks old had a hatching percentage of 88.52 and 84.18% respectively.

TABLE 2. Average values over the age range of hens and the variables analyzed.

Variable	Age range	Mean	N	Variable	Mean age	Mean	N
Surface temperature (ºC)	young	37.41a±0.14	50	Heart weight (g)	mean	0.35a±0.01	10
	mean	36.81b±0.32	10		young	0.32a±0.01	50
Total weight (g)	young	46.2a±1.42	10	GIT weight (g)	mean	2.02a±0.08	10
	mean	41.00b±0.63	50		young	1.88a±0.03	50
Egg yolk free weight (g)	young	40.34a±1.14	10	Duodenal loop weight (g)	mean	0.46a±0.01	10
	mean	36.47b±0.51	50		young	0.43a±0.02	50
Egg yolk weight (g)	young	5.86a±0.39	10	PVV weight (g)	mean	2.61a±0.09	10
	mean	4.53b±0.17	50		young	2.55a±0.04	50

The range of mean age: 48 to 53 weeks; Young range: 33, 34, 35, and 36 weeks; Treatments with different letters are significant ($p<0.05$) by the Tukey test.

TABLE 3. Average overall hatching, hatchability, fertility and Pasgar©score batches of Cobb® genetic strain submitted to thermal stimulation.

Batch	Treat.	Age of female breeders (weeks)	Incub. Stage	Hatching (%)	Waste (%)	Hatch-bility (%)	Fertility (%)	Pasgar©score (%)
200	T2	53	SS	81.15	2.25	84.18	96.39	8.8
201	T1	33	SS	84.45	2.59	89.71	98.59	9.2
201	Control	34	SS	87.98	2.59	89.69	98.09	8.4
201	Control	34	MS	87	2.54	-	-	-
201	T3	35	SS	88.35	1.98	89.18	99.25	9.2
201	T2	48	SS	85.95	2.32	88.52	97.09	8.2
203	T2	36	SS	89.57	1.25	90.57	98.89	8.9

T1 = heat stimuli 1.39ºC (14^{th} to 18^{th} E.D./3h); T2 = reduced heat stimuli 1ºC)(16^{th} to 18^{th} E.D./2 h); T3= cold fixed stimuli 36ºC (14^{th} to 18^{th} .D.E/3h); control MS: no stimuli - multiple stages; control SS: no stimuli - single stage.

Results from the non-hatched eggs which were evaluated using the embryo-diagnosis (Table 4) reveals that treatment T3 did not present high percentage of non-beaked eggs. This occurred probably due to the low temperature inside the incubator. There is a standard embryo mortality rate within each stage of embryo development as a result of inadequate management of the fertile egg production (egg management, genetic strain, and the age of female breeders) (MACARI et al., 2013b). In the present study high values of embryo mortality were found in the group without thermal stimulation in a single stage and T3 (4.6 and 4.7%, respectively), and in T2 for tardy mortality (4.4%).

The embryo temperature which was assessed by the eggshell surface temperature was in average 38.60ºC to 38.75ºC in each treatment in the last days of incubation, independent of the thermal stimulus. Although the embryo temperature fluctuated within 3 hours, it would be back to the control (goal). Similar results using the same measurement technique were obtained by LEKSRISOMPONG et al. (2007). ROMANINI et al. (2013) monitoring the fertile eggs temperature during incubation and found that the eggs temperature varied within the range of 3-6 ºC in the last days of incubation. MOOLENAR (2011) indicates that the eggshell temperature for layers embryo needs to be approximately 37.78ºC since the first day of incubation until transferring to the

hatching. The standard temperature used varies within the threshold of 37.5 to 37.8ºC (WILLEMSEN et al., 2011; YALÇIN et al., 2012). The technical recommendation for single stage incubators is to reach an average eggshell temperature of 38.33ºC in the last three days before transferring to hatching, with the maximum limit of 38.61ºC (PAS REFORM, 2010ab). Under commercial incubation conditions, the challenge remains in maintaining equal eggshell temperature equally distributed in all trays, especially in the beginning and the final of the process due to the eggs heterogeneity characteristic.

TABLE 4. Average values of embryo diagnosis of lots of Cobb® lineage in 150 eggs trays.

Batch	200	201	201	201	201	203
Treatment	T2	T1	Control SS	T3	T2	T2
Age (weeks)	53	33	34	35	48	36
Infertility (%)	3.6	1.4	1.9	0.7	2.9	1.1
Mort. 1-4 days (%)	3.6	3.7	4.6	4.7	2.3	3.6
Mort. 5-14 days (%)	0.7	1.1	0.6	0.9	0	0.2
Mort. 15-21 days (%)	4.4	2.2	2.8	1.9	3.8	2.2
Beaked alive (%)	0.7	1.8	1.1	1	0.9	0.9
Beaked dead (%)	0	0.3	0.1	0.4	0.4	0.4
Rotten (%)	0.7	0	0	0.6	0.2	1.1
Cracked (%)	0.2	0.2	0.6	0.8	0.2	0
Alive and not beaked (%)	0	0.1	0.3	0.1	0	0.2
Fungus (%)	0	0.1	0	0	0	0
Total of incubated eggs	450	900	900	900	450	450
Total of unborn eggs	62	99	107	99	61	44

T1 = heat stimuli 1.39ºC (14^{th} to 18^{th} E.D./3h); T2 = reduced heat stimuli 1ºC)(16^{th} to 18^{th} E.D./2 h); T3= cold fixed stimuli 36ºC (14^{th} to 18^{th} E.D./3h); control MS: no stimuli - multiple stages; control SS: no stimuli - single stage.

Different genetic strains of fowls (broiler, layer, tom turkey) need different incubation temperature to reach the same embryo temperature. Studies indicate different embryo heat production in broilers and layers (NANGSUAY et al., 2011), and amongst distinct broiler genetic strains (TONA et al., 2010). According to LOURENS et al. (2007), the variation in the embryo temperature is a result of the modification in the metabolism during the growth.

Birds under heat thermal stimulation tend to hatch earlier than those not stimulated while birds stimulated by cold tend to reduce the embryo development (WILLEMSEN, 2010, MOHAMMAD & SARDARY, 2016). However, the effect of both the increase and reduction of the incubation temperature during hatching depends on the duration and the temperature amplitude manipulation, and the period the manipulation took place (TZSCHENTKE, 2009; MOHAMMAD & SARDARY, 2016). The hatch windows are the period between the first and last chick hatched. In the present study birds thermally stimulated in T3 and T2 had a hatch window of 10.5 h. The birds in T1 and the control SS with no stimuli - single stage were 16.6 h and 17 h, respectively. All treatments using single stage incubation had similar results, and it was not identified shortened of the hatch windows in the heat thermal manipulation. The response of chicks to the adverse expositions after hatching is highly dependent on the exact moment of the hatching of each bird; that is still not fully understood commercially (ROMANINI et al., 2013).

In Table 5, it is shown the results of three houses were a flock of thermally manipulated broilers were reared. Heat thermal stimulation T1 presented higher efficiency index (EI), mean weight, mean daily weight, and feasibility than the others, as well as less percentage of condemnations, and less feed conversion. The group thermally stimulated by cold (T3) presented inferior results when compared to T1 and the group without stimulation in a single stage.

HULET et al. (2007) report loss post-hatching in body weight and feed conversion in birds under thermal stimulation. The results of the present study mainly in T1 indicate an improvement in the index. The fertile eggs exposition to high-temperature 38.5ºC during few hours (4-6 h),

between the 10th and the 16th day of incubation, might improve the birds resilience to heat in the last week of growth (AKSIT et al., 2010). However, broilers might respond both positive and negatively, depending on the management of the variables involved in the rearing process (LEKSRISOMPONG et al., 2009).

TABLE 5. Batch performance of thermally stimulated from breeders with 33, 34 and 35 weeks of age.

House	Sex	Amount of birds	Treat.	Podo-dermatitis (%)	Condem-nation FIS (%)	Feasibility (%)	FC (kg)	MDG (g)	EI (Unit)	MW (kg)
X	Mixed	21.300	T1	45	1.36	98.4	1.758	59.09	330.8	2.64
Y	Mixed	21.200	SS	53	1.45	97.36	1.776	56.95	3.121	2.43
Z	Mixed	17.000	T3	100	2.48	96.12	1.946	0.5337	2.636	2.34

T1 = heat stimuli 1.39ºC (14th to 18th E.D./3h); T3= cold fixed stimuli 36ºC (14th to 18th E.D./3h); control SS: no stimuli - single stage; FC=feed conversion; MDG = mean daily gain; EI = efficiency index; MW= mean weight;

CONCLUSIONS

Thermal stimulation by heat or cold did not cause embryo mortality and did not alter the chick quality and the hatchability indexes for the Cobb® genetic strain. Heat stimuli with 1.39ºC above the set points from the 14th to the 18th day of embryo development presented EI, mean weight, mean daily weight, and the feasibility higher the others. The percentage of condemnations was smaller than the others as well as the feed conversion. These data suggest that the imprinting of temperatures is a tool available to the incubators to improve production, quality, and performance. It was not clear which is the proper frequency, the intensity and the duration of the thermal manipulation.

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