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Research Article

Effects of Low Temperature During Late Incubation on Incubation Duration, Hatching and Post Hatch Performance

Adnan Jabbar[1]*, Syed Idrees Asim[1], Adnan Yousaf[1], Farhad Shafique[1], Abdul Hameed[2], Farrukh Raza[2], Hamad-ur-Rehman[2], Amjad Riaz[3] and Yasir Allah Ditta[4]

[1]Salman Poultry (Pvt.) Ltd, Rawalpindi, Pakistan

[2]Punjab Department of Livestock and Dairy Development, Kot Adu Punjab, Pakistan

[3]Department of Animal Reproduction, University of Veterinary and Animal Sciences, Lahore, Pakistan

[4]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan

***Corresponding Author**: Adnan Jabbar, Salman Poultry (Pvt.) Ltd, Rawalpindi, Pakistan

Received: February 12, 2020; **Published:** April 29, 2020

Abstract

Temperature manipulation during incubation has effect on embryonic development, hatchability and incubation duration. The incubation temperature of broiler breeder eggs was decreased 3°C and 2°C than standard temperature at day 15 for next 113 hours. The minimum 35°C/95°F is integral to start peeping of chicks. After incubating at 3°C and 2°C less than standard incubation temperature, the incubation temperature was again increased 1.5°C and 1°C respectively up to hatching results delay in hatching for 12 hours. The hatchability (84.38 ± 3.44^{a}, 86.79 ± 2.17^{a}), candling (8.06 ± 2.66^{b}, 7.29 ± 1.39^{b}) and dead in shell (7.56 ± 0.92^{c}, 5.92 ± 0.83^{c}) were insignificant ($P < 0.05$). The temperature manipulation did not affect the hatch window. The hatch window was found same for control and experimental group. The only effect was 12 hours late peeping of chicks compared to control. The chicks transferred to broiler houses and house conditions were kept same for both experimental and control group. We found that feed intake (g) of experimental group was higher than control for first four weeks; while in fifth week the feed intake of control was better than experimental group. Due to high feed intake the weight gain (g) of experimental group was higher for first three weeks and for control group last two weeks. The Feed conversion ratio was found to be better for experimental group for first week, for rest four weeks the FCR of control group was found better than experimental group. The weekly mortality of chicks was found higher for experimental group, with highest for last week of growing. In short by temperature manipulation after d 15 of incubation at 3°C and 2°C less than standard incubation temperature for 113 hours then increasing temperature 1.5°C and 1°C respectively up to hatching results in delay in hatching for 12 hours, with significant ($P < 0.05$) last week mortality.

Keywords: *Hatch Delay; Hatch Window; Incubation Duration; Incubation Temperature*

Introduction

During last few years the artificial incubation has experienced a technological, economic and social revolution. These technologies generated costs related to more sophisticated facilities as well as operational cost such as energy, water expense and maintenance. The concept of artificial incubation was established centuries ago considering time, heat, and moisture and air renewal for incubation as well as turning. The normal range of incubation temperature for most of hatcheries is 37.5°C - 37.8°C [1]. This temperature range is critical to achieve standard hatchability, adequate hatch window and quality chicks. The deviation from standard incubation temperature results

in compromise with hatchability, hatch window and quality of chicks. The egg presents four mechanism of heat transfer: conduction (in egg heat is transferred from embryo to egg shell by conduction, provided their temperature are different), convection (egg loses heat by air current to its surroundings) radiation (the heat gain by eggs through radiations) and evaporation (egg loses heat by diffusion of water vapors through egg shell). However, egg gain or loss heat only when there is temperature difference between environment and eggshell and this is influenced by egg internal composition, eggshell, egg size and incubation conditions [2]. The four mechanisms of egg heating and cooling, the incubation temperature variation may result improper incubation duration along compromise hatchability and chick's quality.

Material and Method

Ethical approval

The experiment was performed considering all rules and regulations regarding Animal rights (SPCA) Society for protection and care of animals, University of veterinary and Animal Sciences Lahore Pakistan.

Experimental site

The experiment was performed at one of the biggest broiler hatcheries of south Asia, SS hatchery Chakri Salman Poultry Pvt. Ltd (Sadiq Group of Companies). The hatchery is situated near M-2 in Chakri region District Rawalpindi Punjab Pakistan. The hatchery is facilitated with latest HVAC system, having ISO (international standard organization) 1900-2000 certified. The hatchery can incubate 8.6 millions eggs/month through single stage incubator Avida-4 (chick Master USA). The hatchery can incubate more than 86 million eggs per month.

Experimental eggs

The (n = 538365) eggs for experimental group A were taken from SSF11-A-1 (Salman sadiq Farm flock no.11 breed Arbor Acre-1 (n = 102951), Arbor Acre-2 (n = 41814), Cobb-2 (n = 51812) Ross (n = 48577), SSF12-R-1 (n = 7870), SSF12-R-2 (n = 7027), SSF13 (n = 71226), SSF14 (n = 21817), SSF10-H1-AI-A (Hubbard Artificial insemination group A n = 22917), SSF10 R-2 (n = 20393), SSF10H-3 (n = 5713), SP131-AI-A (Sadiq Poultry n = 24869), SP131-AI-B (n = 22374), SP140 (n = 45806) and SP141 (n = 20718). For control group B eggs (n = 538365) from same flocks were collected to minimize the flock age and manage mental stress.

Eggs grading and setting

The eggs grading for both groups were performed through eggs grading Machine SANOVO STAALKAT Machine number JB 11786-D. The machine has ability to differentiate the eggs based on eggs weight along hairline crack eggs, leak eggs and dirty eggs detection. The eggs grading was performed as described by Jabbar., *et al* [3].

Eggs fumigation

The fumigation of both groups was performed with $KMNO_4$ and formalin to avoid contamination on eggs surface. The fumigation was performed through automatic fumigation system provided by chick master USA. The fumigation protocol was followed as described by Jabbar., *et al* [4].

Incubation profile

The eggs from both groups were incubated according to Age wise incubation profile described by Jabbar., *et al* [5]. Four setters for each group were selected, two setters with prime incubation profile, one young and one with old age incubation profile provided by chick master (Table 1).

Setter no.	Incubation Profile	Incubation Day	Incubation Time hrs	Heater Hours	Cooling Hours	Cooling water used Gallon
1	Young	14.7	355	44.2	30.2	197.4
2	Prime	14.9	359	55.1	35.6	219.2
3	Old	15	361	56.8	33.8	288.4
4	Prime	14.7	355	53.8	29.9	220.8

Table 1: *The incubation profile, incubation hours and heating and cooling hours and cooling water used by incubators before start of experiment.*

Experimental day

The experiment started at 15th day of incubation at 355 to 361 hours of incubation. The total heating of eggs was 44.2 to 63.8 hours, cooling 29.9 to 35.6 hours and cooling water was used 197.4 to 570.8 gallon before start experiment (Table 1).

Temperature manipulation in setters

On day 15th or 355 hours (setter-1) and 359 hours (setter-2) of incubation the incubation temperature of the incubators containing young and prime flock's eggs was decreased 2°C compared to original temperature to delay the hatch up to 12 hours. Similarly, at 361 hours (setter-3) and 355 hours (setter-4) of incubation the incubation temperature of incubators containing old and prime flock eggs was decreased 3C^{o} compared to original temperature for delay the hatch up to 12 hours (Table 2).

Setter no.	Incubation Profile	Original/control Temperature F	Temperature de-creased up 2°C	Transfer to Hatcher Hrs	Total Time hrs in Setter at low temperature
1	Young	99.1F	95.3F	444	89
		98.9F	95.1F		
		98.7F	95F		
2	Prime	99F	95.3F	444	85
		98.7F	95F		
		98.5F	94.8F		
	Temperature decreased up 3°C				
3	Old	99F	93.5F	444	83
		98.7F	93.2F		
		98.5F	93F		
4	Prime	99F	93.5F	444	89
		98.7F	93.2F		
		98.5F	93F		

Table 2: *Incubation of eggs at 2°C^{0} and 3°C less than original temperature in setters to delay hatch up to 12 Hours.*

Incubation at low temperature

The total time for low temperature incubation in setters was 89 hours and 85 hours for the eggs incubated at 2°C less than original temperature and 83 hours and 89 hours for the eggs incubated at 3°C less than original temperature before transfer of eggs to hatchers table 2.

Eggs transfer to hatchers

The transfer of eggs from setters to hatchers was performed after 444 hours of incubation for both experimental and control groups. During transfer candling was performed to assess the fertility of eggs through automatic transfer table provided by KUHL U.S.A. (Table 3).

Setter	Hatchers Incubation Time	Original/control Temperature	Temperature decreased up 2°C	Egg shell Temperature °C	Eggs Percentage	Hatch Pulling Hrs
1&2	2	98.5	94.8	33+	10	Setter hours 444
	22	98.4	94.6	30 - 33	47	
				25-30	43	
	Temperature increased up 1°C					
	12	98	96			
	6	97.8	95.9	34+	10	
	6	97.5	95.5	31 - 34	47	
	12	97.2	95.3	26 - 30	43	Hatcher hours 70
	5	96.9	95			
	5	96.5	94.6			
	70					70+444=514
	Temperature Decreased up 3°C					
3&4		**Original/control Temperature**	**Temperature decreased up 3°C**	**Eggshell Temperature °C**	**Eggs Percentage**	**Hatch Pulling Hrs**
	2	98.5	93	33+	11.25	Setter hours 444
	22	98.4	92.8	30-33	22.5	
				25-30	66.25	
		Temperature Increased up 1.5°C				
	12	98	95.1	34+	11.25	
	6	97.8	95	31-34	22.5	
	6	97.5	94.6	26-30	66.25	
	12	97.2	94.4			Hatcher hours 70
	5	96.9	93.7			
	5	96.5	93.5			
	70					70+444=514

Table 3: *Temperature manipulations in hatchers and egg shell temperature.*

Water loss

During transfer of incubating eggs from setter to hatchers the water loss was measured according to formula:

$$\frac{\text{Weight at setting-weight at transfer} \times 100}{\text{Weight at setting-Empty tray weight}}$$

Temperature manipulation in hatchers for the eggs incubated at 2°C less

The eggs incubated at 2°C less than original temperature in setters were continued to incubate 2°C less than original temperature in hatchers for next 24 hours.

Egg shell temperature measurements

The eggshell temperature was measured by using laser gun DT-380 (-50°C to 380°C) at the equator of the egg [6]. The individual eggs were selected randomly in Hatchers baskets to measure eggs shell temperature. The eggs were taken out from hatcher along basket to rule out the Hatcher temperature.

Measuring the egg shell temperature (EST) incubated at 2°C less

The eggshell temperature (EST) was taken after 24 hours in hatchers to assess the livability of embryos. Only 10 percent eggs were found with EST more than 33°C and 47 percent with 30 - 33°C and remaining 43 percent with EST 25 - 30°C (Table 3).

Temperature increased up to 1°C

Considering the low EST, the incubation temperature was increased to 1°C for the growing embryos. By increasing the incubation temperature 1°C the EST was also increased 1°C with same percentages for the next 46 hours up to hatch pulling (Table 3).

Temperature manipulation in hatchers for the eggs incubated at 3°C less

The eggs incubated at 3°C less than original temperature in setters were continued to incubate 3°C less than original temperature in hatchers for next 24 hours [7].

Measuring the egg shell temperature (EST) incubated at 3°C less

The eggshell temperature (EST) was taken after 24 hours in hatchers to assess the livability of embryos. Only 11.25 percent eggs were found with EST more than 33°C and 22.5 percent with 30 - 33°C and remaining 66.25 percent with EST 25 - 30°C (Table 3).

Temperature increased up to 1.5°C

Considering the low EST, the incubation temperature was increased to 1.5°C for the growing embryos. By increasing the incubation temperature 1.5°C the EST was also increased 1.5°C with same percentages for the next 46 hours up to hatch pulling (Table 3).

Hatch pulling and chicks grading

Hatch pulling was performed according to Jabbar., *et al* [3,4]. Chicks having shining eyes, legs, feathers and soft nose without any physical abnormality were considered as good quality (A grade), chicks with any physical abnormality specially omphalitis, extended abdomen and joint problem were considered as low quality chicks (B grade).

Chicks transfer to farms

Chicks (n = 30,000) from both groups were transferred to broiler houses through environmental control vehicles. The chicks transfer vehicle were set at 25 - 27°C along 65% humidity to minimize the transportation stress [8]. Salman sadiq Farm AT was selected for experimental group and Salman sadiq Farm Parri was selected for control group. The poultry house conditions were kept same for the chicks of both groups to rule out any effect due to poultry house conditions (Table 4).

Parameters	0 - 7d	8 - 14d	15 - 21d	22 - 28d	29 - 35d
Temperature ^{0}F	32 - 28	28 - 25	25 - 23	23 - 21	21 - 21
Humidity %	65 - 70	60 - 65	60 - 65	60 - 65	60 - 65
Minimum Ventilation(m^3/hour/bird)	0.1	0.2	0.42	0.8	1.9 - 2.20

Table 4: *Broiler house conditions from day-1 to day 35.*

The temperature, humidity and ventilation were kept same for both experimental and control group to minimize any effect due to these conditions. The standard vaccination schedule was applied to protect the birds from diseases. On day 1 the chicks were injected against infectious bursal disease along water spray of infectious bronchitis. On day 7 the chicks were vaccinated with eye drops containing Newcastle and Avian influenza, similarly on day 15 the second shot of Newcastle and infectious bronchitis was given in drinking water (Table 5).

Day	Vaccine	Rout
1	IBD+IB	IBD Injection +IB spray
7	ND+H9	Eye drops
15	ND+IB	Drinking Water

Table 5: *Vaccination schedule of broiler.*

All vaccines were given to recommendation of vaccine suppliers. The vaccines were performed at cooler part of day for better efficiency. Standard broiler sb feed was offered to both poultry houses. Baby crumbs from day one to 10, simple crumbs from 11 - 20 days and pallet from 21 to end of flocks.

Statistical analysis

All data were analyzed by using Statistical Analysis System package software (SAS version 9.2, SAS Institute Inc., Cary, NC, USA). All means were compared using t-test and results were presented as mean ± SEM (standard error of the mean). Results were considered significant if $P < 0.05$.

Results and Discussion

Hatchability, candling and dead in shell

The hatchery parameters for both control and experimental group were compared, the hatchability, candling and dead in shell of experimental group were lower than control group, the Hatchability (84.38 ± 3.44^a, 86.79 ± 2.17^a), candling (8.06 ± 2.66^b, 7.29 ± 1.39^b) and dead in shell (7.56 ± 0.92^c, 5.92 ± 0.83^c) were insignificant ($P < 0.05$) (Table 6).

Parameters	Group B	Group A	P value
Hatchability	84.38 ± 3.44^a	86.79 ± 2.17^a	< 0.05
Candling	8.06 ± 2.66^b	7.29 ± 1.39^b	< 0.05
DIS	7.56 ± 0.92^c	5.92 ± 0.83^c	< 0.05

Table 6*: The hatchability, candling and dead in shell of experimental and control groups.*

Hatch window and hatch delay

The time duration between first and the last chick hatches out [9]. The standard hatch window is 18 to 24 hrs for normal healthy young flock. The proper hatch window is necessary for quality chicks as well as intestinal development [10]. The hatch window was measured through Maestro software provided by chick master. The actual humidity curve relative to standard humidity is good indication of hatch window provided by the software maestro. The humidity start increasing relative to start of wet chicks piping from eggs. The wet chicks were the source of increased humidity. So, from the start to end of humidity curve we can estimate the hatch window of concern hatch along its peak time (Figure 1 and 2). The hatch window can be affected by eggs holding before incubation, broiler breeders age, the hatchability percentage [5]. The hatch window was same (18 - 20 hrs) for both experimental and control group (Table 5). The chciks peeping was started after 54 hours and 58 of eggs transfer to hatchers for the eggs incubated at 3°C and 2°C less than standard incubation temperature respectively.Due to late peepping, the total incubation duration was increased 514 hours compared to control group 502 hours. The standard hatch window is necessary for chicks quality and better FCR [11]. The temperature manipulation of experimental groups at d 15 to 21 results in 12 hours hatch dealy (Table 5). Our results were supported by Willemsen., *et al.* [12] who worked on temperature manipulation during incubation of chicken embryos and found temperature manipulation affects the incubation duration. Incubation at 3°C higher than requirement from embryonic d 16 to up to d 18 resulted reduces embryonic growth yolk consumption higher mortality and reduction in hatchability. Blood glucose, lactate, liver glycogen, plasma triglycerides, and non-esterified fatty acid indicated an altered carbohydrates and lipid metabolism for embryos incubated at high temperature. Although the embryos incubated to lower temperature was also significantly reduced, their embryonic development and growth were strikingly similar to those of control group. Yalçın., *et al.* [13] worked on low temperature incubation and found that 1°C low incubation temperature from d 10 to 18 reduces embryonic growth due to lower egg shell temperature and delay in incubation.

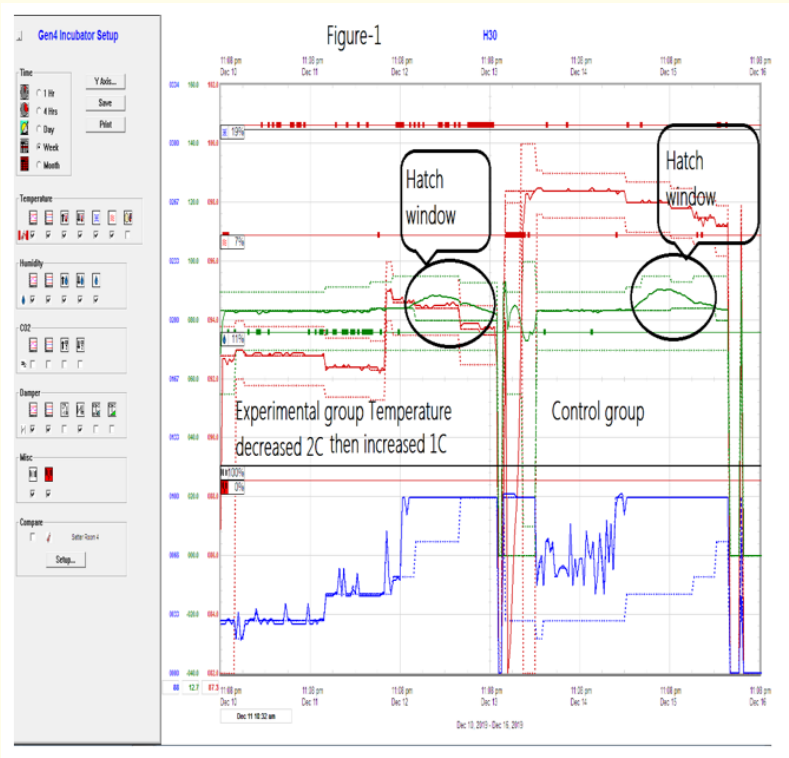

Figure 1

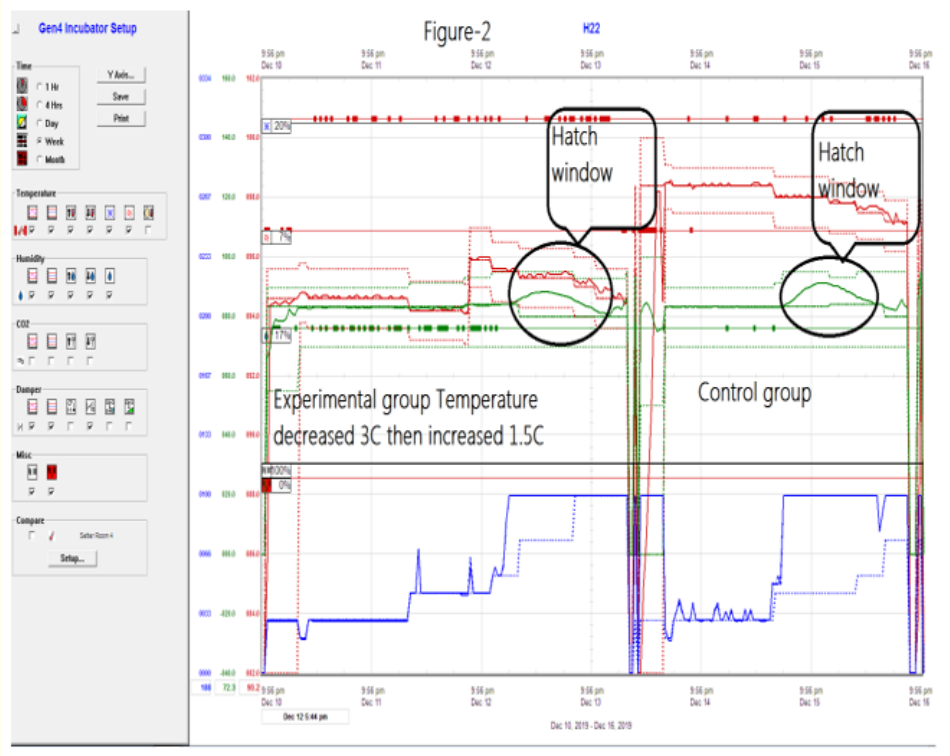

Figure 2

Afsarian., *et al.* [14] found that eggshell temperature manipulation on d 11, 13, 15 and 17 at 15^{0}C for one hour and injected with sterilized distilled water containing 65 ng of T4; injection thyroxin was associated with improve chick quality, reproductive performance of broilers. During our experiment we also found that minimum 95°C is necessary for broiler breeder eggs to be start peeping in hatchers because below 95^{0}C no peeping was found. Considering this we increased the hatchers temperature 2°C and 1.5°C. Yildirim., *et al.* [15] who worked at different hatchers temperatures 36.1°C, 37.2°C, 38.3°C and 39.4°C from 17d of incubation until hatch. The maximum heat production was found with 39.2°C and lowest and maximum hatch abilities were found with 37.2°C and 36.2°C. Bergoug., *et al.* [16] found pre-incubation and incubation condition affects the hatchability and hatch window. According to Nakage ES., *et al.* [17] incubation temperature is inversely proportion to incubation length. Isabel., *et al.* [18] found longest incubation with 34°C and shortest incubation length with 38°C.

Parameters	Control	3^{0}C	2^{0}C
Hatch window	18 - 20 hours	18 - 20 hours	18 - 20 hours
Start of chicks peeping piping	After 30 hours of transfer to hatchers	After 58 hours of transfer to hatchers	After 54 hours of transfer to hatchers
Total incubation time	502 hours	514	514
Hatch Delay	No	12 hours	12 hours

***Table 7**: Incubation hours, hatch window and hatch delay.*

Feed intake, weight gain, FCR and mortality at farms

The feed intake was same for both groups during first week at farm while feed intake was higher for experimental group in second, third and fourth week. The feed intake was much lower for experimental chicks compare to control. Leksrisompong., *et al.* [19] worked and found that temperature manipulation during incubation after d 16 has effect on feed intake and feed conversion ratio. Due to high feed intake compare to control the weight gain of experimental chicks was for first, second and third week while for fourth and fifth week the weight gain of experimental chicks was lower than control (Table 8).

Parameters	Hatch Delay	Control	P Value
Feed Intake(g)	630.2 ± 144.2^{c}	646.6 ± 176.2^{c}	< 0.05
Weight gain(g)	408.8 ± 65.74^{b}	444.0 ± 90.84^{b}	< 0.05
FCR (%)	1.23 ± 0.13^{d}	1.16 ± 0.09^{d}	< 0.05
Mortality	1.86 ± 0.76^{a}	1.12 ± 0.24^{a}	< 0.05

Table 8: *Mortality %, Weight gain (g), Feed intake (g) and FCR%*

The feed conversion ratio was better for control compare to experimental group. The FCR for experimental group was low for each week starting form second week to the end of flock. Fernandes., *et al.* [20] worked on temperature manipulation at last four days of incubation and found significant weight loss and fat percentage.

Similarly, the mortality of experimental group was higher than control from first week to end of flock. The highest mortality was found at the end of flock at fifth week. Sgavioli., *et al.* [21] found that temperature manipulation during incubation results inappropriate metabolism and high mortality of chicks. The change in skin thickness and vascularity, as well as change in thyroid and growth hormones level, are due to temperature manipulation during incubation [22]. The feed intake, weight gain, FCR and mortality was better for control group compare to experimental group but statistically ($P < 0.05$) insignificant [23].

Recommendation

To delay hatch up to 12 hours, the eggs can be incubated 2 - 3°C less than original temperature after d 15 of incubation. The minimum 95°F is necessary to start peeping of eggs. The temperature manipulation will not affect the hatch window or hatchability, but we have to compromise with slightly high mortality at farm.

Conclusion

The temperature manipulation during late incubation by decreasing the incubation temperature can be used to delay the hatch. The temperature manipulation after d 15 of incubation at 3°C and 2°C less than standard incubation temperature for 113 hours then increasing temperature 1.5°C and 1°C respectively up to hatching results delay in hatching for 12 hours, with significant ($P < 0.05$) last week mortality.

Conflict of Interest

The authors declare that they have no conflict of interest with respect to the research, authorship and/or publication to this research.

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Volume 5 Issue 5 May 2020