

Original Research

The influence of electric stimulation shock on the embryonic development and behavioral traits in chicks embryo

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ABSTRACT:

The aim of this experiment was to study the effect of dormancy during the incubation period, the hypothesis behind this concept was giving some stimuli to develop embryonic growth. This study was conducted at the experimental field of the Department of Animal Production, College of Agriculture, University of Anbar, Iraq. 450 eggs (Ross 308) were spread to four treatments each with three duplicates. A voltage device was used to shock the egg, after patterning the eggs with a line of iron filings to confirm electrical conductivity. The eggs were shocked at different times; three times a day that started from one day of hatching. The results showed that a significant changes were noted in the percentage of embryonic weight, percentage of albumin and the percentage of the shells at seventh day post incubation for experimental treatments. A significant change was seen in the percentage of embryonic weight and amniotic sac and liquid, percentage of albumin and yolk was noted at 14 days post incubation for experimental treatments. Significant changes in the percentage of embryonic weight and percentage of yolk at 17 days post incubation for experimental treatments were also noted. There was a significant increase (P<0.01) in the percentage of membrane penetration, the percentage of tucking and the number of motility for experimental treatment compared with the control. So, it can be concluded that the electrical stimulation develops embryonic growth and adjusts behavioural traits to obtain the best position for successful hatching.

Keywords:

Electric shock, Embryonic development, Behavioral traits, Embryo chicks.

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INTRODUCTION

Chick embryo needs more care and requires increasing feeding for its development, by utilizing the nutrients and other substances inside the egg. During an embryonic development stage, the embryo undergoes seizures from hibernation which make it unable to utilize food. Hence, embryonic stimuli are essential for optimal embryonic growth. There are numerous researches which show that the versatile demonstrations of grown-up living beings are joined by stamped moves in the electrical action of the mind, from the fundamental change of biopotentials to fine improvements of the working of neurons and cell populace (Borrelli *et al.*, 2008; Sudakov, 2010). In the meantime, no data exists concerning the neurophysiological relationship of versatile movement in early ontogeny. Investigations of the arrangement of background electrical manifestations of brain activity amid early times of development (Emery and Clayton, 2005; Hameroff, 2006), have just revealed the times at which electrical activity arises.

The electrical movement of the mind ought to be considered as an exceptional component joining the nerve centres amid the association of adaptive activity of the developing organism, which parameters of the Electroencephalogram (EEG) end up being most characteristic in such manner, and this kind of standard is the reason for the formation of controlling impacts in the everyday activities of the functional systems of the organisms in early ontogeny. According to Barros *et al.* (2009) the end of embryogenesis in chicks are set apart at intra-and inter-structural contrasts in the nature of the brain biopotentials. Moreover, there are vital roles, in early ontogeny, for primary rhythms of excitation which are distinguishable from the lists of electrical activity in the brain and which apply a considerable impact on lower lying nervous structures and change their hereditarily determined activity (Belich and Konstantinova, 2003; Corner and Togt, 2012).

The reason for suspecting that early in ontogeny

there exist electrographic that relates the entry of sensory signals into the brain, and procedures associated with their rearrangement, additionally of the effector output. This, in turn offers an occasion to theorize that the spontaneous electrical activity of the brain is a reflection of a mechanism, already in early times of ontogeny which sorts out the impacts which control the functional systems of the living being. The inquiries showed have coordinated our enthusiasm toward the investigation of the electrical activity of the embryonic brain in the formation of directed, adaptive shifts in motor analyzer activity amid the terminal time of embryogenesis (Scanes, 2015). So inspired by the idea, that embryo was shocked with an electric shock, makes it able to break the hibernation and thus aid in getting of feed. The successful hatching is predicated on a characteristic hatching position which the embryo assumes more than 24 h before hatching; the beak has penetrated into the air chamber and is directed obliquely against the shell; the neck is tightly coiled; the right side of the head facing the air space is covered by the right wing; the tarsal joints are braced against the shell near the pointed pole (De Smit *et al.*, 2008). If the embryo fails to attain this position its chance of successful hatching is greatly reduced, unless there is a stimulus that helps the embryo to complete these behavioral traits and thus successful hatching (Oppenheim *et al.*, 1978). The major events in this sequence are: first, the process of the tucking of the beak and the right side of the face under the right wing (process referred to as tucking), this remarkable feat is followed by a rotatory shift of the body toward the air chamber, whereby the hatching position is attained (Provine, 1972). The next event is the penetration of the beak through the membrane separating the embryo from the air space (membrane penetration), this process does not require coordinated movements, several hours later, pipping occurs; it involves movements similar to those performed during the hatching process (Bellairs *et al.*, 1981). There are similar co-ordinated movements in-

cluding a rotatory part that instrumental in a few other action designs which are preparatory to hatching. They include the lifting of the head out of the yolk sac; the tucking of the head under the right wing and pipping, preparatory step, meaning the penetration of the membrane separating the embryo from the airspace (Tong *et al.*, 2013). The objective of this study that stimulation of chick embryo to consumption of substances inside the egg for getting high production and take the best position for successful hatching.

MATERIALS AND METHODS

Animal study

The study was carried out according to the protocol approved by the University of Anbar, Ethics-Committee, Iraq. Fertile eggs from Ross (308) strain broiler breeder hens were procured from a commercial farm to run the experiment.

Experimental study

Embryonic test

The embryonic test was conducted in seven days from the incubation where we had broken the eggs shell, took out the contents of the egg and the traits such as embryo weight, albumin and shell were measured. Third embryonic test was conducted in 14 days from the incubation where we had broken the eggs shell, took out the contents of the egg and the following traits were measured. Embryo weight, yolk, amniotic sac and liquid and albumin. Fourth embryonic test conducted in 17 days from the incubation where we broke the eggs shell, took out the contents of the egg and the embryo weight and yolk were measured (Orlov, 1987).

Behavioural traits

The behavioral traits were measured on day 17 of hatching because of the coordinated movements that appear to originate. They do not seem to be related to the random movements which are characteristic of embryos. For the study of membrane penetration as a percentage (%) of range penetration. Tucking (the tucking

of the beak and the right side of the face under the right wing) as a percentage (%) of the situation and head and beak motility (number of motility in 30 sec) before the hatching process (Hamburger and Hamilton, 1951), an opening was also made in the air chamber. All other observations were made through windows of varying size and position which were prepared in the following way: the egg was candled and the contour of the air chamber was marked on the surface. After membrane penetration, the position of the beak can also be ascertained in candling. Before membrane penetration, we could not find a reliable landmark that would indicate the position of the embryo and one had to depend on the chance and good luck when placing the window in the desired position. The window was prepared with a pair of watchmaker forceps (Hamburger and Oppenheim, 1967). The shell was pierced, usually near the air chamber, since head, wings, thigh and toes are positioned near the air chamber. The shell membrane was moistened and carefully peeled off the chorioallantoic membrane. Great efforts were made to avoid serious loss of blood, though small hemorrhages could hardly be avoided. Once a particular part of the embryo was identified, the window was enlarged in the desired direction by chipping off shell pieces and removing the shell membrane, thus exposing the chorioallantoic membrane (Hamburger and Hamilton, 1992). This resulted in the arrest of flow of blood in the capillaries and smaller vessels of the exposed chorioallantoic membrane which could then be cut with a minimum of hemorrhage. Figure 1 shows a window of average size placed at a frequently used location: the beak, part of the left wing, the right thigh and part of the right toes are visible. After the observations were completed, the window was sealed with parafilm. Embryos with windows survive for many hours or days (Quigley and Armstrong, 1998).

Statistical analysis

This experiment were carried out by using Complete Randomized Design (CRD) and the data were ana-



Figure 1. The window of hatching in the egg

lyzed by using SAS program for statistical analyzing (SAS, 2001). The means for each treatments were compared by using Duncan's polynomial with 0.05 and 0.01 significance level to determine the significant differences between the averages (Duncan, 1955).

RESULTS AND DISCUSSION

Embryonic test

Table 1 shows the effect of electric shock on embryonic development at seven day from incubation. There seen a significant increase ($P<0.01$) in the embryonic weight for T_4 (1.87 g) compared with the other treatments, while there was a significantly increase ($P<0.01$) in embryonic weight for T_3 and T_4 (1.47 and 1.87 g) consecutively, compared with T_1 (CO) it was (1.23 g), However there was not significant in amniotic weight+fluid between the experimental groups and con-

trol group. But there was a significant increases ($P<0.01$) in allantoic weight+fluid to T_2 , T_3 and T_4 it were values (4.16, 6.76 and 6.84 g) consecutively, compared with T_1 (CO) it was (4.18 g). While there was a significant decrease ($P<0.01$) for albumin weight to T_4 the values was (10.1 g) compared with the other treatments, also there were no different between experimental group and control group in the yolk weight, but there was a significant decrease ($P<0.01$) in the shell weight to T_4 the value was (8.45 g) compared with the other treatments T_1 , T_2 and T_3 values were (9.45, 9.88 and 9.77 g) consecutively.

Table 2 presents the effect of electric shock on embryonic development at 14 day from incubation, there was a significantly increase ($P<0.01$) in the embryonic weight for T_4 (19.46 g) compared with T_2 and T_3 (18.06 and 17.53 g) consecutively, which increased than T_1 (CO) (13.14g). There was a significantly increase ($P<0.01$) in amniotic weight+fluid to T_4 (17.53 g) compared with the other treatments except T_3 (17.16 g) there aren't any difference between them, also there aren't any different between T_1 and T_2 (15.66 and 16.00 g). The allantoic weight+fluid showed significant increase ($P<0.01$) to T_2 , T_3 and T_4 (11.83, 12.23 and 13.16 g) consecutively compared with T_1 (CO) (8.80). The results showed the significant decrease ($P<0.01$) for albumin weight to T_2 , T_3 and T_4 (5.60, 5.23 and 5.03 g) consecutively compared with T_1 (7.60 g), while there

Table 1. Effect of electric shock on embryonic development at 7th day post incubation

As a percentage of the egg weight at the test (%)							
S. No	Traits (g)	Treatments				Mean	P-value
		T_1	T_2	T_3	T_4		
1	Embryonic weight	1.23 ± 0.1 ^c	1.34±0.3 ^{bc}	1.47±0.5 ^b	1.87±0.6 ^a	1.47	0.01
2	Amniotic weight +fluid	3.35±0.03	3.54. ± 0.08	3.44±0.09	3.45±0.11	3.44	N.S.**
3	Allantoic weight +fluid	4.16±0.1 ^c	4.18±0.2 ^b	6.76±0.4 ^a	6.847±0.6 ^a	5.48	0.01
4	Albumin weight	10.11±0.5 ^a	10.21±0.5 ^{ab}	9.31±0.3 ^{bc}	10.1±0.5 ^{ab}	9.93	0.01
5	Yolk weight	16.89±0.4	17.63±0.04	18.00±0.4	17.45±0.4	17.49	N.S.
6	Shell weight	9.45±0.7 ^a	9.88±0.70 ^a	9.77±0.60 ^a	8.45±0.40 ^b	9.39	0.01

* SEM: Standard Error Mean

** N.S.: Non Significant

a, b, c: means in the same Rows with different superscripts differ significantly at probability value 0.01 and 0.05.

Table 2. Effect of electric shock on embryonic development at 14th day post incubation

As a percentage of the egg weight at the test (%)							
S. No	Traits (g)	Treatments				Mean	P-value
		T ₁	T ₂	T ₃	T ₄		
1	Embryonic weight	13.14±0.3 ^c	18.06±0.5 ^b	17.53±0.6 ^b	19.46±0.7 ^a	17.0475	0.01
2	Amniotic weight +fluid	15.66±0.4 ^c	16.00±0.4 ^{bc}	17.16±0.6 ^{ab}	17.53±0.7 ^a	16.58	0.01
3	Allantoic weight +fluid	8.80±0.3 ^b	11.83±0.6 ^a	12.33±0.6 ^a	13.16±0.8 ^a	11.53	0.01
4	Albumin weight	7.60±0.5 ^a	5.60±0.4 ^b	5.23±0.4 ^b	5.03±0.5 ^b	5.865	0.01
5	Yolk weight	14.10±0.6 ^a	13.00±0.4 ^b	13.66±0.3 ^b	12.16±0.4 ^c	13.23	0.01
6	Shell weight	8.42±0.7 ^a	8.06±0.7 ^{ab}	7.72±0.5 ^b	6.53±0.4 ^b	7.6825	0.01

* SEM: Standard Error Mean

** N.S.: Non Significant

a, b, c: means in the same Rows with different superscripts differ significantly at probability value 0.01 and 0.05.

was a significant decrease (P<0.01) for yolk weight to T₄ the value was (12.16 g) compared with the other treatments T₁, T₂ and T₃ (14.10, 13.00 and 12.16 g) consecutively, while there are significant decrease (P<0.01) between T₂ and T₃ compared with T₁ but there aren't any difference between them. The shell weight was significantly decreasing (P<0.01) in T₃ and T₄ (7.72 and 6.53 g) compared with T₁ (8.42 g), while there aren't any difference between them and T₃ (8.06 g).

Table 3 shows the effect of electric shock on embryonic development at 17 day from incubation, the results presented a significant increase (P<0.01) in the embryonic weight for T₄ (27.56 g) compared with the other treatments T₁, T₂ and T₃ (24.00, 25.86 and 27.26 g) consecutively while there was significant increase (P<0.01) to T₂ and T₃ compared with T₁. Whereas there was a significantly increase (P<0.01) in amniotic

weight+fluid to T₃ and T₄ (13.33 and 14.90 g) consecutively, compared with the other treatments except T₂ (13.03 g) there aren't any difference between T₃ but difference that T₄, also T₂ difference than T₁ (10.33 g). The allantoic weight+fluid were significantly increased (P<0.01) to T₂, T₃ and T₄ (12.83, 13.33 and 14.16 g) consecutively compared with T₁ (6.80 g). There was a significant decrease (P<0.01) for yolk weight to T₂, T₃ and T₄ (6.00, 7.66 and 6.61 g) consecutively compared with T₁ (11.10 g) and there was a significant decrease (P<0.01) to T₄ compared with T₂ and T₃. There was a significant decrease (P<0.01) to T₃ and T₄ (5.72 and 5.53 g) in the shell weight compared with T₁ (6.42 g) but there wasn't differences between T₂ and T₁ (CO) and T₂ with T₃ and T₄.

In nature, the mother hen works on the brood of the eggs and provide the embryo with all the necessary

Table 3. Effect of electric shock on embryonic development at 17th day post incubation

As a percentage of the egg weight at the test (%)							
S. No	Traits (g)	Treatments				Mean	P-value
		T ₁	T ₂	T ₃	T ₄		
1	Embryonic weight	24.00±0.4 ^c	25.86±0.7 ^b	27.26±0.7 ^b	27.56±0.9 ^a	26.17	0.01
2	Amniotic weight +fluid	10.33±0.3 ^c	13.03±0.5 ^b	13.33±0.6 ^{ab}	14.90±0.9 ^a	12.89	0.01
3	Allantoic weight +fluid	6.80±0.5 ^b	12.83±0.7 ^a	13.33±0.6 ^a	14.16±0.8 ^a	11.78	0.01
4	Yolk weight	11.10±0.9 ^a	6.00±0.6 ^b	7.66±0.5 ^b	6.16±0.5 ^c	7.73	0.01
5	Shell weight	6.42±0.6 ^a	6.06±0.4 ^{ab}	5.72±0.3 ^b	5.53±0.2 ^b	5.93	0.01

* SEM: Standard Error Mean

** N.S.: Non Significant

a, b, c: means in the same Rows with different superscripts differ significantly at probability value 0.01 and 0.05.

growth requirements necessary for its survival and non-mortality this is called maternal care, one of these maternal care is to release a sound from hen to the embryo as there is vocal communication between the embryo and the hen, this is a type of stimulation of embryo growth and development (Abdulateef, 2017). On the other hand, there is a potential difference of voltage is generated from the friction between eggshell and feathers. The feathers contain some of the elements that generate the charge, while containing the egg on some other elements, creating a kind of effort that can stimulate the embryo (Abdulateef *et al.*, 2018). There are the extra embryonic membranes that contribute to the generation of the egg potential. Stern *et al.* (1985) indicated that the potential difference between blastoderm (negative) and albumen (positive) may reach 8 mV after 24 h of incubation, potential difference between embryo and albumen increases during the first four days of incubation and decays afterwards. As the size of the embryo at this stage is a few millimeter, the shell contacting the electrode acts as a spatial average, which drastically attenuates the amplitude of the surface potentials.

The rapid increase of the upside negative surface potential reflects not only the change of the absolute magnitude of the embryonic potential but primarily the growth of the embryo, the weight of which increases between the first and fourth day approximately 100 times and attains 0.1, 1.1 and 3.5 g on days 4, 7 and 10 respectively (Pitsillides, 2006). The slowing of the dipole movement on day five coincides with the sinking of the continually heavier embryo into the yolk. Between days 5 to 9, the embryo turns on its left side and later on its back. It is gradually moved by contractions of amnion into the large end of the egg while albumen collects in the small end. Vince (1966) showed the reversal of the embryonic potential observed on days 7-10 is evidently not due to a reversal of the embryo albumen potential gradient but to changed distribution of the sinks and sources of current within the egg (Wu *et al.*,

2001). But in the artificial hatching these stimuli weren't found, so the stimulation of embryo is necessary. Electrical stimulation works on the growth and development of nerves, especially in the first stage of growth, and this will develop neurological synapse in the brain and promotes the development of nerves and thus increases the expression of the protein in the nucleus of cells (Covell and Noden, 1989), as well as the increased neurological synapse, leads to increased secretion neurotransmitters and hormones are essential for growth from brain, The diencephalon is said to have a nervous control of the anterior lobe, because of the facts that injury of the nucleus or fibers of the diencephalon results in atrophy or degeneration of the anterior lobe of the pituitary, that hormonal secretion from the anterior lobe increases by stimulating those nervous tracts (Groef *et al.*, 2008). However, electrical stimulation of the hypothalamus caused a release of a substance which passes through the pituitary stalk and the portal blood vessels to the pituitary and stimulates to release the hormones are essential for growth, also neurotransmitters support growth regulatory and morphogenetic functions (Lauder and Schambra, 1999). One of these neurotransmitters is called acetylcholine, ACh released from growing axons, regulates growth, differentiation and plasticity of developing central nervous system neurons, also plays a key role in the regulation of morphogenetic cell movements, cell proliferation, growth, and differentiation in avian also, it has the role in the transmission of nerve impulses within the sympathetic system (Laasberg *et al.*, 1987). While ACh promotes survival of chicken spinal motoneurons that would otherwise undergo programmed cell death when deprived of trophic factors (Messi *et al.*, 1997; Groef *et al.*, 2008). The results obtained showed that the improvement of versatile developments is joined by a considerable reproduction of brain electrical action, which stays stable over the span of the whole time of working of the engine analyzer in the new administration. Assessing the character of the

EEG as indicated by subjectively unique files (histograms, autocorrelation and cross-relationship analyses) (Emery and Clayton, 2005), one may presume that another, steady administration of development is built up against a foundation of synchronization of the electrical procedures of the cerebrum and an expansion in the relative importance of variances in the overwhelming EEG rhythms. As authenticated via autocorrelation investigation there happens a noteworthy enhancement of the occasional parts of the EEG (with the insignificant coefficient of extinction) (Bogdanov *et al.*, 1984; Scanes, 2015). The high voltage of Electric Stimulation (ES) seemed to depend more on myofibrillar fragmentation than just on metabolic acceleration as was the case with low voltage. This was the reason given for the greater improvement in tenderness observed with the high voltage ES (Thompson *et al.*, 1987). While the high voltage ES of broilers reduced the activity of at least m-calpain (p-calpain was completely autolyzed in both ES and control muscles) (Walker *et al.*, 1995). In contrast, that ES increased calpain activity but had no effect on m-calpain (Alvarado and Sams, 2000). It is a protein having a place with the group of calcium-

dependent, non-lysosomal cysteine proteases (proteolytic catalysts) communicated pervasively in animal and numerous different living beings, it is dynamic members in procedures, for example, cell portability and cell cycle movement, and in addition cell-type particular capacities, for example, long-haul potentiation in neurons and cell combination in myoblasts. Under these physiological conditions, a transient and confined convergence of calcium into the cell actuates a little nearby of calpains (for instance, those near Ca²⁺ channels), which at that point advance the flag transduction pathway by catalyzing the controlled proteolysis of its objective proteins (Glass *et al.*, 2002). So the stimuli of electric according to above working in to direct during two way, the first do reduce of hibernation, makes the embryo able on the utilization of food. Secondly it stimulates the nervous system, thus increasing of bio-operations in the body which improved the weight of chick (Abizaid and Andrews, 2015).

Behavioural traits

Figure 2 show the influence of stimulation of electric shock on behavioural traits of chick embryos in 17 days of hatching. There was significantly increase

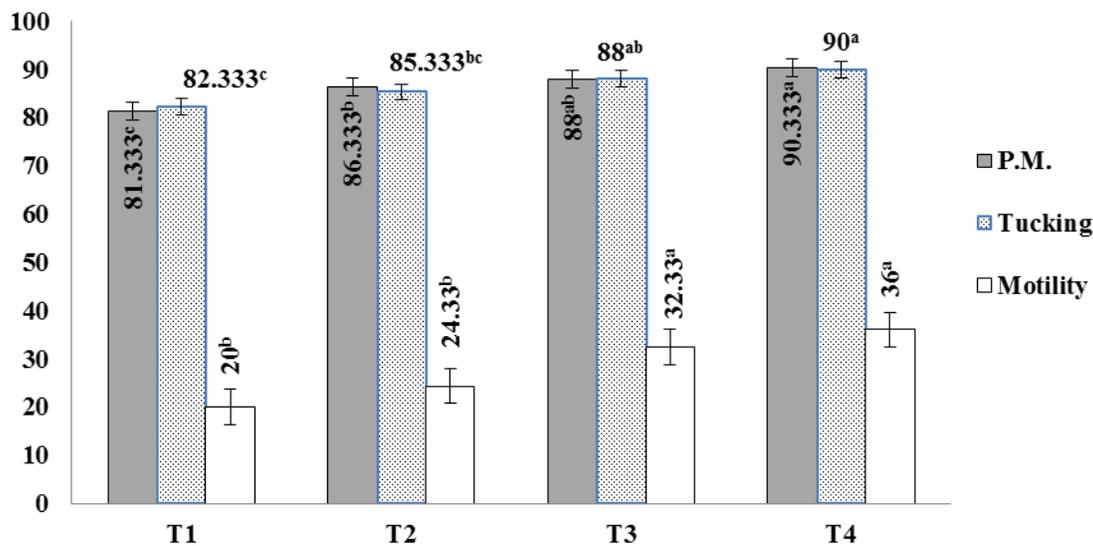


Figure 2. The influence of stimulation electric shock on the behavioural traits of chick embryos in 17 days of hatching

SEM P.M.: 2; SEM Tucking: 3.9166; SEM Motility: 9.666; a, b, c: means in the same rows with different superscripts differ significantly at probability value 0.05.

($P < 0.05$) in the percentage of membrane penetration to T_4 compared with the T_1 and T_2 , while there was no difference between T_4 with T_3 , but there was no difference between T_2 and T_3 , while there was a significant increase ($P < 0.05$) in the percentage of membrane penetration to T_2 and T_3 compared with T_1 control (without shock). However, Figure 2 presents a significant increase ($P < 0.05$) in the percentage of tucking to T_4 compared with the T_1 and T_2 , while there was no a difference between T_4 with T_3 , but there was no a difference between T_2 and T_3 while there was a significant increase ($P < 0.05$) in the percentage of tucking to T_3 compared with T_1 (without shock), even though they was not different between T_1 and T_2 . About the number of motility of head and beak Figure 2 show a significant increase ($P < 0.05$) to T_3 and T_4 compared with the T_1 and T_2 , while there was no the difference between T_3 and T_4 also there was no difference between T_1 and T_2 .

The theory holds that the bioelectric possibilities present in developing nerve tissue apply a situating impact on the bearing of development of nerve fibers. Neural development and synaptic connections happen between fibres that are invigorated (unexpectedly or something else) in the meantime or in close temporal contiguity (Tona *et al.*, 2004). The electrical field speculation contrasts in their expectations concerning the impacts of the electrical action on axons and dendrites. So the axons of developing nerve cells develop a similar way taken by the electric current that emanates from the developing nerve package (i.e., axons develop with the current) (Covell and Noden, 1989). Increased neurological development and neurological synaptic improves the vital state of the embryo and thus increases its well-being and makes it able to take the proper position in hatching (Groef *et al.*, 2008). If the movement of the body embryo in the right position and take the appropriate position for hatching, it is the result of the improved embryo status (Tong *et al.*, 2013). However, the electrical stimulation to increase the strength of muscle move-

ment and these muscles are the hatching muscle, this muscle helps to take the penetration position (PM) and break the shell of the egg and thus contribute to the process of hatching (Abdulateef, 2017). The position of the head under the right wing is one of the correct behavioural positions (tucking), so the electrical stimulation on the flexibility of the muscles and increase the strength of the nerves and make the movement of head and light better and thus take the appropriate situation of hatching.

CONCLUSION

In concluding that electrical stimulation works to develop embryonic growth and thus increases body weight as well as adjusts behavioural traits to obtain the best position for hatching for successful hatching.

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