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Embryonic Growth of Chicks under the Influence of Electric Field

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Abstract: Eggs from layer-type breeder flocks (Leghorn and Baladi, King Saud University) between 40 and 45 weeks of age were used in 2 trials to study the effects of electric field (EF) during incubation on embryonic growth and egg water loss. The incubator was divided into two compartments for the control and EF treatments. Two aluminum plates were fitted to the inside walls, face to face, in the EF compartment and connected to a step up electric transformer. The level of the EF was 30 kV/m, 60Hz. Eggs were set in an incubator on trays either in the control or EF during 18 d of incubation. EF incubation increased egg weight loss, and embryonic weight when expressed on an absolute value, percentage of egg weight, and daily weight gain by approximately 14.3, 50, 49.4, and 46.2%, in Leghorn, and 9.6, 14.7, 14.6, and 14.9%, in Baladi eggs, respectively. There were significant interactions between incubation treatment and age of the embryo on egg weight loss and embryo weight. EF incubation had significantly higher percentage of water loss and embryo weight at 16 and 14 d of incubation and older in Leghorn eggs, respectively and at 15 d of incubation and older in Baladi eggs, when compared with their counterparts of the control incubation treatment. It was concluded that the exposure of chicken eggs to EF of 30 kV/m, 60Hz, during incubation increased the egg water loss, and embryonic growth.

Key words: Layer-type breeder, electric field, embryonic growth

Introduction

Life of chicken embryo is sustained within a relatively narrow range of well-defined environmental parameters such as temperature, humidity, turning and ventilation (Tullet, 1990). The proper conditions are met only within a thin of eggshell. For embryo life to be maintained these factors must be kept within their "normal" limits; even minor deviations produce immediate physiological and sensory effects (Marshall and Cruickshank, 1938; Crittenden and Bohren, 1961; Smith and Bohren, 1975; Tullett, 1990; Vick *et al*., 1993; Christensen and Nestor, 1994; Kalita, 1994; Veldkamp *et al*., 2002). Modern incubators are designed with these four basic principles in mind to provide an optimum environment for embryo development and successful hatching.

There are other environmental factors, which can influence the development, growth and hatchability traits of incubated eggs. Shafey (2004) and Shafey *et al*. (2005) reported that including light into the internal environment of the incubation of chicken eggs improved embryonic growth, and hatchability performance. Additionally, there has been considerable work into the effects of electric field (EF) in embryology and morphogenesis (Jaffe and Nuccitelli, 1977; Jaffe, 1981 and 1986; Nuccitelli and Erickson, 1983; Robinson, 1989). A variety of natural endogenous EF exists within living organisms where they serve to transmit information at a basic level (Walleczek, 1992; McCoy *et al*., 1995). Nuccitelli (2003) measured the EF across the primitive streak of chick embryo to be 10-20 mV mm^{-1}. Results from different investigations suggest that the bioelectric field guides morphogenesis, growth, and cell division (Marsh and Beams, 1952; Jaffe and Stern, 1979; Nuccitelli, 1988; Hotary and Robinson, 1992; McCaig and Zhao, 1997; Nuccitelli, 2003). They provide polarity information, including the ability to orient the plan of cell division (Song *et al*., 2002). The electric signals and chemical signals can enhance each other in growth control. For example, growth factors and extracellular calcium are required for EF-induced directional migration of human keratinocytes. EF in turn can induce the expression of transforming growth factor (Kimura *et al*., 1998), as well as cause asymmetric distribution of growth factor receptors and other membrane proteins.

There is a lack of information regarding the effects of external EF on the production traits and welfare of poultry in general. The possibility that induced EF might influence embryonic growth and consequently hatchability performance of eggs has not been examined in details. The objectives of this study were to investigate the effects of exposing chicken eggs during incubation to EF on embryonic growth.

Materials and Methods

A total of 168 freshly laid eggs produced by a layer-type breeder flock (Leghorn, King Saud University), at 45-

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Table 1: Mean of embryo weight between d 7 and d 18 of incubation (D) expressed both on an absolute and percentage basis (embryo weight *100/egg weight) and daily gain of a layer-type breeder eggs (Leghorn) incubated under control and continuous electric field (EF) in trial 1[1]

Main effect means	Egg weight (g)	Egg weight loss (%)	Embryo weight (g)	Embryo weight (%)	Daily weight gain (mg/d) (g/day)(g *100/day)
Incubation treatment (T)					
Control (42)	55.9±0.61	8.4±0.37	5.0±0.73	9.1±1.32	340.4±39.80
EF (42)	55.7±0.58	9.6 ± 0.59**	7.5±1.01**	13.6±1.85**	497.8±53.32**
Day of incubation (D)					
7 (14)	56.1±0.30	5.4±0.25^{d}	0.60 ± 0.02^{e}	1.1±0.04^{e}	85.7±3.35^{e}
9 (14)	56.2±1.02	6.7±0.29^{cd}	1.9 ± 0.07^{d}	3.4±0.09^{ed}	213.6±7.77^{d}
11 (14)	54.9±1.21	7.7±0.39^{c}	2.5 ± 0.20^{d}	4.5±0.40^{d}	224.1±18.47^{d}
14 (14)	55.4±1.02	9.6±0.32^{b}	7.1 ± 0.60^{c}	12.9±1.14^{c}	506 .4±43.07^{c}
16 (14)	56.2±1.05	12.4±0.95^{a}	10.9 ± 0.97^{b}	19.6±1.69^{b}	683.4±60.46^{b}
18 (14)	54.8±1.25	12.1±0.51^{a}	15.3 ± 0.99^{a}	28.0±1.86^{a}	851.6±54.97^{a}
Source of variation	---------- Probability ----------				
T	NS	**	**	**	**
D	NS	**	**	**	**
T X D	NS	**	**	**	**

[1]Values are Means ± SEM of the number of replicates given in parentheses.

[a,b,c,d,e] Means within column not sharing a common superscript differ significantly (P<0.05).

** Significant difference (P<0.01). NS= Not significant (P>0.05).

week of age were used in the first trial. Eggs were numbered and weighed individually. Eggs weighing between 54 and 59 g were used. Eggs were assigned to fourteen replicates of twelve eggs each. Seven replicates were randomly assigned to each of the two incubation treatments (control and EF) and evenly distributed into the incubator trays. Eggs were set in a Maino, force-draft incubator (Model II, Maino Enrico, Co., Rome, Italy) and incubated at 99.5°F and 55% relative humidity. The egg compartment of the incubator (85 cm deep, 50.5 cm width and 83.5 cm height) was divided into two compartments with a frame of thin sheet of wire mesh for the EF and control treatments. Two aluminum plates were fitted face to face on the sidewalls of the EF compartment of the incubator (Fig. 1). The distance between the two plates was 50 cm. Each plate was fitted with a cable and connected to a step up electric transformer (Cat. No. 721-411, Jefferson Electric Company, Illinois, USA) to convert 110 V to 15000 volt. The frame of wire mesh was used to eliminate the electric wave from crossing into the compartment of the control treatment. Additionally, a three wire grounded power plug was used for the EF compartment, so that the earthy pin of the plug carries any extra current to earth potential and consequently eliminate any current to cross into the control compartment. The EF was on constantly during the 18 d of the experimental period. The level of EF was 30 kV/m with a frequency of 60-Hz. Eggs were turned every 2 h. Eggs were examined by candling at d 6 and d 12 of incubation and infertile eggs and eggs containing dead embryos were removed. Seven eggs per treatment were removed for the weight of embryos on d 7, 9, 11, 14, 16, and 18 of incubation. Eggs were broken open and embryos were separated and weighed individually after removing the yolk sac and placing it on a paper tissue to dry. The trial was repeated with the same number of eggs obtained from a different layer-type flock (Baladi, King Saud University), at the age of 40-week. Eggs weighing between 42 and 47 g were used. Ten eggs per treatment were removed for the weight of embryos on d 7, 12, 15 and 18 of incubation. Embryos at d 18 from trials 1 and 2 were photographed. Measurements were made of egg weight loss as a percentage of egg weight, embryonic weight expressed on an absolute value, percentage of egg weight (embryo weight*100/EW) and daily gain (mg/d) from d 7 to d 18 of age. Data from trials 1and 2 on embryonic growth were subjected to analysis of variance as 2 x 6 and 2 x 4, respectively factorial arrangement with incubation treatment, and age of embryo as main effects and their two-way interaction fitted into the model. All per cent data were transformed using arc sine square root percentage transformation before analysis. When significant variance ratios were detected, differences between treatment means were tested using the least significant difference (LSD) procedure. All statistical analysis was performed using the Statistical Analysis System (SAS Institute, 1985).

Results

The effects of EF incubation of Leghorn and Baladi eggs and day of incubation on egg weight loss and embryo

Table 2: Mean of embryo weight between d 7 and d 18 of incubation (D) expressed both on an absolute and percentage basis (embryo weight *100/egg weight) and daily gain of a layer-type breeder eggs (Baladi) incubated under control and continuous electric field (EF) in trial 2[1]

Main effect means		Egg weight (g)	Egg weight loss (%)	Embryo weight (g)	Embryo weight (%)	Daily weight gain (mg/d) (g/day) (g *100/day)
Incubation treatment (T)						
Control	(40)	44.9±0.28	9.4±0.55	6.8 ± 0.94	15.1 ± 2.07	433.9±48.52
EF	(40)	44.8±0.33	10.3±0.66**	7.8 ± 1.02**	17.3 ± 2.26**	498.5±53.59**
Day of incubation (D)						
7	(20)	44.6±0.60	4.9±0.14[d]	0.55±0.03[d]	1.3±0.08[d]	79.28±4.71[d]
12	(20)	44.8±0.38	8.8±0.27[c]	3.7±0.11[c]	8.2±0.25[c]	304.6±8.85[c]
15	(20)	44.8±0.48	10.8±0.27[b]	8.32±0.29[b]	18.6±0.66[b]	555.3±19.36[b]
18	(20)	45.1±0.20	14.9±0.44[a]	16.7±0.37[a]	36.9±0.68[a]	925.7± 0.39[a]
Source of variation		---------- Probability ----------				
T		NS	**	**	**	**
D		NS	**	**	**	**
T X D		NS	**	**	**	**

[1]Values are Means ± SEM of the number of replicates given in parentheses.
[a,b,c,d]: Means within column not sharing a common superscript differ significantly (P<0.05).
** : Significant difference (P<0.01). NS=Not significant (P>0.05).

weight during 18 d of incubation are shown in Table 1 and 2, for trial 1 and 2, respectively. EF incubation of Leghorn and Baladi eggs significantly (P<0.01) increased egg weight loss, and embryo weight expressed on an absolute value, percentage of egg weight or daily weight gain (mg/day) when compared with those of the control incubation of eggs (Fig. 2). Day of incubation significantly (P <0.01) increased embryo weight of Leghorn and Baladi eggs when expressed as an absolute value, percentage of EW or daily gain basis. There was no significant difference in egg weight between incubation treatments, and among days of incubation. Also, there was no significant difference in embryo weight gain of Leghorn between d 9 and 11 of incubation when expressed on an absolute value, percentage of egg weight or daily weight gain, and between d 7 and 9 of incubation when expressed as a percentage of egg weight.

There were significant (P<0.01) interactions between incubation treatment and day of incubation on egg weight loss, and embryo weight in Leghorn and Baladi eggs (Fig. 3 and 4, respectively). EF incubation had significantly higher percentage of water loss and embryo weight when expressed as an absolute value, percentage of egg weight or daily weight gain at 16 and 14 d of incubation and older in Leghorn eggs, respectively and at 15 d of incubation and older in Baladi eggs, when compared with their counterparts of the control incubation treatment. There was no significant difference between the two incubation treatments in the percentage of water loss, and embryo weight at 14 and 11 d of incubation and younger, respectively in Leghorn eggs and 12d of incubation and younger in Baladi eggs.

Discussion

Results from this study indicate that exposing chicken eggs to EF of 30 Kv/m, 60 Hz, during 18 d of incubation increased embryonic growth and egg water loss by approximately 14.3 and 49.4 %; and 9.6 and 14.6% for Leghorn and Baladi eggs, respectively, when compared with their counterparts of the control incubation (Table 1 and 2). The development of chicken embryo is affected by various aspects of environments to which the egg is exposed to, although the rate of embryonic development is closely regulated within a species (Ricklefs and Starck, 1998). Biological effects of EF *in vitro* have been reported by many investigators (Marino and Becker, 1977; Marino, 1993; Misakian *et al.*, 1993). Living cells carry out their metabolic processes through charge transfers; they generate electric currents and bioelectric impulses whose form, frequency, and amplitude depend on the metabolic process and type of cells involved. EF acts on matter through forces on electric charges (Adair, 1999).

The EF used in this study operated at a frequency of 60 Hz, which is in the extremely low frequency (ELF) portion of the electromagnetic spectrum (Marino and Becker, 1977; Marino, 1993). Electromagnetic waves at these low frequencies contain relatively small amounts of energy and are referred to as non-ionizing radiation. These low frequency waves or particles have not enough energy to eject electrons from molecules, and can not damage the structure of cells. The mechanisms by which EF may interact with biomolecular systems and tissues are incompletely understood. Kaune and Gillis (1981) formalized a number of concepts that simplify the description of the interaction between an animal and an EF. The biological effects of EF may be caused perturbation of an operating system and, or by direct cellular effects. In principle, an EF of sufficient magnitude could have a direct effect on biological tissues by acting directly on the free ions in the extracellular milieu, on the charged portions of the biomolecules, or by interaction with electric moments of

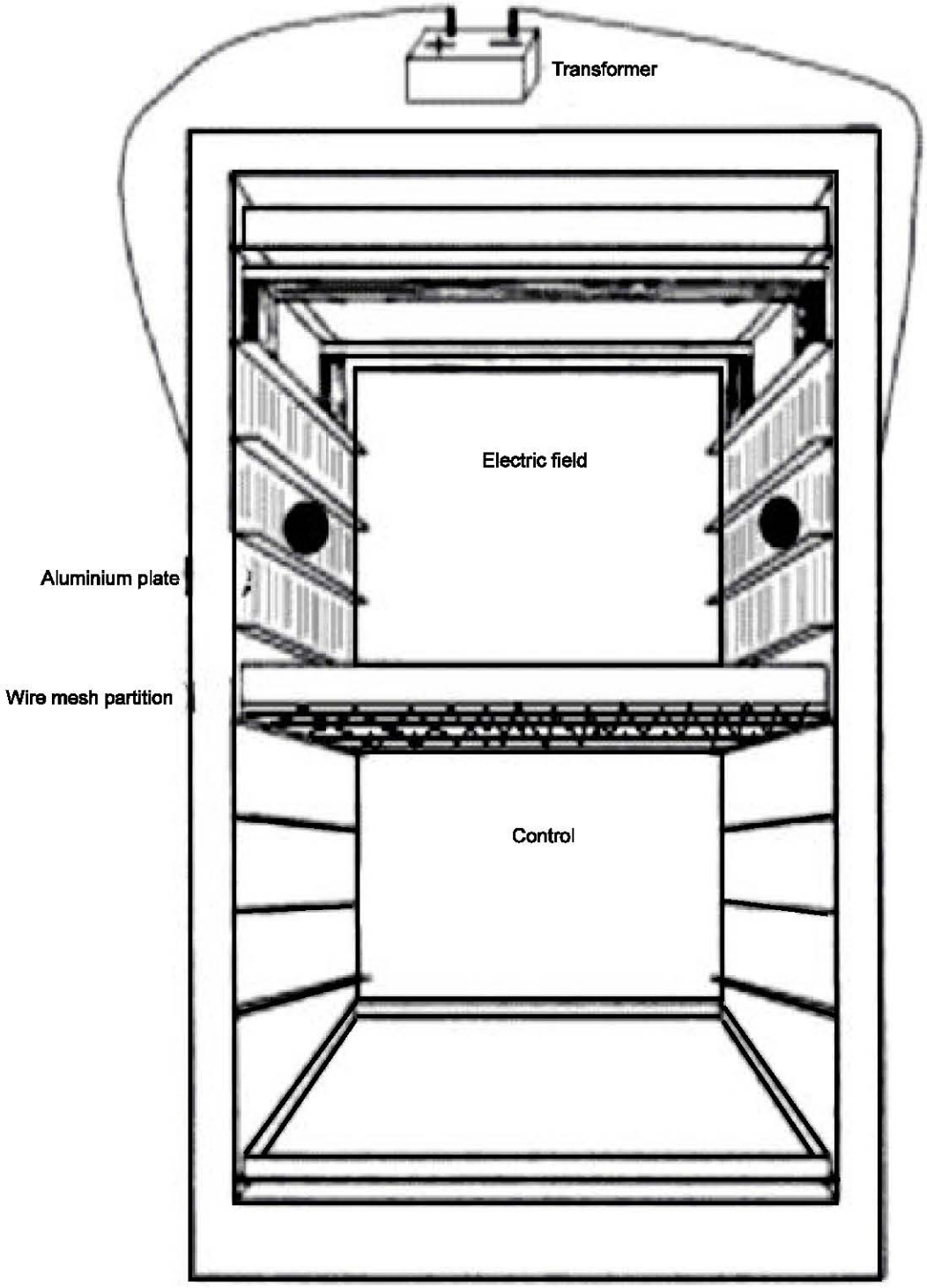

Fig. 1: Electric field arrangement in the incubator

molecular electronic structure. Additionally, the EF effects can occur only at resonance frequencies (Ghannam *et al.*, 2002). When an external EF is applied with a frequency similar to one of the bioelectric fields, resonance interference between the two pulses can occur. The resultant of this interaction between the two pulses is the final effect of the applied field on the metabolic process involved. If the applied resonant impulse is equal to or a little higher than the bioelectric impulse, energization or the metabolic processes will

Fig. 2: Embryos on the right side are from eggs incubated under the influence of electric field. Note the difference in size and feather development. Embryos were from comparable weight eggs and at 18-day of age.

occur as a result of enhancement of the charge motion involved. If the amplitude or the applied impulse is much larger than the bioelectric impulse, destructive metabolic processes will probably occur. Therefore, one may conclude that the effect of EF on cell function may lead to either the change in the character of the resonating

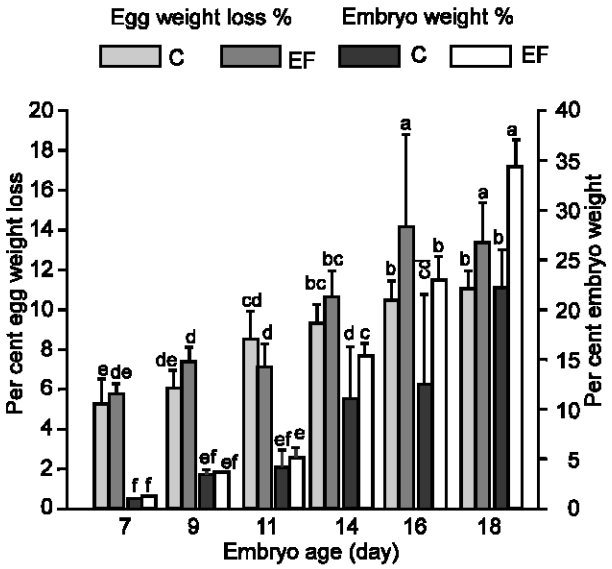

Fig. 3: Egg weight loss (egg weight *100/initial egg weight) and embryo weight (embryo weight *100/egg weight) between d 7 and d 18 of incubation (age) of a layer-type breeder eggs (leghorn) incubated under control (C) and continuous electric field (EF)

[a,b,c,c,e,f]Means within column not sharing a common superscript differ significantly (P<0.05)

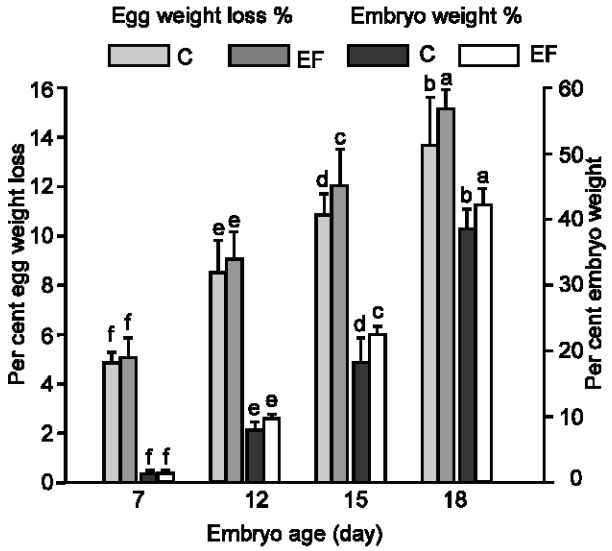

Fig. 4: Egg weight loss (egg weight *100/initial egg weight) and embryo weight (embryo weight *100/egg weight) between d 7 and d 18 of incubation (age) of a layer-type breeder eggs (Baladi) incubated under control (C.) and continuous electric field (EF)

metabolic process of the cell involved. In addition, EF effects could be attributed to either activation of enzymes (e.g., Na, K-ATPase) or increase of certain protein concentrations (Berg, 1999). Factors influencing egg water loss from incubating eggs include incubation conditions (humidity, air circulation, and temperature), eggshell characteristics (surface area, and thickness), and internal conditions of the egg (albumin and yolk), the most important of which is the amount of heat produced by the embryo (Benton and Brake, 1996; French, 1997). However, eggs of comparable physical dimensions and eggshell characteristics were used in this study and incubated under two different incubation environments (EF and control environments). It is possible that EF incubation of eggs influenced the internal environment of the incubated eggs and that increased water loss from the egg. The improvement of evaporation rate of water from the incubated egg may improve the capacity of eggshell to vital gas exchange and consequently, support oxygen consumption, survival and growth of the embryo (Taylor *et al*., 1956; Peebles *et al*., 1987). However, more research is needed to determine the effects of EF on the internal environment of incubated eggs, and survival of the embryo.

The body of the animal depends upon electricity to keep the movement and balance of all chemical responses. This concept has proven to be of considerable value in understanding many of the life functions that are poorly explained when viewed solely within the framework of biochemistry. It was concluded that the exposure of chicken eggs to EF of 30 kV/m, 60Hz, during 18 d of incubation accelerated embryonic growth, and increased water loss from the egg.

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