Impact of the electric shock on the embryonic development and physiological traits in chicks embryo

Article *in* The Indian journal of animal sciences · November 2020

1 author:

Mohammed Qais
University of Anbar
74 PUBLICATIONS **26** CITATIONS

SEE PROFILE

Indian Journal of Animal Sciences **90** (11): 1541–1545, November 2020/Article

Impact of the electric shock on the embryonic development and physiological traits in chicks embryo

S M ABDULATEEF[1]✉, O K ATALLA[1], M Q A L-ANI[2], T H T MOHAMMED[1], F M ABDULATEEF[3] and O M ABDULMAJEED[1]

College of Agriculture, University of Anbar, Iraq

Received: 26 October 2019; Accepted: 29 March 2020

ABSTRACT

The objective of this study was to investigate the impact of stimulating the embryo during the dormancy in the incubation period. 450 eggs (Ross 308) were allocated in four treatments each with three replicates. The treatments were as follows: T1 control (without shock), T2 Shocked (40) Millivolts (mV), T3 Shocked (50) (mV), T4 Shocked (75) (mV). A different voltage device was used to shock the egg, after marking the eggs with a line of iron filings to ensure electrical conductivity, eggs were shocked at different times three times a day. The results showed that the percentage of embryonic weight increased significantly and the percentage of albumin decreased significantly and the percentage of shells for experimental treatments during the seven days of incubation compared to the control treatment. The significant increase in the percentage of embryonic weight and amniotic sac and liquid and a significant decrease in the percentage of albumin and yolk compared to the control treatment at 14 and 17 days of incubation for experimental treatment. Significant increase in neurophysiological traits of neurons, brain weight for T2, T3 and especially T4 concluded that electrical stimulation had a positive effect on the embryo.

Keyword: Electric Shock, Embryo chicks, Embryonic development, Physiological traits

Chick embryos such as the embryos of another animal, need more care because it grows outside the body of the hen (Marteinson *et al.* 2016), so chick embryo needs more developmental feeding. The embryo undergoes seizures from hibernation during an embryonic phase of growth which makes it unable to use food, therefore, embryonic stimuli are essential for optimal embryonic growth.

Many publications suggest that the adaptive actions of adult organisms are accompanied by marked changes in the brain's electrical activity, from the systematic conversion of biopotentials into good neuronal and cellular functional rearrangements (Borrelli *et al.* 2008, Sudakov 2010). At the same moment, data on the neurophysiological correlation of adaptive activity in early ontogeny is hardly available. Early developmental studies of the creation of background electrical manifestations of brain activity have only revealed the periods during which electrical activity arises (Emery and Clayton 2005, Hameroff 2006). There are important roles, in early ontogeny, for main excitation rhythms detectable from indices of electrical brain activity, and which affects the lower nervous systems substantially and changes their hereditarily determined activity (Belich and Konstantinova 2003, Corner and van der Togt 2012).

These are reasons to believe that electrographic signals occur early in ontogeny that correlates the entry of sensory signals into the brain, and the rearrangement processes concerned, including the effector production (Brooks *et al.* 1991). This, in turn, provides an opportunity to assume that the spontaneous electrical activity of the brain reversal a mechanism, early ontogenic periods that organize the factors that regulate the organism's functional processes. The issues stated focused our interest in the research of the electrical activity of the embryonic brain in direct formation, adaptive changes in engine analyzer activity during the terminal stage of embryogenesis (Scanes 2015). So, inspired by the concept the embryo was given an electrical shock, so that it can break the hibernation and thus feed. The effective hatching is based on a distinctive hatching situation that is assumed by the embryo more than 24 h before hatching: the beak enters into the air chamber and is coordinated sideways against the shell; the neck is coiled; right side of the head facing the airspace is covered by the right-wing; Tarsal joints are fastened against the shell close the point (De Smit *et al.* 2008). The aim of this study was stimulation of chick embryo to enhance consumption of substances inside the egg for getting high production and to take the best position for successful hatching.

Present address: [1]Department of Animal Production, College of Agriculture; [2]Department of Biology, College of Science, University of Anbar, Iraq. [3]Ministry of Agriculture, Directorate of Anbar Agriculture, Iraq. ✉Corresponding author e-mail: ag.salwan.mahmood@uoanbar.edu.iq

MATERIALS AND METHODS

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 obtained from a commercial farm.

Experimental study: The study was carried out in the experimental farm of Animal Production Department, College of Agriculture, University of Anbar, Iraq. 450 eggs were utilized (ross 308) appropriated to five treatments; every treatment with 3 replicate. The research treatments were: T1= control (without shock), T2= Shocked (40) (mV) Millivolts, T3= Shocked (50) (mV), T4= Shocked (75) (mV). A different voltage device was used to shock the egg, after marking the eggs with a line of iron filings to ensure electrical conductivity.

Embryonic test: The embryonic test was conducted 7 days from incubation where the eggs shells were broken and egg content taken out to measure the traits viz. Embryo weight, Albumin and shell. Third embryonic test was conducted in 14 days from incubation where embryo weight, yolk, amniotic sac and liquid and albumin were measured in egg content. Fourth embryonic test was conducted in 17 days from incubation where the following traits were measured Embryo weight and yolk (Orlov 1987).

Tissue collection: The chicks were treated with ether (anesthetic or hypnotic fluid) and after anesthesia, chicks were killed (Euthanasia). They were executed and the brain alongside the brainstem was expelled from the skull by cutting off all the cranial nerves and vessels at the base, and after extraction, the brain was weighed (Wolkowitz *et al.* 1999).

Tissue processing: Immediately after the tissue was obtained, it was immersion fixed in 4% of paraformaldehyde at 4°C for 2 weeks. The brains were dehydrated, infiltrated and the blocks were prepared by embedding in paraplast. Serial coronal sections of 7 µm thickness were cut with a rotary microtome. The sections were mounted on egg albumin-coated glass slides and subsequently stained for Nissl substance with 1% buffered thionin. A comparison of the size of sections of the brain of experimental and control groups at a distance of 2 mm from the rostral end of the brain was done (Kesar 2014).

Quantification: The measurement of the neuronal nuclear area was determined using an image analyzing system (AXIO ZEIZZ). The measurements were made under a × 100 objective lens such that pixel size was 0.51 µm.

Statistical analysis: This experiment were carried by using Complete Randomized Design (CRD) and the data were analyzed by using SAS program for statistical analyzing (SAS 2001). The means for each treatments were compared by using Duncan's polynomial by using 0.05 and 0.01 significance level to determine significant differences between the averages (Duncan 1955).

RESULTS AND DISCUSSION

Embryonic test: Table 1 shows the effect of electric shock on Embryonic Development at 7 day from incubation. There was significant increase (P<0.01) in embryonic weight for T4 compared with the other treatments, while there was a significant increase (P<0.01) in embryonic weight for T3 and T4 consecutively, compared with T1 (CO). However there was no significant difference in amniotic weight + fluid between experimental group and control group. But there was a significantly increase (P<0.01) in allantoic weight + fluid to T2, T3 and T4 consecutively, compared with T1 (CO). While there was a significant decrease (P<0.01) for albumin weight to T4 compared with the other treatments, there was no difference between experimental group and control group in yolk weight, there was a significant decrease (P<0.01) in Shell weight to T4 compared with the other treatments. Table 2 presents the effect of electric shock on embryonic development at 14 day from incubation, there was increase (P<0.01) in embryonic weight for T4 compared with T3 and T4 consecutively, which increased than T1 (CO). There was a significant increase (P<0.01) in amniotic weight + fluid to T4 compared with the other treatments. The allantoic weight + fluid significantly increased (P<0.01) to T2, T3 and T4 consecutively compared with T1 (CO). The results show decreasing (P<0.01) albumin weight to T2, T3 and T4 consecutively compared with T1 (CO), while there was a significantly decreasing (P<0.01) yolk weight to T4

Table 1. Effect of electric shock on Embryonic Development at 7 days from incubation

Trait (gm.)	As a percentage of the egg weight at the test %						
	Treatment				SEM*	Mean	*P*-value
	T1	T2	T3	T4			
Embryonic weight	1.23^{c}	1.34^{bc}	1.47^{b}	1.87^{a}	0.222	1.47	0.01
Amniotic weight +fluid	3.35	3.54	3.44	3.45	0.238	3.44	N.S.**
Allantoic weight +fluid	4.18^{b}	4.16^{c}	6.76^{a}	6.847^{a}	0.637	5.48	0.01
Albumin weight	10.11^{a}	10.21^{ab}	9.31^{bc}	10.1^{ab}	0.619	9.93	0.01
Yolk weight	16.89	17.63	18.00	17.45	1.11	17.49	N.S.
Shell weight	9.45^{a}	9.8^{a}	9.77^{a}	8.45^{b}	0.667	9.39	0.01

*SEM, Standard Error Mean; **NS, 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 14 days from incubation

Trait	As a percentage of the egg weight at the test %						
	Treatment				SEM*	Mean	*P* value
(gm.)	T1	T2	T3	T4			
Embryonic weight	13.14^{c}	18.06^{b}	17.53^{b}	19.46^{a}	0.741	17.0475	0.01
Amniotic weight +fluid	15.66^{c}	16.00^{bc}	17.16^{ab}	17.53^{a}	0.901	16.58	0.01
Allantoic weight +fluid	8.80^{b}	11.8^{a}	12.33^{a}	13.1^{a}	1.02	11.53	0.01
Albumin weight	7.60^{a}	5.60^{b}	5.23^{b}	5.03^{b}	0.846	5.865	0.01
Yolk weight	14.1^{a}	13.00^{b}	13.66^{b}	12.16^{c}	0.844	13.23	0.01
Shell weight	8.42^{a}	8.06^{ab}	7.72^{b}	6.53^{b}	0.423	7.6825	0.01

* SEM, Standard Error Mean;** NS, Non-significant; a, b, c means in the same Rows with different superscripts differ significantly at probability value 0.01 and 0.05.

compared with the other treatments, except T2 and T4 there was no difference between them. The shell weight decrease (P<0.01) in T4 compared with the other treatments.

Table 3 shows the effect of electric shock on embryonic development at 17 day from incubation, and the results presented a significant increase (P<0.01) in embryonic weight of T4 compared with the other treatments, whereas there was a increase (P<0.01) in amniotic weight + fluid to T3 and T4 consecutively, compared with the other treatments. The allantoic weight + fluid increased (P<0.01) to T2, T3 and T4 consecutively compared with T1 (CO). There was a significant decrease (P<0.01) for yolk weight to T2, T3 and T4 consecutively compared with T1 (CO). There was a significant decrease (P<0.01) in T4 in the shell weight compared with the other treatment, but there wasn't different between T2 and T1 (CO).

In nature, the mother hen works on the brood of the eggs and provide the embryo with all the necessary 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 for embryo growth and development (Abdulateef 2017). On the other hand, 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). Extraembryonic membranes lead to egg prospective generation. Stern *et al.* (1985) demonstrated that the potential contrast between blastoderm (negative) and albumen (positive) may achieve 8 mV following 24 h of hatching. The potential difference between embryo and albumin increased over the first four days of incubation and subsequently decay. Since the size of the embryo is only a few millimetres at this point, the shell reaching the terminal runs like an ordinary spatial shell, which radically weakens the adequacy of the surface possibilities. The quick increment of the upside negative surface potential reflects not just the difference in the outright greatness of the embryonic potential however fundamentally the development of the embryo, the weight of which rises roughly 100 times between the first and fourth day and reaches 0.1, 1.1 and 3.5 g respectively on days 4, 7 and 10 (Pitsillides 2006). The moderation of the dipole motion on day 5 corresponds with the sinking of the ceaselessly heavier incipient organism into the yolk. The embryo turns on its left side between days 5 and 9 and later on its back. It is gradually shifted into the big end of the egg by contractions of the amnion, while albumen gathers in the tiny end. Vince (1966) demonstrated the inversion of the embryonic potential observed on days 7–10 is clearly not because of an inversion of the developing life egg whites potential inclination however to changed dispersion of the sinks and wellsprings of current inside the egg (Wu *et al.* 2001). But in the artificial hatching these stimuli aren't found, so the stimulation of embryo is necessary. However, electrical hypothalamus stimulation triggered the release

Table 3. Effect of electric shock on embryonic development at 17 days from incubation

Trait (gm.)	As a percentage of the egg weight at the test %						
	Treatment				SEM*	Mean	*P*-value
	T1	T2	T3	T4			
Embryonic weight	24.00^{c}	25.86^{b}	27.26^{b}	27.5^{a}	0.52	26.17	0.01
Amniotic weight +fluid	13.03^{b}	10.33^{c}	13.33^{ab}	14.9^{a}	0.688	12.89	0.01
Allantoic weight +fluid	6.80^{b}	12.83^{a}	13.33^{a}	14.16^{a}	0.804	11.78	0.01
Yolk weight	11.10^{a}	6.0^{b}	7.66^{b}	6.16^{c}	0.906	7.73	0.01
Shell weight	6.42^{a}	6.06^{ab}	5.72^{b}	5.53^{b}	0.246	5.93	0.01

*SEM, Standard error mean; **NS, Non-significant; a, b, c means in the same rows with different superscripts differ significantly at probability value 0.01 and 0.05.

of a substance through the pituitary stalk, and the portal of blood flow to the pituitary and stimulates to release the hormones which are essential for growth, also neurotransmitters support growth regulatory and morphogenetic functions (Lauder and Schambra 1999). One of these neurotransmitters is ACh, known as acetylcholine, released from increasing axons, controls the development, differentiation and plasticity of central nervous system cells, additionally assumes a main role in organizing morphogenetic cell developments, cell multiplication, development, and differentiation in avians too. It plays a part in nerve impulses movement in the sympathetic system (Laasberg *et al.* 1987). ACh encourages the survival of spinal motoneurons in chicken that would otherwise suffer programmed cell death without trophic variables (De Groef *et al.* 2008). The findings acquired show that a significant reconstruction of brain electrical activity accompanies the growth of adaptive movements, stays neutral throughout the engine analyzer's entire operating period in the new system. Assessment of EEG rating by qualitatively distinct indices histograms, autocorrelation and cross-correlation analyses (Emery and Clayton 2005) showed that a fresh, stable motion system is developed in the context of the synchronization of the electrical mechanisms of the brain and the boost in the relative importance of changes in the dominant EEG rhythms. As evidenced by the autocorrelation assessment, the regular EEG elements are significantly amplified (with the insignificant extinction coefficient) (Scanes 2015). The high voltage of electrical incitement (ES) appeared to be more reliant on myofibrillary discontinuity than on metabolic increasing speed, similarly as with low voltage. This is the reason for the increased affection with the high voltage ES (Thompson *et al.* 1987). While broilers have a high-voltage ES decreased at least m-calpain activity "p-calpain was completely autolysis in both ES and control muscles" (Walker *et al.* 1995). ES, on the other hand, enhanced calpain activity but had no impact on m-calpain (Alvarado and Sams 2000). It is a protein of the calcium-dependent, non-lysosomal cysteine proteases (proteolytic enzymes) family that are omnipresent in mammals and many other organisms, and it is involved in processes such as cell mobility and the growth of cell cycles, In addition to cell-specific tasks such as long-term neuronal potency and myoblast cell fusion. At these physiological circumstances, a temporary and localized calcium flow into the cell activates a tiny local calpain population for example, those close to Ca^{+2} channels, then progress the signal transduction path by catalyzing the proteolysis regulated by the target proteins (Glass *et al.* 2002). So the stimuli of electric shock according to above working directs in two ways, tha first being reduction of hibernation, which makes the embryo able for the utilization of food and; the second it stimulates the nervous system, thus increasing bio-operations in the body which improved weight of chick (Abizaid and Andrews 2015).

Physiological traits: Fig. 1 indicates the effect of electric shock on neurophysiological traits in chick embryo, which significantly increase (P<0.01) in neurons for T2, T3 and T4 (40.34, 41.33 and 44.13 µm) consecutively, compared with T1 (35.29 µm). However, Fig. 2 demonstrated the effect of electric shock on Brain weight, there was no difference between T3 and T4 (0.87 and 0.89 g.) consecutively

Electrical stimulation helps the growth and development of nerves, especially in the first stage of growth, and this develops 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 of neurotransmitters and hormones which 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, and hormonal secretion from the anterior lobe increases by stimulating those nervous tracts (De Groef *et al.* 2008). This mechanism of electric stimulation develops during the terminal period of embryogeny and coincides in time with such essential transformations of the brain's functional properties as the establishment of stable, rhythmic electrical activity and the ability to assimilate a rhythm. The existence of these properties may be considered a sufficient criterion of the

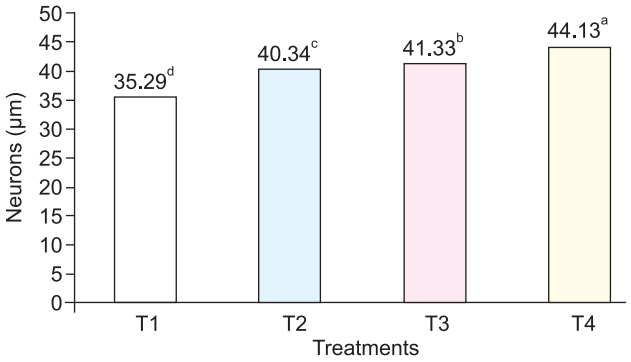

Fig. 1. Effect of electric shock on Neurons (µm) in the chick embryo SEM = 1.78; a, b, c: means in the same rows with different superscripts differ significantly at probability value 0.05.

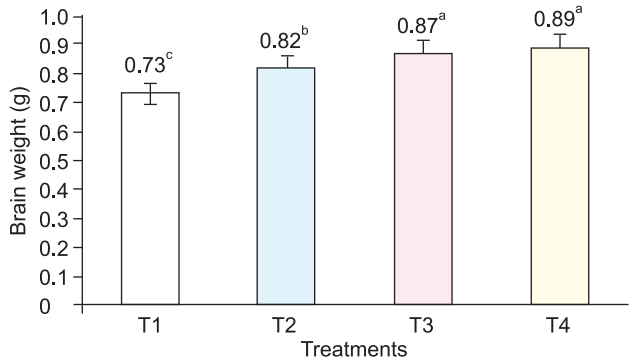

Fig. 2. Effect of electric shock on brain weight g in the chick embryo SEM = 0.1; a, b, c: means in the same rows with different superscripts differ significantly at probability value 0.05.

functional maturity of the brain in early ontogeny (Falk *et al.* 2007). The electrical stimulation stimulates the cells to secrete glucocorticoid, which at the same time stimulate their receptors to the brain. Glucocorticoid actions in the brain are mediated by glucocorticoid receptors and mineralocorticoid receptors. Glucocorticoid receptors occur throughout the brain but are most abundant in the hippocampal, hypothalamic and pituitary area (Belyi 1980).

It can be concluding that electrical stimulation works to develop embryonic growth and thus increases brain weight that helps to complete biological processes in the body, as well as adjusts physiological traits to obtain the best position for successful hatching.

REFERENCES

Abdulateef S M. 2017. The effect of maternal care (acoustic stimulus) on embryonic development and hatching muscle of broiler chickens. *The Iraqi Journal of Agricultural Science* **48**: 1263–74.

Abdulateef S M, Rashid S and Mutlab M. 2018. The effect of stimulation electric shock on embryonic development in broiler chicks. *Poultry Science* **97**: 146.

Abizaid A and Andrews Z. 2015. *Neuroendocrine mechanisms that connect feeding behavior and stress*, pp 143–145. Frontiers Media SA.

Alvarado C Z and Sams A R. 2000. The influence of postmortem electrical stimulation on rigor mortis devel-opment, calpastatin activity, and tenderness in broiler and duck pectoralis. *Poultry Science* **79**: 1364–68.

Belich A I and Konstantinova N N. 2003. Formation of coordinational function of the central nervous system: Phylo- and Ontogenetic aspects. *Journal of Evolutionary Biochemistry and Physiology* **39**: 375–87.

Bely-$ V P. 1980. Qualitatively similar effects of micro-iontophoretic applications of corticotropin and hydrocortisone in the hippocampal and hypothalamic neurons of rabbits. *Biulleten'eksperimental'noi biologii i meditsiny* **89**: 390–92.

Borrelli E, Nestler E J, Allis C D and Sassone-Corsi P. 2008. Decoding the epigenetic language of neuronal plasticity. *Neuron* **60**: 961–74.

Brooks R J, Bobyn M L, Galbraith D A, Layfield J A and Nancekivell E G. 1991. Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Canadian Journal of Zoology* **69**: 2667–76.

Corner M and van der Togt C. 2012. No phylogeny without ontogeny– A comparative and developmental search for the sources of sleep-like neural and behavioral rhythms. *Neuroscience Bulletin* **28**: 25–38.

Covell J and Noden D. 1989. Embryonic development of the chick primary trigeminal sensory motor complex. *Journal of Comparative Neurology* **286**: 488–503.

De Groef B, Grommen S V and Darras V M. 2008. The chicken embryo as a model for developmental endocrinology: development of the thyrotropic, corticotropic, and somatotropic axes. *Molecular and Cellular Endocrinology* **293**: 17–24.

De Smit L, Bruggeman V, Debonne M, Tona J K, Kamers B, Everaert N and Decuypere E. 2008. The effect of nonventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. *Poultry Science* **87**: 551–60.

Duncan B D. 1955. Multiple range and multiple F test. *Biometrics* **11**: 1–24.

Emery N J and Clayton N S. 2005. Evolution of the avian brain and intelligence. *Current Biology* **15**: R946–R950.

Falk J, Drinjakovic J, Leung K M, Dwivedy A, Regan A G, Piper M and Holt C E. 2007. Electroporation of cDNA/Morpholinos to targeted areas of embryonic CNS in *Xenopus*. *BMC Developmental Biology* **7**: 107.

Glass J D, Culver D G, Levey A I and Nash N R. 2002. Very early activation of m-calpain in peripheral nerve during wallerian degeneration. *Journal of the Neurological Sciences* **196**: 9–20.

Hameroff S. 2006. Consciousness, neurobiology and quantum mechanics: The case for a connection (pp. 193–253). *The Emerging Physics of Consciousness*. Springer, Berlin, Heidelberg.

Kesar A G. 2014. Effect of prenatal chronic noise exposure on the growth and development of body and brain of chick embryo. *International Journal of Applied and Basic Medical Research* **4**: 3–6.

Laasberg T, Pedak A and Neuman T. 1987. The muscarinic receptor-mediated action of acetylcholine in the gastrulating chick embryo. Comparative biochemistry and physiology. *Comparative Pharmacology and Toxicology* **86**: 313–16.

Lauder J M and Schambra U B. 1999. Morphogenetic roles of acetylcholine. *Environmental Health Perspectives* **107**: 65–69.

Marteinson S C, Drouillard K G and Verreault J. 2016. Short-term fasts increase levels of halogenated flame retardants in tissues of a wild incubating bird. *Environmental Research* **146**: 73–84.

Orlov M V. 1987. Biological Control in Incubation, 3rd edn. Moschow, Russcellezgat (in Russian).

Pitsillides A A. 2006. Early effects of embryonic movement: 'A shot out of the dark. *Journal of Anatomy* **208**: 417–31.

SAS Institvte. (2001. SAS Usen Guide: statistics version 6th edn, SAS Institute Iuc, Cary, NC.

Scanes C G. 2015. *Sturkie's Avian Physiology*. Sixth Edition. Academic Press is an imprint of Elsevier. ISBN: 978-0-12-407160-5.

Stern C D, Manning S and Gillespie J I. 1985. Fluid transport across the epiblast of the early chick embryo. *Development* **88**: 365–84.

Sudakov KV. 2010. *System mechanisms of mental activity*. (Eds) Korsakov S S *Neurology and Psychiatry* **110**: 4–14.

Thompson L D, Janky M D and Woodward S A. 1987. Tenderness and physical characteristics of broiler breast fillets harvested at various times from post-mortem electrically stimulated carcasses. *Poultry Science* **66**: 1158–67.

Vince M A. 1966. Potential stimulation produced by avian embryos. *Animal Behaviour* **14**: 34–43.

Walker L T, Shackelford S D, Birkhold S G and Sams A R. 1995. Biochemical and structural effects of Rigor mortis-accelerating treatments in broiler pectoralis. *Poultry Science* **74**: 176–86.

Wolkowitz O M and Reus V I. 1999. Treatment of depression with antiglucocorticoid drugs. *Psychosomatic Medicine* **61**: 698–711.

Wu K C, Streicher J, Lee M L, Hall B K and Müller G B. 2001. Role of motility in embryonic development I: Embryo movements and amnion contractions in the chick and the influence of illumination. *Journal of Experimental Zoology* **291**: 186–94.