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STUDIES ON THE EFFECT OF THE INSECT GROWTH REGULATOR LUFENURON ON EMBRYOGENESIS OF CHICK *GALLUS domesticus* (WHITE LEGHORN STRAIN)

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Research Article

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ABSTRACT

An attempt was made to evaluate the effect of an Insect Growth Regulator, Lufenuron (Match ®, 5.4% EC) on vertebrates' embryogenesis using Gallus domesticus as a model. Lufenuron (10µl and 100µl of 54ppm) when injected in pre incubated eggs (96hr, Hamburger and Hamilton 22-23) of G. domesticus was found to induce structural and skeletal anomalies in the developing embryos. At the dose of 10µl of Lufenuron, 100% survival of the embryo was obtained but the chicks failed to hatch. At the higher dose of 100µl of Lufenuron, retardation of the embryogenesis and induction of teratogenesis was observed. The morphological and skeletal anomalies reported were abdominal hernia, macropthalmia, macrocephaly, caudal regression syndrome and deflection of hind limb digits. In conclusion, Lufenuron (100µl of 54ppm) is embryotoxic and teratogenic to a non target organism, chick. Thus exposure to Lufenuron in the food chain may leads to undesirable consequences in vertebrates.

KEYWORDS: Insect Growth Regulator, Lufenuron, Chick embryogenesis, teratogenesis

Introduction

In a variety of pest control activities around the world, synthetic chemicals continue to play a significant role. In India, insecticides account for 80% of the total pesticides used while the herbicides usage is insignificant[1]. In modern farming and food production, non-approved use of pesticides increasingly contributes to environmental pollution[2]. Indiscriminate use of DDT was brought into focus in the book '*Silent* Spring'[3]. It was reported that bio-magnification of pesticides in an ecosystem has resulted in mortality and reproductive impairment of embryos and adult birds[4, 5]. Therefore possibility of entering these pesticides through food chain and producing undesirable effects on non target organisms including humans cannot be ruled out. Moreover the eventual fate of pesticide residues and their potential damage to human health generally remains unknown.

Chickens are excellent table birds and consumed by people all over the world in large quantities. Hens fed with pesticide contaminated feed are likely to transport the pesticides and their metabolites through eggs. The protective mechanisms are not fully functional in the fetus as compared to that of adults[6]. Hence it is important to study the effect of pesticides on developing embryos. Chick embryo is an excellent model system for studying vertebrate embryogenesis. This model

is cheaper, easy to monitor and gives quick results. The sequence of chick development has been well illustrated[7, 8], which is useful for standardization of chick development.

Insect Growth Regulators are chemicals which are use for killing pests[9], and they also act on chitin synthesis causing an inhibition of insect molting[10]. Lufenuron is an efficient Insect Growth Regulator (IGR), a potent chitin synthesis inhibitor and also interferes with the biosynthesis and deposition of chitin in the cuticle during insect molting process and mainly used in the control of the cat flea, *Ctenociphalides felis*[11]. It is well documented that commercial pesticides have induced adverse effects on avian development[12, 13, 14]. Research findings reported the number of intoxications with pesticides on eggs and birds by ingestion, absorption or inhalation[15]. However the effect of Lufenuron on chick embryogenesis has not been reported so far. The studies of the effect of Lufenuron on chick embryogenesis felt to be fruitful for understanding its mode of action on vertebrates, since the developmentally regulated genes are seemingly conserved in vertebrates. Since Lufenuron is used as an IGR, the studies of secondary effects of this insecticide in developing chick are of great importance. Therefore, in the present investigation, an attempt has been made to study the effect of Lufenuron on chick embryogenesis.

Materials and Methods

Dose response for Lufenuron:

Freshly laid fertilized eggs (0hr stage) of *Gallus domesticus* (White Leghorn Strain) were obtained from IVBP (Indian Veterinary Biological Products), Pune. Eggs were washed with distilled water to remove the stains (blood and fecal) and allowed to air dry before wiping with 70% ethanol. Eggs were then incubated at 37.5^{0}C with a relative humidity of 70-80% for 24 hrs in BOD incubator. Freshly prepared 54ppm stock solution of Lufenuron (5.4% EC, m/s Syngenta Company, Bombay) in distilled water was used for experimentation. Different doses of stock solution were used for in ovo treatment of pre incubated eggs of *G. domesticus* to observe the dose response. The doses at which the embryo remains viable were selected for experimentations.

To study effect of Lufenuron on chick embryogenesis:

A dose of 10µl and 100µl of stock solution was injected in air sac of pre incubated eggs (96hr, HH, 22-23) with micropipette. Entire procedure was carried out under aseptic conditions in laminar flow. The control group of eggs was injected with same quantity of distilled water. Treated and controlled eggs were sealed with an adhesive tape, labeled and incubated at 37.5^{0}C with 70-80% relative humidity in a BOD incubator for 21 days (full term development). The eggs were observed every day for fungal infection, contamination and embryonic death. Contaminated eggs were discarded. Five replicates of ten each per dose were prepared. After 21 days control and treated embryos were dissected out in 0.9% saline. Observations were made on phenotypic deformities in treated embryos.

Studies on the Skeletal Anomalies:

For visualizing musculoskeletal development, un hatched embryos were isolated on day 21, deskinned, eviscerated and stained with Alcian blue and Alizarin red S[16]. Microphotography was done using Nikon DSLR Camera attached to a binocular stereo zoom microscope at 2X and 4X magnification.

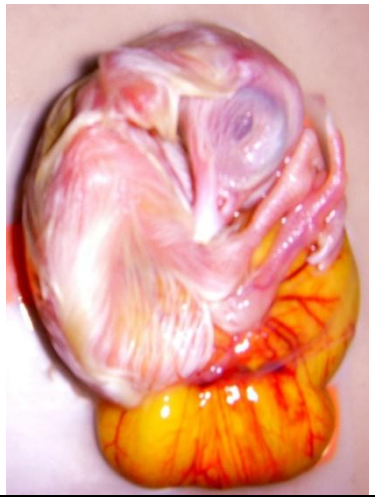
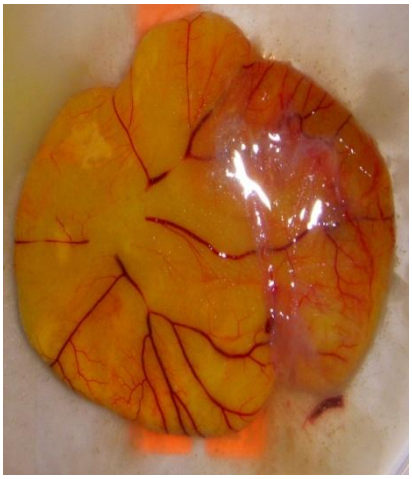
Results:

In ovo treatment of fertilized eggs of chicks with 10µl of 54ppm Lufenuron resulted

in retarded growth and development of embryos as chicks failed to hatch on 21st day of incubation following the treatment. Normal hatching of chicks on 21st day of incubation was obtained in control eggs. It was observed that an unutilized yolk with profuse vasculation remains attached with the embryos developed from Lufenuron treated eggs (**Fig.1 A, B**). However 100% survival of the embryos was observed in eggs treated with 10µl, 54ppm Lufenuron.

Injection of 100µl of 54ppm Lufenuron in ovo to fertilized chick eggs was shown to affect embryonic in all the treated eggs. Also, at higher dose (100µl) of Lufenuron, embryonic growth was retarded, morphogenesis of the embryo was incomplete and chicks failed to hatch. Further it was observed that the utilization of yolk by growing embryo was affected resulting in weakly ingested yolk with profusely branched blood vessels extended on its outer extremities was found to attached with the embryo. (**Fig.2A, B, C**). Structural anomalies such as abdominal hernia, haematomia of vitelline blood vessels, hemorrhagic chorioallantoic membrane (CAM) and macropthalmia (increased size of eye), macrocephaly (disproportionately large head) (**Fig.2 B, C**) were also observed. In ovo treatment of fertilized chick eggs with 100µl of 54ppm Lufenuron was found to induce skeletal system anomalies as revealed by Alizarin red S and Alcian blue staining of chick embryo developed on 21st day of incubation. The skeletal anomalies developed were caudal regression syndrome in which caudal region was reduced or entire tail was lacking as revealed by the absence of caudal vertebrae (**Fig.3A**).Also, Lufenuron (100µl of 54ppm) treated eggs were found to develop embryos which lost their bilateral symmetry because of incomplete ossification and calcification in sacral spine as compared to that of control (**Fig.3D, E**). Further, it was observed that the chicks developed from Lufenuron treated eggs have reduced number of vertebral ribs (**Fig.3A, B**) and deflected hind limb digits (**Fig.3C**).

Fig.1: Photographs showing effect of Lufenuron (54ppm, 10µl) on chick embryo, on 21st day of incubation.

A. Unhatched chick with weakly utilized mass of yolk.	**B.** Isolated unutilized mass of yolk from chick embryo showing profuse vasculation on the surface of yolk sac.
	

➢ **Fig. 2: Photographs showing effect of Lufenuron (54ppm, 100µl) on chick embryo, on 21st day of incubation.**

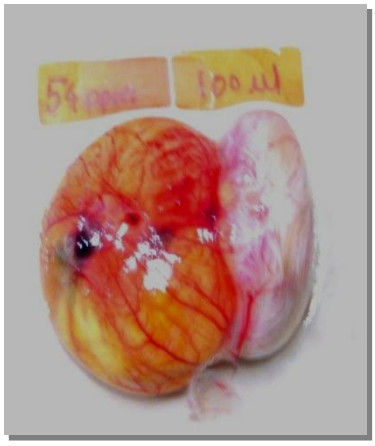
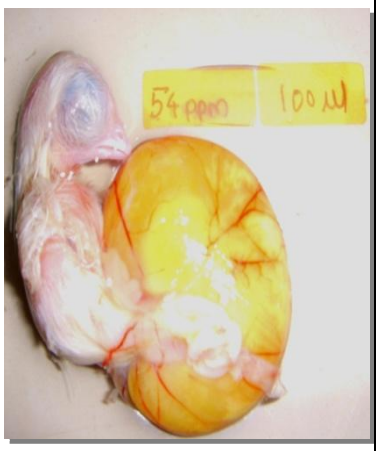
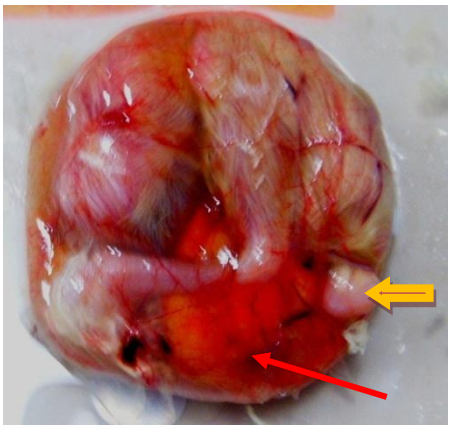
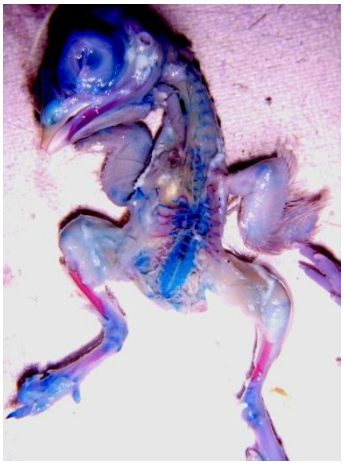
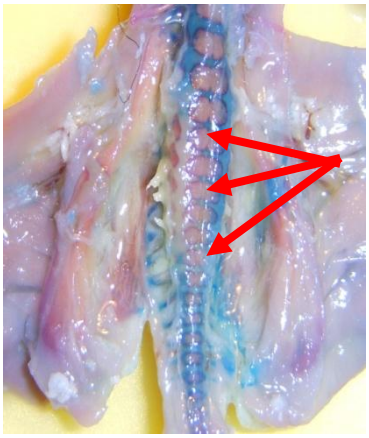
➢ **A.**Incompletely developed embryo with intense vascularization on yolk sac.	➢ **B**. Chick embryo with unutilized mass of yolk.	➢ **C.**Haemorrhagic Chorioallantoic Membrane (indicated by red arrow), haematoma in yolk sac and hernia (yellow arrow).
		

Fig.3: Photographs showing skeletal anomalies in embryo stained with Alizarin Red S and Alcian Blue developed from Lufenuron (54ppm, 100µl) treated eggs, on 21st day of incubation

A. CRS (Caudal Regression Syndrome)and macrocephaly	**B**.Normal Skeletal development in Control Embryo	**C**. Thickened Synsacrum showing CRS and deflection of hind limb digits (Indicated by arrow).
		

➢ **D**. Enlarged view of vertebral column showing unilateral chondrogenesis of Synsacrum (Sacral Spine) (Indicated by arrow).	➢ **E**. Enlarged view of photograph **B**.
	

Discussion

The present studies revealed that Lufenuron has embryo lethal and teratogenic effect on chick embryos. Further, there was dose dependent effect of Lufenuron on chick embryogenesis. At lower dose (10µl), 100% survival was observed but it affects hatchability of the chick. Similar observations were made in Cypermethrin and Chlorpyrifos treated fertilized Rhode Island Red eggs[14]. Injection of higher dose of Lufenuron (100µl) resulted in morphological and skeletal anomalies in chick embryo.

Teratogenic changes involved abdominal hernia, macrocephaly and macropthalmia. However induction of macrocephaly and macropthalmia by Lufenuron (100µl) was in contrast to observations in Cypermethrin treated chick embryos in which micropthalmia or absence of eyes and reduction in size of head that is microcephaly was observed[17] and induction of micropthalmia in embryos of eggs of chick treated with enrofloxacin[18] and chlorpyrifos and cypermethrin treated chick embryos[14].

Our observations of induction of skeletal anomalies such as deformed ribs and vertebrae due to Lufenuron (100µl) treated eggs are in conformity with those reported in the developing chick by RPR-V, an organophosphate[19]. In the present studies the caudal regression syndrome was observed in which there was lack of caudal vertebrae. Similar results were observed in embryos of eggs obtained by maternal treatment with enrofloxacin[18].

Thus our data concludes that an Insect Growth Regulator, Lufenuron at a very low dose (100µl of 54ppm) was embryotoxic and teratogenic to a non target organism, chick.

The studies revealed that exposure to Lufenuron in the food chain may leads to undesirable consequences in vertebrates. It is therefore recommended that Lufenuron should be used with caution as it can be hazardous to domestic animals and human beings. Further studies at the molecular level evaluating the effect of pesticides on specific genes that regulate development in vertebrates are essential.

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