Antibiotic residue in eggs of laying hens following injection with gentamicin

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Abstract:

1) Thirty two hens were assigned to four equal groups (n = 8) and injected intramuscularly or subcutaneous with either 2 or 4 mg/kg gentamicin for 3 successive days.
2) The transfer of gentamicin into eggs was determined separately from albumen, yolk and whole egg daily during dosing and withdrawal periods by high performance liquid chromatography (HPLC).
3) Drug excretion was usually over a long period and with a high dose in the yolk compared to the albumen due to the incorporation and storage of drug in preovulatory yolks during the dosing period.
4) Residues in the whole eggs were detected after the last injection for 12 and 15 days when hens injected with 2 and 4 mg/kg gentamicin for 3 successive days respectively.
5) The concentrations of drug residues were closed to the limit of the assay's sensitivity (0.01µg/g).

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Key words: Antibiotic residue – gentamicin – yolk – albumen – whole egg.

Introduction:

It is almost impossible to produce food animal origin which is completely free from traces of drugs or chemicals (Campbell, 1978). Nowadays antibiotics are used on a large scale in poultry farms to cure or prevent diseases to promote growth. For example, gentamicin is an aminoglycoside antibiotic which is active against many strains of Gram-negative bacteria, including Pseudomonads, also against many Staphylococcus aureus strains, whereas Enterococci and Streptococci are insensitive to gentamicin alone (Richard and Clarsonce, 1986). Even if no additional drug transfer occurred after the dosing period, hens would still store drug residues in egg yolks slated for ovulation days to weeks in future (Donoghue *et al.*, 1997). These residues consist of the parent compound or compounds derived from the parent drug (or both), including metabolites, conjugates and residues bond to macromolecules (Weber 1979). Residues appeared in human food of animal origin and certain greatly affected the life and health of human causing many troubles as allergic phenomena, sensitization, teratogenic, carcinogenic effects and antibiotic resistance (Mitchell *et al.*, 1998). Also make adversely effect consumer confidence in animal product. Therefore, before any drug can be marketed for use in food-producing animals, three considerations that pertain to its safety assessment must be addressed: 1) identify and measuring residues in edible tissues, 2) determining from toxicity tests the conditions for the safe use of the drug with regard to the persistence of the residues, 3) assuring in the use that residues don't exceed the amount regarded as safe. From here appear the importance of our study investigates the presence of gentamicin residues in albumen, yolk and whole egg during and after therapeutic treatment via intramuscular and subcutaneous routs and determine how many days-weeks still taken eggs contaminated with drug residue after the dosing period (withdrawal period) to reduce the potential for unintentional contamination of this important food commodity. Also the withdrawal period can be defined with the time required for the residue of toxicologic concern to reach safe concentration as defined by the tolerance.

Materials and Methods:

Animals:

A total number of 32 Single Comb White Leghorn hens, 38 weeks of age, with weights ranging from 1.6 to 1.8 Kg, were used in his study. Hens were individually caged and had ad libtium access to standard laying hen feed and water and subjected to

14 hour of light daily. Hens were fed for a week and their eggs were collected and analyzed to be certain that no gentamicin residues contaminated the eggs before beginning he experiment.

Experimental procedure:

Thirty two hens were assigned to 4 groups (n=8). Groups 1 and 3 injected intramuscularly into the pectoral muscles with 2 and 4 mg gentamicin/Kg body weight/day for 3 days respectively. Groups 2 and 4 injected subcutaneously with 2 and 4 mg gentamicin/Kg body weight/day for 3 days respectively. Injection of drugs eliminates the confounding influences of variation in drug exposure due to differences in hens feeding or water consumption tendencies (Donoghue *et al.,* 1996). On the day of the study, hens were monitored every 5 minutes to establish the time of oviposition. Injection was given one hour after the daily oviposition to synchronize time of injection to the daily phase of yolk formation. Because ovulation occurs within approximately 30 minutes after oviposition (Johnson, 1986). To insure that each group had eight hens, additional hens (4 hens per group) has been used at the start of the study and maintained as a spare to use if any hen at any group didn't lay an egg or died during the experimental period.

Analytic mehods:

The residues of genamicin in the eggs were detected by modifying HPLC method recommemded by the European Union (Heitzman 1994). Five grams of the yolk and 5 grams of the albumen were taken from the egg and the remaining part of the egg was mixed and then 5 grams was taken from this mixture. Thus he quantity of gentamicin in yolk, albumen and whole egg was analyzed. Trichloracetic acid (5%w/v)/1mM EDTA solution was added to the samples in the tube; the samples were homogenized and then centrifuged to precipitate the proteins. At he end of this process, pH was adjusted to 0.7 and they were filtered using a Sephadex G25 (Bakerbond Spe Kolon) ion exchange column. The samples were analyzed in the HPLC with post-column derivation (Phenomex Luna C-18 column; 5 µm, 250 mm × 4.6 mm). The o-phthalaldehyde (.8% containing 0.2% 2-mercaptoehanol and 0.1% Brij) was pumped at 0.5 ml per min and fluorescent peaks were detected at 400 nm. The total concentration of the gemtamicin was calculated.

Recovery studies:

The gentamicin sulphate analytic standard (Sigma G1914, Lot:070K1038), with purity of 665 µg/mg, was added to the yolk, albumen and the whole egg whose concentration were 0.01, 0.02, 0.03, 0.05 and 0.07 µg/ml, and it was homogenized to allow the extraction of the gentamicin from the eggs and to establish the limit of detection. It was extracted with the above-stated analytic method and was applied to the HPLC.

Statistical analysis:

Data were analyzed by analysis of variance using the Statistical Analysis System (SAS® software, 1994) general linear models program. Treatment means were partitioned by least square means (LS MEANS) analysis. A probability of $P \leq 0.05$ was required for statistical significance.

Results:

Tables 1, 2 and 3 demonstrate gentamicin residues detected in albumen, yolk and whole egg during the dosing and withdrawal periods when hens injected in different dosages via different routes respectively. From dosing began on day 1, the first egg contained gentamicin residues in the albumen but the first yolk (day 1) didn't contain drug residues and appeared from the second yolk (day 2). As shown in Table (1) gentamicin residues in the albumen started to decrease slightly after day 1 in the withdrawal period but in he yolk and whole egg (Table 2 and 3) the residues increased gradually and the highest concentrations towards the middle of the withdrawal period then the residues started to fall rapidly. Results from this study demonstrate that gentamicin residues were incorporated into albumen for 6 and 8 days for dosing 2 and 4 mg/kg life body weight within the withdrawal period respectively but in either yolk or whole egg for 12 and 15 days respectively, after that gentamicin residues disappeared completely (<0.01µg/g). The gentamicin residues tolerance levels reported by U.S.A.; Germany; France and Holland were (0.1-0.4; 0.2; 0.1-0.2 and 0.1 mg/kg) respectively (Lavzquez *et al.,* 1990). It is clear that the drug remained in the yolk for a long period and at high concentrations compared to albumen.

Table (1) Residues of gentamicin in the albumen (µg/g) during and after injection for 3 successive days.

	Days	i/m 2 mg/kg	s/c 2 mg/kg	i/m 4 mg/kg	s/c 4 mg/kg
Dosing period	1	0.09 ± 0.02	0.11 ± 0.03	0.17 ± 0.04	0.16 ± 0.03
	2	0.12 ± 0.03	0.13 ± 0.02	0.23 ± 0.06	0.20 ± 0.05
	3	0.17 ± 0.04	0.16 ± 0.04	0.32 ± 0.08	0.31 ± 0.07
Withdrawal period	1	0.20 ± 0.04	0.21 ± 0.05	0.36 ± 0.05	0.37 ± 0.06
	2	0.18 ± 0.06	0.18 ± 0.04	0.31 ± 0.08	0.33 ± 0.05
	3	0.15 ± 0.04	0.13 ± 0.03	0.24 ± 0.07	0.23 ± 0.04
	4	0.09 ± 0.02	0.09 ± 0.03	0.18 ± 0.04	0.17 ± 0.03
	5	0.04 ± 0.01	0.03 ± 0.01	0.12 ± 0.03	0.13 ± 0.003
	6	0.01 ± 0.01	0.02 ± 0.01	0.07 ± 0.02	0.09 ± 0.01
	7	<0.01	<0.01	0.05 ± 0.02	0.05 ± 0.01
	8	<0.01	<0.01	0.02 ± 0.01	0.03 ± 0.01
	9			<0.01	<0.01
	10			<0.01	<0.01
	11				

The values in the same column or row are insignificant (P≤0.05).
i/m = intramuscular; s/c = subcutaneous.

Table (2) Residues of gentamicin in the yolk (µg/g) during and after injection for 3 successive days.

	Days	i/m 2 mg/kg	s/c 2 mg/kg	i/m 4 mg/kg	s/c 4 mg/kg
Dosing period	1	0.0	0.0	0.0	0.0
	2	0.49 ± 0.08	0.51 ± 0.11	0.91 ± 0.21	0.92 ± 0.18
	3	0.56 ± 0.12	0.55 ± 0.09	0.99 ± 0.18	0.98 ± 0.15
Withdrawal period	1	0.65 ± 0.14	0.64 ± 0.10	1.11 ± 0.22	1.08 ± 0.20
	2	0.74 ± 0.10	0.75 ± 0.16	1.26 ± 0.14	1.22± 0.27
	3	0.81 ± 0.17	0.83 ± 0.14	1.39 ± 0.22	1.38 ± 0.23
	4	0.88 ± 0.21	0.89 ± 0.18	1.50 ± 0.26	1.49 ± 0.30
	5	0.95 ± 0.14	0.97 ± 0.23	1.60 ± 0.23	1.58 ± 0.22
	6	0.99 ± 0.19	1.01 ± 0.17	1.69 ± 0.27	1.67 ± 0.21
	7	1.02 ± 0.21	1.04 ± 0.21	1.74 ± 0.30	1.70 ± 0.19
	8	0.88 ± 0.19	0.87 ± 0.18	1.63 ± 0.16	1.76 ± 0.25
	9	0.62 ± 0.10	0.64 ± 0.13	1.51 ± 0.23	1.53 ± 0.17
	10	0.35 ± 0.06	0.37 ± 0.06	1.29 ± 0.20	1.32 ± 0.23
	11	0.19 ± 0.04	0.18 ± 0.04	1.01 ± 0.16	1.05 ± 0.20
	12	0.07 ± 0.02	0.06 ± 0.01	0.52 ± 0.09	0.54 ± 0.08
	13	<0.01	<0.01	0.20 ± 0.04	0.25 ± 0.04
	14	<0.01	<0.01	0.09 ± 0.02	0.11 ± 0.02
	15			0.03 ± 0.01	0.05 ± 0.01
	16			<0.01	<0.01
	17			<0.01	<0.01

The values in the same column or row are insignificant (P≤0.05).
i/m = intramuscular; s/c = subcutaneous.

Table (3) Residues of gentamicin in the whole egg (µg/g) during and after injection for 3 successive days.

	Days	i/m 2 mg/kg	s/c 2 mg/kg	i/m 4 mg/kg	s/c 4 mg/kg
Dosing Period	1	0.06 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.02
	2	0.21 ± 0.05	0.23 ± 0.05	0.46 ± 0.07	0.44 ± 0.08
	3	0.28 ± 0.06	0.31 ± 0.07	0.49 ± 0.09	0.48 ± 0.11
Withdrawal period	1	0.31 ± 0.05	0.34 ± 0.08	0.56 ± 0.13	0.54 ± 0.10
	2	0.35 ± 0.07	0.36 ± 0.07	0.60 ± 0.11	0.61 ± 0.11
	3	0.38 ± 0.06	0.37 ± 0.05	0.67 ± 0.08	0.65 ± 0.13
	4	0.41 ± 0.08	0.43 ± 0.10	0.72 ± 0.11	0.74 ± 0.12
	5	0.42 ± 0.07	0.45 ± 0.08	0.81 ± 0.10	0.85 ± 0.19
	6	0.44 ± 0.10	0.46 ± 0.10	0.85 ± 0.12	0.87 ± 0.15
	7	0.47 ± 0.09	0.45 ± 0.07	0.91 ± 0.15	0.88 ± 0.13
	8	0.38 ± 0.07	0.36 ± 0.09	0.87 ± 0.14	0.90 ± 0.18
	9	0.19 ± 0.04	0.24 ± 0.06	0.39 ± 0.08	0.43 ± 0.10
	10	0.12 ± 0.02	0.13. ± 0.03	0.30 ± 0.07	0.32 ± 0.06
	11	0.06 ± 0.02	0.08 ± 0.02	0.23 ± 0.05	0.22 ± 0.04
	12	0.02 ± 0.01	0.02 ± 0.01	0.15 ± 0.03	0.17 ± 0.03
	13	<0.01	<0.01	0.06 ± 0.02	0.08 ± 0.02
	14	<0.01	<0.01	0.03 ± 0.01	0.04 ± 0.01
	15			0.01 ± 0.01	0.01 ± 0.01
	16			<0.01	<0.01
	17			<0.01	<0.01

The values in the same column or row are insignificant (P≤0.05).
i/m = intramuscular; s/c = subcutaneous.

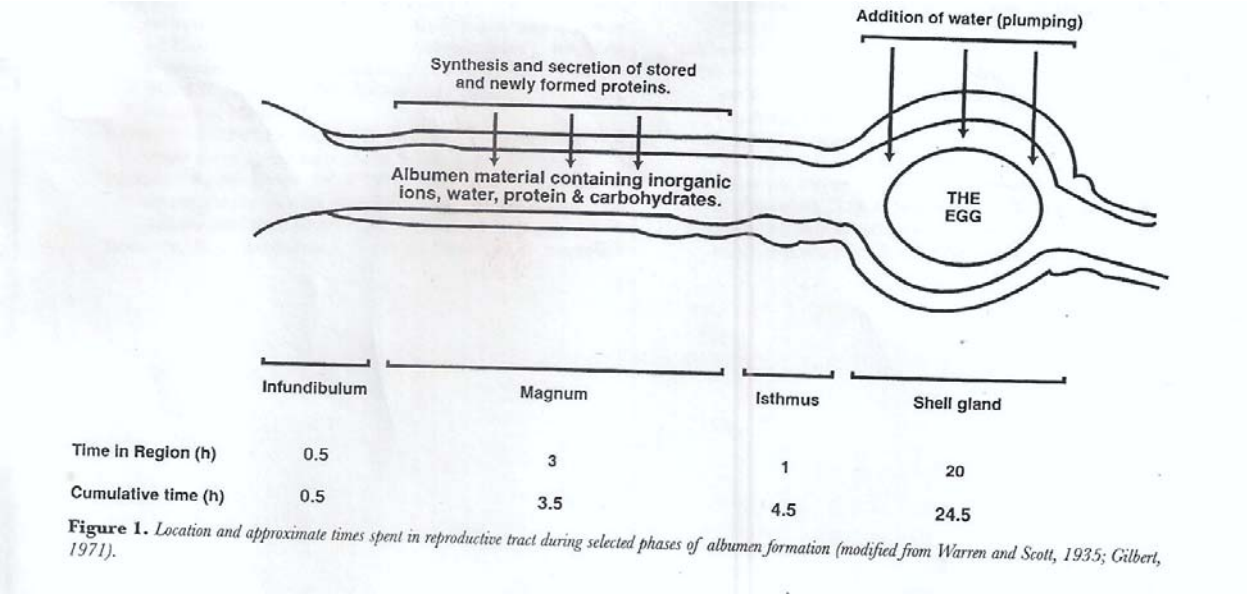

Figure 1. *Location and approximate times spent in reproductive tract during selected phases of albumen formation (modified from Warren and Scott, 1935; Gilbert, 1971).*

Discussion:

According to hens were dosed one hour after oviposition and hens ovulate approximately 30 minutes after oviposition (Johnson, 1986). For this the first egg would not contain any (0.0%) drug residues because the first yolk had completed formation and ovulated before drug treatment even through yolk was still in the respective tract for the process of albumen secretion. Concerning to albumen, egg white formation occurs in 3 general phases. . These are 1) Synthesis and storage of albumen proteins prior to ovulation, 2) Secretion of stored proteins and synthesis and secretion of new proteins during passage of the ovum (yolk) down the reproductive tract (preplumping), 3) Addition of water (plumping phase) (Warren and Scott, 1935; Gilbert, 1971 and Solomon, 1983). These phases are depicted in the Figure (1). The drug residues are incorporated during the later two phases of albumen formation because the hens were dosed one hour after oviposition. For this, the drug residues would be present in the first egg albumen collected after the first day of dosing. As general drug residues are contained in eggs for days after drug withdrawal or continued drug transfer after drug withdrawal could be higher or lower depend on 1) drug dosing; 2) route of administration; 3) drug half-life; 4) age of hens; 5) health of the hen; 6) rate of egg production and 7) drugs with a propensity to be stored in body tissues (such as the fat or liver) and released back to the blood stream will enhance daily carry-over accumulation of drugs in developing yolks (Donoghue *et al.,* 1996). Gentamicin diffuses mainly into extracellular fluids, and appears to be accumulated in the body tissues to some extent, with slow release from these sites and in small amounts for long period (Games *et al.,* 1996). Our results indicate that incurred gentamicin residues are contained in yolk for a long period and at a higher concentration than albumen. Concerning for a long time, drug residues were incorporated during the dosing period and stored in the preovulatory yolks for a number of days before ovulation. At higher concentrations, the majority of yolk formation occurring within the last 2 weeks prior to ovulation, during this period, the individual yolks increase in size from approximately 0.2 to a final mature weight of 17 g, in addition, there are approximately 20 small yolks (0.1 to 0.2 g) maintained within individual ovarian follicles waiting to inter rapid growth phase that is approximately 2-6 weeks from ovulation (Griffin *et al.,* 1984). Gentamicin will incorporate into all these yolks and accumulate residues; the amount of residue accumulation will depend on the developing stage. The smaller yolk (include drug residues towards the inner layer of the growing yolks) had the greatest total drug content more than the larger yolk (include drug residues in the outer layer of development) and it demonstrates the phenomenon of drug residue actually increasing in eggs following termination of drug expose to the hen (Donoghue *et al.,* 1996 and 2001). Many chemicals are preferentially deposited in the yolk rather than in the albumen (Nagata *et al.,* 1992; Donoghue *et al.,* 1994 and Ridley *et al.,* 1998). Concerning to albumen, the presence of drug was at lower concentration and for a shorter period may be according to the hypothesis of the passive diffusion (from denser apace to less dense apace) of the drug from blood plasma to the egg white glandular epithelium until the equilibrium of the drug concentration between the blood plasma and the albumen present in the magnum. (Filazi *et al.,* 2005). We can conclude that when hens injected with gentamicin one hour after oviposition, the first egg will contain drug residues. And recommended the producers that the eggs which produced during the withdrawal period which reach up to 12 and 15 days for doses 2 and 4 mg/kg gentamicin respectively must be discarded because it was evident that eggs were contained gentamicin residues which have harmful effect on the consumers. Obey the legislation regarding drug residue withdrawal period is essential to protect consumer health.

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