International Journal of Fauna and Biological Studies
Available online at www.faunajournal.com

E-ISSN 2347-2677
P-ISSN 2394-0522
https://www.faunajournal.com
IJFBS 2024; 11(6): 01-05
Received: 01-09-2024
Accepted: 06-10-2024

Batool A Majeed
Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq

Wasan Moaed S Haker
Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq

Samar S Ghazi
Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq

Bahaa A Alsereah
Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq

Corresponding Author:
Batool A Majeed
Department of Veterinary Public Health, Veterinary Medicine College, University of Basrah, Iraq

Injecting Japanese quail eggs with florfenicol and determining its effect on egg embryos

Batool A Majeed, Wasan Moaed Shaker, Samar S Ghazi and Bahaa A Alsereah

Abstract
The experiment used 180 quail eggs with florfenicol in some production and egg hatchring distributed into four groups, at a rate of 45 egg for each treatment, inject the eggs before the first day of Incubation at the wide end of the egg with the following compounds. The first group: There is no injected group (control), The second group: a group of injections with phosphate-buffered saline solution. The third and fourth groups whose members were injected with florfenicol injectable solution at a dose of 10 and 20 mg/kg of egg weight, respectively. The results indicated that notice the highest hatching rate was in the third and fourth treatments, respectively. As for the average weights of hatched birds, they increased significantly in the third and fourth treatments, respectively, compared to the other study treatments. there was a numerical increase in the mortality of birds in the first and second treatments compared to the rest of the study treatments, as there were no deaths during the breeding period, that there was a significant increase in the average weights of perished embryos during the various stages of embryonic development. As for the differences between the percentages of perished embryos during stages of embryonic development and the percentage of quantitative embryonic deaths were not significant. As significant increase for all ages compared to the live weight character of the birds treated 2, 3, 4 and Control group. No significant difference appeared between the study treatmentson weight gain. We conclude that injecting hatching eggs with florefenicol on the first day before incubation, at a dose of 20 mg/kg eggs, improved the final weight of the birds and eggs hatcharing.

Keywords: Florefenicol, quail, egg, embryo, weight

Introduction
Bacterial diseases have posed a major threat in the poultry industry and have led to economic losses in the industry. Therefore, utmost efforts were made to reduce these losses. Among the methods used is the use of several types of Antibiotics. Antibiotics have been used widely and for various purposes. In the veterinary field, it has been used to treat birds. To prevent diseases, or used as a food additive to feed in order to enhance growth. Florfenicol a synthetic broad-spectrum antibacterial agent effective against most Gram-positive and Gram-negative bacteria isolated from birds. Florfenicol It belongs to the amphenicol family, and is used in treatment. Serious infections caused by susceptible strains of pathogens. The action of florfenicol is bacteriostatic by preventing protein synthesis within the bacteria. (Khalil *et al*. 2012) [5]. Florfenicol works as a preventive aspect to reduce the mortality rate in birds during their growth period. Birds treated with florfenicol show a clear improvement in growth rate, better increase in body weight, and better utilization of feed compared to those that were not treated with florfenicol. Florfenicol has an effective effect against many bacterial strains resistant to thiamphenicol (Ronette 2012) [6]. The results of a study conducted by (Hadi, *et al*. 2014) [7] showed that injecting the antibiotic florfenicol into chicken eggs at concentrations of 20 mg and 30 mg did not show any toxic effects on chicken embryos in terms of macroscopic and pathological infections, and that injecting the antibiotic at these concentrations is considered safe for the birds. The safety of hatching eggs in hatcheries is achieved by injecting the eggs with an antibiotic into the egg in order to get rid of bacterial infection, prevent infection, and eliminate pathogens. In this regard, we conducted this study by injecting a florfenicol solution into the embryos of Japanese quail eggs at different doses, believing that the results we will obtain will contribute and help in our understanding of the safety and security of using antibiotics in bird embryos.

Materials & Methods

Management of Experiment

Hatching eggs: One hundred and eighty fertile quails eggs (Cotrnix Japonica) with the average egg-weight of 14-17 g and with the same age were purchased from the general authority for agricultural research / abu ghraib, Baghdad. In this farm, birds were kept and grown up under the standard condition of breeding.

Egg injection method: The manual method was used by using a 1 ml insulin syringe with a 29 gauge needle. The eggs were injected before inserting it into the incubator (Bertin, *et al*., 2009) [8] in the air space area (Weber, *et al*., 2004) After wiping the area to be injected with 10% formalin, the resulting holes were closed injection process. Then with beeswax place the eggs in the incubator.

Preparation of Drugs for use: Florfenicol injectable solution was obtained from a pharmaceutical company. Each milliliter of drug contains 300 mg florfenicol. It was diluted in phosphate buffered saline solution. A volume of 0.5 mL of phosphate buffered saline solution with 10 and 20 mg florfenicol was inoculated per Kg egg-weight.

First Stage: One hundred and eighty eggs quail birds were used in the study, eggs were divided into 4 groups randomly, each group containing 3 replicates (15) egg / replicate. Inject the eggs before the first day of Incubation at the wide end of the egg according the following groups: T_1: There is no injected group (control), T_2: a group of injections with phosphate-buffered saline solution. T_3: a group with injected with florfenicol injectable solution at a dose of 10 mg/kg of egg weight. T_4: a group with injected with florfenicol injectable solution at a dose of 20 mg/kg of egg weight.

Second Stage: After the eggs hatched and the chicks emerged at one day old, the birds were divided into 4 groups (according to the previous egg division procedures) and rearing was carried out until the age of 42 days, and the food and water provided to the birds was free.

Nutrition: The birds were fed from hatching until the twenty-first day of their lives on a starter diet and then fed on a growth and production diet. These diets were prepared from one of the feed factories in northern Iraq. Table (1) shows the components of these diets and their chemical analysis.

Table 1: Components of diet with their chemical analysis.

Ingredients	Starter diet (%)	Growth diet (%)
Yellow corn	50	53
Soybean meal	28	28
Protein concentrated (50% protein)	10	8
Wheat bran	8	4
Limestone	2	3
Salt	0.5	0.5
Vegetable oil	1	3
Mixture of vitamins and antibiotic	0.5	0.5
Total	100	100
Metabolic energy (kcal/kg)	2918.5	3095,8
Protein (%)	23.02	21,69
Calcium (%)	0.80	1.04
methionine%	0.60	0.48
Phosphorus (%)	0.37	0.52

*NRC 1994

The criteria studied in the two stages are

1. Hatching rate: Hatching percentage of fertilized eggs = number of hatched birds / number of fertilized eggs x 1.

2. Weight at hatching: The hatched chicks were weighed by taking four samples from each treatment. Each sample consisted of six chicks using Precisin type (Blancce Precisin) sensitive balance for two decimal places after sorting.

3. Percentage of losses: The numbers of deaths in each replicate were recorded weekly and calculated as a percentage of the total number of chicks in each replicate. It was replicated and treated at the end of the experiment according to the following equation:

Percentage of deaths = number of chicks that died / number of chicks quantity x 100 Al-Zubaidi (1986) [12].

4. Determining the stage of fetal death: The stage of embryonic lethality was determined according to Pedroso, *et al*. (2006) [13] indicated, by breaking eggs those that did not hatch and remained in the incubator after 18 days of incubation, as there were embryonic deaths in the early stage of incubation (1-5) day, in the middle stage of incubation (6-14) days and in the late stage of incubation (15-18) days, and the weight of the perished fetus was determined according to was stated in Khalil, (2009) [14].

5. Percentage and weight of perished embryos

The perished embryos were weighed for each stage of embryonic mortality using a scale sensitive to two decimal places after sorting. The percentage of perished embryos was extracted by following the following equation:

Percentage of destroyed embryos = number of destroyed embryos / number of fertilized eggs x 100.

6. Average of living body weight and weight gain (gm): The birds of each treatment were weighed individually at the ages of 30 and 42 days by weighing the birds of each replicate collectively. Then, weight gain rates were calculated according to the following equation:

Weight gain = average live body weight at the end of the week - average live body weight at the beginning of the

week). (Al-Fayyad and Naji, 1989) [15]

Statistical analysis

In order to determine the statistical significances among different variables (SPSS 2009) [16] was used. Analysis of variance tests were applied to analyze the obtained results.

Results

Hatching rate, hatched bird weight, and breeding mortality rate: Table (2) shows the effect of injecting eggs with florefenicol on the hatching rate, the weight of the hatched bird, and the breeding mortality rate, as the highest hatching rate was in the third and fourth treatments, respectively. Note that there were no significant differences between the different study treatments. As for the average weights of hatched birds, they increased significantly ($p > 0.05$) in the third and fourth treatments, respectively, compared to the other study treatments. There were no significant differences in the percentage of breeding deaths, but there was a numerical increase in the mortality of birds in the first and second treatments compared to the rest of the study treatments, as there were no deaths during the breeding period, which extended for more than seventy days after hatching in each of the third and fourth treatments.

Table 2: The effect of injecting eggs with florifenicole on the hatching rate, the weight of the hatched bird, and the percentage of breeding deaths (mean ± standard error)

Group	Hatching Rate	The weight of the hatched Bird	The breeding mortality Rate
Control	77.29	7.70 ±0.10	4.64 ±0.33
Normal Saline	77.45	7.31 ±0.32	4.66 ±0.45
Florefenicol 10%	83.34	9.33 ±0.03	-
Florefenicol 20%	83.69	9.41 ±0.09	-
			N.S

*Different letters vertically mean there are significant differences at the significance level (0.05)

Average weights and percentages of perished fetuses during the various stages of embryonic development

It is clear from Table (3) that there was a significant increase ($p > 0.05$) in the average weights of perished embryos during the various early, middle, and late stages of embryonic development for the third and fourth treatments, respectively, compared to the control and saline solution treatments, the first and second. As for the differences between the percentages of perished embryos during The early, middle and late stages of embryonic development and the percentage of quantitative embryonic deaths were not significant ($p > 0.05$) regarding the percentage of quantitative embryonic deaths.

Table 3: The effect of injecting eggs with florifenicole in average weights and percentage of perished fetuses during different stages of embryonic development (mean ± standard error)

Group	Average weights of perished embryos during the different stages of embryonic development (gm)			Percentage of perished fetuses during the different stages of embryonic development (%)			Percentage of total perished fetuses (%)
	Early	Middle	Late	Early	Middle	Late	
Control	1.01 ±0.01	2.55 ±0.15	6.40 ±0.33	5.82	6.87	7.93	20.62
Normal Saline	1.07 ±0.10	2.40 ±0.15	6.39 ±0.35	6.85	7.83	8.78	23.46
Florefenicol 10%	1.60 ±0.14	3.45 ±0.15	8.35 ±0.37	4.38	5.47	6.56	16.41
Florefenicol 20%	1.69 ±0.15	3.59 ±0.14	8.35 ±0.37	2.70	4.73	6.79	14.22
				N.S	N.S	N.S	N.S

*Different letters vertically mean there are significant differences at the significance level (0.05)

Live weight of quails hatched at the ages of 32 and 42 days

It was observed from Table (4) the effect of egg incubation with florfenicol on the live weight characteristic of hatched Japanese quail birds at the ages of 32 and 42 days, as there was a significant increase ($p > 0.05$) for all ages compared to the live weight character of the birds treated 2, 3, 4 and Control group.

Table 4: The effect of egg incubation with florfenicol on the live weight characteristic of hatched Japanese quail birds at the ages of 32 and 42 days (mean ± standard error),

Period/day	Control	Normal Saline	Florefenicol 10%	Florefenicol 20%	Mean
32	123.66 ±6.85	126.00 ±6.86	139.66 ±6.87	143.00 ±6.87	133.08±6.86
42	130.42 ±14.03	132.33 ±14.03	173.04 ±14.08	175.33 ±14.08	152.78 ±14.05

*Different letters vertically mean there are significant differences at the significance level (0.05)

Weight Gain of hatched quails during the period (32-42) day: It was noted in Table (5) the effect of injecting eggs with florfenicol on weight gain during the period between (32-42 days), as no significant difference ($p > 0.05$) appeared between the study treatments during this period depending on the injection with florfenicol.

Table 5: The effect of injecting eggs with florfenicol on weight gain during the period between 32-42 days (mean ± standard error)

Period/day	Control	Normal Saline	Florefenicol 10%	Florefenicol 20%	Mean
32-42	17.00 ±1.36	23.33 ±1.86	8.87 ±1.17	12.16±1.29	15.34±142

*Different letters vertically mean there are significant differences at the significance level (0.05)

Discussion

Despite the rapid and significant development that the poultry industry has witnessed during recent decades, pathogens, whether bacterial, viral or fungal, have posed a great danger and become an obstacle to the development of this industry, and have caused huge losses in the field of animal production in general and in general. Poultry health in particular. In order to improve the productive performance of birds, many researchers have used many different compounds of antibiotics in order to eliminate and impede the growth of pathogens and eliminate them, quail (Rigobelo *et al*. 2013) [17], Turkeys (Buscaglia 2013) [18], broilers, layers (Nemati 2013) [20] and poultry farmers (Jones *et al*. 2013) [21]. Amphenicol drugs have played an important role as prophylaxis or treatment against many pathogens in poultry because they have a large and broad spectrum of efficacy as therapeutic agents (Sweetman *et al*. 2009) [22]. Amphenicol compounds have been widely used in many countries around the world it has proven its efficiency and success in the poultry field for several years. Florfenicol is a drug that belongs to the Amphenicol group of drugs and has been approved by the European Union as it allows its use in Treatment of poultry as well as large field animals (Ronette 2012) [23]. In recent decades, flufenicol has been widely used in poultry, despite the lack of information about the effect of its use as an injection in eggs and the extent of its effect on embryos and hatchability rates.

The results showed that there was an arithmetic superiority that did not reach a significant level in the hatching rate of eggs of treatments 10% florfenicol and 20% florfenicol compared with the rest of the study's treatments, the reason may be attributed to Improving the health condition of the embryos as a result of eliminating pathogens that affect the embryos inside the egg, which has a positive impact on the improve hatching rate. The results of the current study did not agree with the results of the study (AL-Shahrani and Naidoo, 2015) [24] conducted, in which they obtained a low hatching rate as a result of injecting florfencol into the egg hen. Perhaps the reason is due to the difference in the type of eggs that were injected or the high concentrations of the injected substance. As for the weight rates of the hatched birds, they increased significantly in the 10% and 20% florfenicol treatments compared to the rest of the study treatments, and the reason may be due to increase metabolic rates (Buchanan, *et al*. 2003) [25], which led to increased rates of embryonic growth at hatching compared to hatched birds due to eliminate pathogens which led to an increase in the weight of the hatched birds (Martin and Schwabl, 2008) [26]. The results indicated that there were no fatalities during the study period, the birds were treated with 10% and 20% florfenicol. The reason is that the elimination of pathogens due to the injection of florfenicol into the eggs led to a reduction in the risk of oxidation during the stages of embryonic development and beyond. The results indicated a significant increase in the live weight rates of birds treated with 10% and 20% florfenicol for all ages. The reason for this may be attributed to the role of florfenicol as a growth promoter and increasing protein production rates and muscle building. The results of the current study agreed with the results (Agricultural Science Paper 2012) [27], considering that florfenicol enhances growth and also improves the nutritional efficiency of feed (Agricultural Science Paper (2013) [28]. The results indicated that there were no significant differences in the average weight increases according to the injection with florfenicol and for both periods. The reason for this may be attributed to the birds reaching physical and sexual maturity during this period, as the weight gain during this period is similar.

Conclusion

The research results showed an improvement in production performance as a result of using safe concentrations of the antibiotic florfenicol injected into hatching eggs, according to recommendations.

References

1. Swayne D, Glisson JR, McDougald L, Nolan LK, Suarez DL, Nair VL. Diseases of poultry. Wiley-Blackwell; 2013.
2. Tavakkoli H, Derakhshanfar A, Gooshki SN. A short preliminary experimental study on teratogenic effect of methenamine in embryonic model. Int J Adv Biol Biomed Res. 2013;1(12):1523-8.
3. Tavakkoli H, Derakhshanfar A, Salandari S. Investigation on the using of linco-spectin solution for in ovo administration in chicken embryo. Int J Adv Biol Biomed Res. 2014;2(1):110-6.
4. Tavakkoli H, Derakhshanfar A, Salandari S. Toxicology of urotropine in chicken embryo model. Open J Vet Res. 2014;18(2):109-15.
5. Khalil S, Hamed E, Hassanin O. Residue withdrawal of florfenicol from the serum and edible tissues of broiler chickens. J Am Sci. 2012;8(12):112-23.
6. Ronette G. Pharmacokinetics and bioequivalence of florfenicol oral solution formulations (Flonicol® and Veterin® 10%) in broiler chickens. J Bioequivalence Bioavailability. 2012;4(1):1-5.
7. Hadi T, Amin D, Samaneh NG. The effect of florfenicol egg-injection on embryonated chicken egg. Int J Adv Biol Biomed Res. 2014;2(2):496-503.
8. Bertin A, Richard-Yris MA, Möstl E, Lickliter R. Increased yolk testosterone facilitates prenatal perceptual learning in Northern bobwhite quail (*Colinus virginianus*). Horm Behav. 2009;56(4):416-22.
9. Weber FH, Genteman KC, Lemay MA, Lewis DO, Evans NA. Immunization of broiler chicks by in ovo injection of infective stages of Eimeria. Poult Sci. 2004;83:392-9.
10. National Research Council (NRC). Nutrient requirements of poultry. 9th ed. Washington, D.C.: National Academy Press; 1994.
11. Razouki WH, Hussein S, Ismail Abdel Reda AH. The effect of egg position during hatching on hatching characteristics and subsequent performance of broilers. Ibaa Res Group. 2000;15(2).
12. Al-Zubaidi SS. Poultry management. Basra University Press; 1986.
13. Pedroso AA, Café MB, Leandro NSM, Stringhini JH,

Chaves LS. Desenvolvimento embrionário e eclodibilidade de ovos de codornas armazenados por diferentes períodos e incubados em umidades e temperaturas distintas. Rev Bras Zootec. 2006;35:2344-9.
14. Khalil S, Hamed E, Hassanin O. Residue withdrawal of florfenicol from the serum and edible tissues of broiler chickens. J Am Sci. 2012;8(12):112-23.
15. Al-Fayyad HA, Nagy SA. Poultry products technology. 1st ed. Baghdad: Directorate of Higher Education Printing Press; 1989.
16. SPSS. SPSS Statistics for Windows, version 18.0. Chicago: SPSS Inc.; 2009.
17. Rigobelo EC, Blackall PJ, Maluta RP, Ávila FA. Identification and antimicrobial susceptibility patterns of Pasteurella multocida isolated from chickens and Japanese quails in Brazil. Braz J Microbiol. 2013;44(1):161-4.
18. Buscaglia C. Influence of the addition of antibiotics on survival of herpevirus of turkeys. Avian Dis. 2013;52(2):437-46.
19. Tavakkoli H, Derakhshanfar A, Salandari S. Investigation on the using of linco-spectin solution for in ovo administration in chicken embryo. Int J Adv Biol Biomed Res. 2014;2(1):110-6.
20. Nemati M. Antimicrobial resistance of porteus isolates from poultry. Eur J Exp Biol. 2013;3(6):499-500.
21. Jones K, Thornton J, Zhang Y, Mauel M. A 5-year retrospective report of Gallibacterium anatis and Pasteurella multocida isolates from chickens in Mississippi. Poult Sci. 2013;92(12):3166-71.
22. Sweetman SC, Pharm B, PharmS F, editors. Martindale: The complete drug reference. London: Pharmaceutical Press; 2009.
23. Ronette G. Pharmacokinetics and bioequivalence of florfenicol oral solution formulations (Flonicol® and Veterin® 10%) in broiler chickens. J Bioequivalence Bioavailability. 2012;4(1):1-5.
24. Al-Shahrani S, Naidoo V. Florfenicol induces early embryonic death in eggs collected from treated hens. BMC Vet Res. 2015;11:213.
25. Buchanan KL, Evans MR, Goldsmith AR. Testosterone, dominance signalling and immunosuppression in the house sparrow, Passer domesticus. Behav Ecol Sociobiol. 2003;55:50-9.
26. Martin TE, Schwabl H. Variation in maternal effects and embryonic development rates among passerine species. Philos Trans R Soc Lond B Biol Sci. 2008;363:1663-74.
27. Agricultural Science Paper. The determination method of thiamphenicol and florfenicol residues and their elimination in chicken tissues. Anim Husbandry Vet Med. 2012.
28. Agricultural Science Paper. Immunomodulatory effects of Bufonis parenteral on immunosuppression induced by florfenicol in mice. Animal Husbandry Vet Med. 2012.