

THE EFFECT OF USING A NEW LOCAL OILY INJECTABLE CADMIUM COMPLEX (CLC) IN LAYERS HENS' (LOHMAN BROWN) TO CONTROL OVARITIS AND SALPINGITIS CAUSED BY (*E.coli*) AND HEN DAY EGG PRODUCTION

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Abstract

An oily injectable administration CLC, chemically named [Dinitrato- bis {(p-methyl anilino) phenyl acetonitrile} Cadmium (II)]. (2) Hydrate, its chemical formula as [Cd (HL)₂(NO₃)₂]. 2H₂O was manufactured as a complex, and tested in vitro against *E.coli* (sensitivity test) was showed inhibitory zone of 12 mm(+++).The CLC was injected subcutaneously in the layer hens neck (*Lohman brown*) at different levels ,T₁ 0.0% , T₂ 0.25% , T₃ 0.50% and(T₄ ,T₅)1.0%.After five days *E.coli* was orally administrated by drinking water at concentration of 8500x10⁶cell \ ml, (highly pathogenic) as causative agent of Salpengitis and Ovaritis ,after another five days direct smears of *E.coli* from ovaries and oviducts , which had been cultured on MacConkey Red agar (differential agar for *E.coli* diagnosis) to identify *E.coli* colonies , also it tested biochemically by(Indole &Methylene Red) tests which were both + ve. The Results after four months are revealed that the treated chickens showed a resistance against *E.coli* infection; moreover the bacterial counts results of this study are associated with significant decreases of *E.coli* counts as compared to normal control (T₁), while there were significant increases in (T₅: doesn't inject CLC but administrated *E.col* orally by drinking water), that showed a pathological case. When a pathological anatomy had been mead there was ascitis, gaseous ovary, atrophied oviduct which are the main pathological symptoms of ovaritis and Salpengitis. The protective effects of CLC in respect to decreases *E.coli* counts in egg, ovaries and oviducts in T₃ (0.050). But chickens hen day showed significant decreases and the lowest egg production drop were in T₃ (0.50%) followed by T₂, T₄ and T₁ respectively. Then confirmed by measuring the residual concentration of Cd in liver and egg of treated chickens by using the atomic absorption, which revealed that livers and egg are free from any Cd residues.

The obtained results refer to the safety use of CLC successfully as synthetic chemical prophylactic agent against *E.coli* infection during production period in layers, in addition to its inexpensive cost.

Key word: Cadmium Ligand Complex. \ treatment of ovaritis.lohman brown. E.coli.

Introduction

The CLC was synthesized from α - aminonitrile with Cd (II) ion (Rawaa and Al-Hassan ,2010) , which are well known as biologically active compounds, and used in many pharmaceutical preparations for its routes as biologically inhibitors (Favata *et.al.*1998 ;Kalir *et.al.* ,1969)

The aminonitrile compound is used to explain the biological reactions, by acting as biological inhibitors and chelating agents (Foust *et. al.*, 1972). The ligands and its metal complexes have become very important in many fields including biological and industrial studies (Fatema, 2008). Cadmium (Cd) as a heavy metal, known to be highly toxic to both human and animal. Cd is widely distributed in the environment due to its various industrial uses, always there is naturally some Cd in agricultural areas may

reach to 2mg\ kg. (Aruna and Anisa, 2004). The role of Cd in biology has been recently discovered it has the same job as zinc in its biological activity, but half life of zinc is lowest than Cd in the environment, thus biologically has taken Cd rather than Zn and put it to work (Park and Chun, 2000; Lane and Morel. 2000). Some of toxic effects of Cd due to its interfere with ions metabolism, renal dysfunctions, hepatic damage and anemia (Nordberg *et. al.*, 1985). Because Cd caused oxidative damage in different tissues by enhancing the per oxidation of membranous lipids in the tissues and altering the cells antioxidant system by interaction with the cell organelles (Sarkar *et. al.*, 1995).The synthesis of new complex of Cd(II) as a ligand (CLC) gave it a biological activity against a number of microorganisms (an operation that protect tissues from deleterious effect of free radicals and protect liver from toxicity of Cd if it used alone, and *E. coli* pathogen toxins) (Vaidya *et. al.*, 1996).

E.coli caused many pathological cases to layer chickens for e.g. Cystic degeneration of ovarian follicles,Salpingitis are the commonest causes for layer chickens death, also caused Egg yolk peritonitis , *E. coli* septicemia of a respiratory origin. *E. coli* septicemia as a secondary to enteritis, Panophthalmitis (inflammation of all tissues of the eyeball), Arthritis, Osteomyelitis and Osteonecrosis (inflammation of joints, bone marrow and bone necrosis, respectively), Coligranuloma (Hjarre's disease), Bursitis sternalis (inflammation of the sternal bursa). Egg lying is predisposing factor for *E. coli* infection which associated with estrogen activity (Ivan, 2010). Therefore *Escherichia coli* infections are widely distributed among poultry of all ages and categories. They are primarily related to poor hygienic conditions, neglected technological requirements or to respiratory and immunosuppressive diseases. A common sequel of all infections before will cause local or diffuse peritonitis. Azusa *et. al.* (2007) suggested that certain *E. coli* virulence genes and host factors, such as initiation of egg lay may be associated with occurrence of *E.coli* infection.

The present work is studying the CLC toxicity and its protective role against *E.coli* infection as synthetic prophylactic antibacterial agent.

Material and Methods

CLC was synthesized as antibacterial agent in layers. CLC is a yellowish powder, never been produced before, and manufactured for first time in Basic science Department, College of Agriculture, and University of Baghdad. It's a [Dinitrato- bis {(p-methyl anilino) phenyl acetonitrile} Cadmium (II)] .(2) Hydrate, with a molecular formula $[Cd(HL)_2(NO_3)_2].2H_2O$ (Rawaa and Al- Hassan, 2010), it related to organic compound of aminonitrile which prepared by (Al Timeemey, 2000).The molecular weight for that complex was 716, and 263°C melting point and the Cd percent in CLC about 17.31 % . The oily material used to dilute CLC to various concentrations, which were performed in the department of bacteriology, at Al-Kindy Company, as following:

Liquid paraffin (90) ml; Arlacil (9) ml; Twin80 (1) ml

(CLC has been used at 0.25 %, 0.5 % and 1.0% concentrations)

CLC was identified depending on FT-IR, atomic absorption, & U.V visible spectrophotometers. The magnetic susceptibility and electrical conductivity of CLC were also measured by (Rawaa and Al- Hassan, 2010)

60 healthy layer hens' (*Lohman brown*), of 49 weeks age, weighing 1800-2200 gm (mean weight 2000 gm), had been used in this study. All birds were housed in optimal conditions in Poultry Field of Animal resources Department, Agriculture College, University of Baghdad during the period.May-1 to -August- 30 -2011. They were fed on crushed diet with a formula shows in table (1) according to what layers need. These chickens were divided into five main groups (T₁, T₂, T₃ T₄ and T₅), each group divided in to 3 subgroups each one contain (4 chickens \ subgroup) and injected

subcutaneously in the neck by a dose of oily CLC for T₂, T₃, T₄ and T₅ with concentrations of 0.25%, 0.50% ,1.0% and 1.0% respectively , while T₁ doesn't inject to be as normal control.

The (*Escherichia coli*) was obtained from laboratory of microbiology Department, Veterinary Medicine College, and University of Baghdad. The *E.coli* is G+ve bacteria (Carter, 1973) used as a pathogenic causative agent of egg peritonitis, Salpengitis and Ovaritis (Carter, 1973) has been given to the layers T₂,T₃ T₄ and T₅ by the drinking water at 8.500×10^6 cell \ ml concentration, after five days of injection , and clinical observations were recorded in all groups during the period of study (four months).

After five days of induced infection , 48 eggs had been collected and a chicken from each subgroup had been slaughtered to identify *E.coli* infection and it's count from a direct smears from eggs' surfaces, chickens' ovaries and oviducts , smears had been taken by autoclaved swabs then stepping into a nutrient broth culture and incubated at 37 °C for 48h.Untill it become turbid ,shake it carefully to ensure equal suspension of *E.coli* and a procedure of William (2003) was followed to estimate *E.coli* count in each sample.T₅ was important only for to identify *E.coli* and its count in direct smears from eggs' surfaces, chickens' ovaries and oviducts in infected not injected chickens .

From nutrient broth ,*E.coli* had been cultured on MacConkey Red agar (differential agar of *E.coli*) to identify *E.coli* colonies, also it tested biochemically by(Indole &Methylene Red) tests (Carter ,1973).Hen day production (H.D) was calculated to observe how much CLC influence H.D.before, during and after induced infection by the formula:

$$(H.D. \%) = [\text{no. of egg produced in a period} \div (\text{no. of chickens} \times \text{no. of days})] \times 100$$

(Al-Fayyad and Al- Najii. 1989).

For determination the residual concentration of Cd in body. Samples of liver and eggs were collected 10, 30, 85 days after injection of oily preparations (CLC) respectively. The specimens ashed at 600° C for 48 hr. Then the ash was gave to the serving Lab., Dept. of Chemistry, College of Science to estimate Cd residues in the samples by atomic absorption.

The data were statistically analyzed by using complete randomized design (CRD). The analysis were tested by the SAS programme (SAS, 2001), Also Duncans Multiple Range Test (DMRt) used for showing the significant differences between experimental groups at (P<0.05) probability level (Duncan, 1995).

Table 1. Composition of the experimental diet .

Ingredient	%
Yellow corn	30.8
Wheat	37
Soya bean (48%)	18
Concentrated animal protein (40%)	5
Vitamins & minerals	0.1
Soya bean oil	0.3
DCP stone	7.5
Nacl	0.03
DCP (18%)	1

Mathematical chemical analysis of diet Ingredients according to (NRC,1994) was (metabolized energy:2752K calorie\K.; mathematical crude protein:17.50%; lycine:1.10%;methionine:0.41%; Methionine + Cystine :0.75%;calcium:3.40%;available phosphor:0.42%;linolenic acid:1.05%.

Results and Discussion

The preset study observed that CLC when tested in vitro against *E.coli* gave an inhibitory zone of 12 mm when measured. The affected birds occasionally showing Pathological changes that revealed perihepatitis, pericarditis, ascitis, gaseous ovary, atrophied oviduct which are the main pathological symptoms of ovaritis and Salpengitis (Azusa *et. al.*,2007) .Also *E.coli* cultured on MacConkey Red agar to identify it, that had gave a large mucoid , circular, convex , smooth, white to yellowish white and sometimes translucent colonies (Coles,1974) .Also *E.coli* tested biochemically by Indole gave brilliant red coloring below in the test tube &Methylene test will give a distinct red color (Carter, 1973)

Table 2. Effect of CLC on *E.coli* colonies counts indirect smears from eggs' surfaces, chickens' ovaries and oviducts of layers chickens (*Lohman brown*).

<i>E.coli</i> colonies Counts(cell\ ml)	Experimental groups				
	T ₁ (0.0%)	T ₂ (0.25%)	T ₃ (0.50%)	T ₄ (1.0%)	T ₅ (1.0%)
1× 10 ²	- ve	- ve	- ve	- ve	+ ve
1× 10 ⁴	- ve	- ve	- ve	- ve	+ ve
1× 10 ⁶	- ve	- ve	- ve	- ve	11.3×10 ⁶
Eggs' surfaces <i>E.coli</i> Counts	6.3 ×10 ⁶ ±0.17 a	2.5 ×10 ⁶ ± 0.29 c	2.1 ×10 ⁶ ± 0.06 b	1.3 ×10 ⁶ ± 0.17 b	9.6×10 ⁶

T₁:(0.0%) ,T₂(0.25%) ,T₃(0.50%),T₄(1.0%) .

Small litters' means: presence of significant differences between groups.

The *E.coli* colonies counts for all Experimental groups (Table 2) were all negative to *E.coli* culture .The fifth treatment showed higher bacterial count of *E.coli* 11.3×10⁶ cell\ ml which mean is an acute pathological case (Coles, 1974), while the same table showed eggs' surfaces *E.coli* Counts decreases from T₁ to T₄. But the fifth treatment showed The highest count for *E.coli* too, that because the CLC reduce numbers of *E.coli* by its role as an excellent active antibacterial.

Experimental groups %	Reproductive periods(wks)					Significance Level
	49-52	53- 56	57- 60	61- 64	Mean	
T ₁ (0.0)	A81.91c ±77.64	B73.87 c ± 6.3	C67.43 d ± 6.83	D 66.83 d ± 6.67	72.51 d ± 2.98	**
T ₂ (0.25)	A84.86 b ± 2.19	C81.11 b ± 8.54	B82.03 b ± 8.54	D80.04 b ± 8.35	82.04 b ± 2.85	*
T ₃ (0.50)	A87.12 a ± 1.2	B86.03 a ± 3.38	C84.77 a ± 3.38	D81.01 a ± 2.99	84.73 a ± 4.82	**
T ₄ (0.50)	A85.60 b ±0.028	B81.46 b ± 8.41	C 78.45 c ± 8.41	D76.77 c ± 8.71	80.57 c ± 3.44	**

Significance Level	**	*	**	**	**	
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Table 3. The effect of inject oily CLC to layers chickens (*Lohman brown*) in the mean of hen day production %(H.D %) during reproductive periods 49- 64 wks. of chicken.

Small litters' means: presence of significant differences between groups.

Captile litters' means: presence of significant differences between different reproductive periods.

* Presence of significant differences at ($P \leq 0.05$) probability level

** Presence of significant differences at ($P \leq 0.01$) probability level

Table (3) shows that there was significant differences in (H.D. %) between all treatments and Reproductive periods, T₃ (0.50%) were recorded the highest values in all Reproductive periods, then T₂ (0.25%), T₄ and T₁ respectively. The means when statistically analyzed showed significant differences, T₃ (0.50%) also showed significant increases that followed by T₂, T₄ and T₁.

The reproductive periods revealed significant increases in, T₃ (0.50%) followed by T₂ (0.25%), T₄ and T₁ respectively. CLC as antibacterial agent and increase the immunity of chickens which confirmed the healthy state of the chicken that showed a significant superiority in egg production. Sturkie (1986) mentioned that steroids hormones increase the metabolism that increase proteins formation and decrease proteolysis (Kumagi et. al., 1957). While Jeyaprakash and Chinnaswamy. (2005) mentioned that the administration of Cd disrupted the metabolism of Ca, Fe, Cu, Zn, Se ions and decrease their levels in the organisms, and that doesn't agreed with our results above, because CLC mode of action doesn't disrupt the metabolism of Ca, Fe, Cu, Zn, Se ions and they still in their normal ranges inside chickens body which is clear via their egg production as in figure (1) declared.

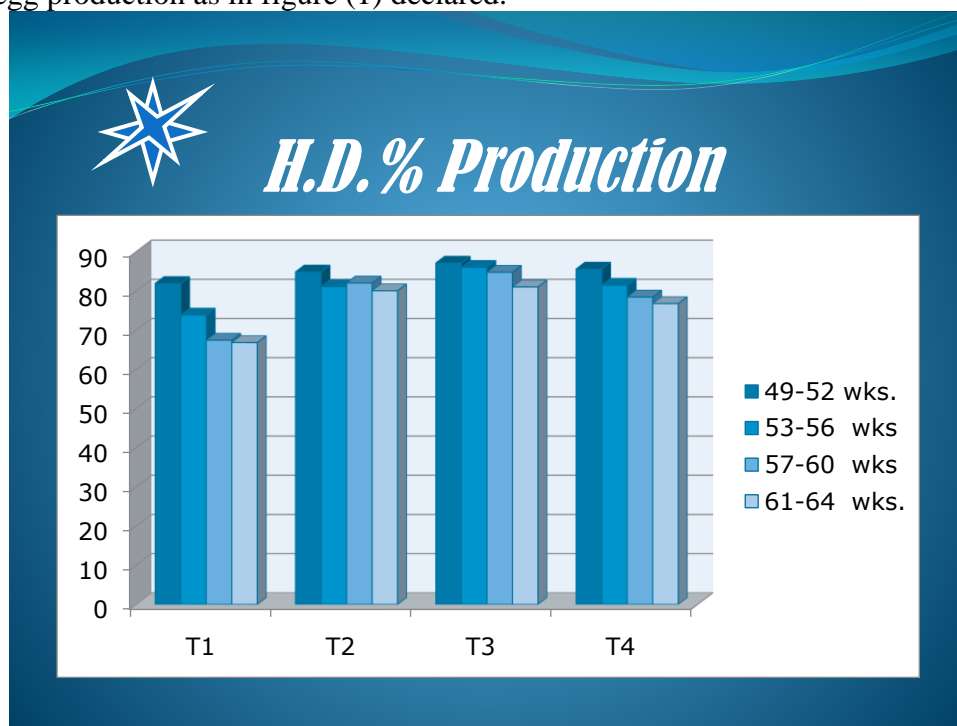


Figure 1. The effect of inject oily CLC to layers chickens (*Lohman brown*) in the mean of hen day production %(H.D %) during reproductive periods 49- 64 wks. of chicken .

Table 4 Concentration of cadmium in ashed eggs and liver.

Samples	Period of collection after injection/day	Samples numbers	Cadmium $\mu\text{g/ml}$
Egg	10	18	-0.027

Egg	30	18	0.006
Egg	85	18	-0.027
Liver	30	18	-0.027
Liver	85	18	-0.029

Results from table (5) mention that ashed livers and egg were empty from Cd, which is very important for human health. Besides , CLC started material is cheap and available which is cost 1.35\$/1gm, which is enough for 100 birds (Daived *et. al.*,1989) .We can conclude from present study that CLC at concentration (0.50% in T₃) can be used successfully to reduce the pathological changes during infection of *E.coli* in layers by using CLC as synthetic prophylactic antibacterial agent.

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تأثير حقن معقد الكاديوم الجديد و المصنع محليا في دجاج بيض المائدة (لوهمان البني) للسيطرة على التهاب المبايض وقناة البيض المتسبب عن الاصابة ببكتريا الايشريشيا القولونية ومعدل انتاج البيض اليومي

أريج عباس مجيد رواء عباس مجيد سناء عبدالحسن محمد.

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المستخلص

CLC مركب تركيبه الكيميائي $[Cd (LH)_2(N_3O)_2] \cdot H_2O$ ، تم اختياره مختبريا على زرع لبكتريا *E. coli* وكان قطر تشييطه للبكتريا 12 ملم (+ev+++)، حضر CLC بشكل محلول زيتي قابل للحقن اذ حقن تحت الجلد في منطقة الرقبة للدجاج البياض (لوهمان البني) وبمستويات مختلفة 0.01%، 0.25%، 3%، 0.50% (T₁: 15، T₄: 15) بينما تركت المعاملة الاولى بدون حقن و عدت معاملة مقارنة . وبعد خمسة أيام جرع القطيع جرثيم *E. coli* مع ماء الشرب بتركيز 8.500×10^6 خلية/مل (عالية الأمراض) كمسبب مرضي لأنهاب قناة البيض والمبايض. وبعد خمسة أيام أخرى أخذت مسحات مباشرة من مبايض الدجاج المصاب. وزرعت على الوسط الزراعي المكوني الاحمر (وسط زرع تفريقي لتشخيص جرثيم *E. coli*)، كما اجريت على هذه المسحات الفحوصات الكيموحيوية وهما فحصي (Indole & Methylene Red). ولما اظهرت النتائج مقاومة الدجاج للاصابة ببكتريا الايشريشيا القولونية طول فترة التجربة التي استمرت أربعة أشهر، حيث اظهرت النتائج انخفاض واضح في اعداد جرثيم الـ *E. coli* في طيور المعاملات الاربعة مقارنة بمجموعة السيطرة T₁، بينما اظهرت T₅ (هذه المجموعة لم تحقن بمادة CLC ولكنها جرعت ببكتريا الـ *E. coli* حيث بدأت العلامات المرضية بالظهور وشخصت اصابة التهاب المبايض من خلال اجراء التشريح المرضي و لوحظ تجمع السوائل الالتهابية في التجويف البطني، تجبن المبايض وضمور قناة البيض التي تعتبر من العلامات الرئيسية لأنهاب قناة البيض والمبايض. ان فعالية CLC جاءت متوافقة مع انخفاض اعداد بكتريا الـ *E. coli* في مسحات البيض، قناة البيض والمبايض في جميع التراكيز لـ CLC وافضلها كانت المعاملة الثالثة (0.50% CLC) بينما ظهر انخفاض معنوي في معدل انتاج الدجاج (H.D. %) في كل المعاملات نتيجة استحداث الاصابة و اقل قيمة لنزول انتاج البيض للطيور كان في (0.50%) T₃ تلتها T₂، T₄ واخيرا T₁. عززت هذه النتائج بنتائج استخدام جهاز الامتصاص الذري اللهبى لمعرفة نسبة الكاديوم المتبقي في الكبد والبيض المرمدين حيث اثبت الفحص خلوهما من الكاديوم تماما.

اثبتت النتائج اعلاه أن CLC ناجح و آمن لأستخدامه كمادة دوائية مصنعة للوقاية من الاصابة ببكتريا *E. coli* خلال فترة الانتاج للدجاج البياض إضافة الى رخص ثمنه.