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STUDY OF IMMUNE RESPONSE IN BROILER CHICKS VACCINATED AGAINST INFECTIOUS BRONCHITIS IN CONCURRENCE WITH THE USE OF DEXAMETHASONE

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ABSTRACT

Dexamethasone is one of the strong synthetic steroids. It has a multipotent effect on the body's cells and tissues, including the immune system, as it induces immune suppression, but sometimes can modulate the immune response, which is still vague and unclear. Although the poverty of studies about their action when administered in parallel with the vaccination program. We aimed to use poultry as a model to study the effect of dexamethasone administration in parallel with the infectious bronchitis vaccination regime. Sixty one-day-old chicks were divided into four groups; the first group was treated with a full dose of dexamethasone ED100 with infectious bronchitis vaccine, while the second group was treated with ED50 of the drug with IB vaccination, whereas the third group was used as a positive control treated with the IB vaccine, and the fourth group was used as a negative control kept without vaccination. The ELISA test was used to detect antibodies against infectious bronchitis as a result of vaccination, and levels of CD4 and CD8 T-cells as indications for the effect of dexamethasone on vaccination. Results showed that the low dose of dexamethasone (ED50) provided a significant increase in antibodies against infectious bronchitis, with an increase in the levels of CD4 and CD8 compared to the others. We conclude that a low dose of dexamethasone may positively affect the immune response to the vaccination program when administered with the infectious bronchitis vaccine.

Keywords: Dexamethasone, IB vaccine, CD4 and CD8

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INTRODUCTION

Synthetic corticosteroids, such as dexamethasone, are very important medications for many diseases, such as anti-inflammatory and analgesic drugs (Ciobotaru *et al*., 2019). Glucocorticoids can modulate immune response *in vivo* and release cytokines from stimulated blood mononuclear cells *in vitro* (Schuld *et al*., 2006). It can regulate immune response either by inhibiting or activating anti-inflammatory and pro-inflammatory cytokines; more studies suggested that glucocorticoids have potent anti-inflammatory effects (Borghetti *et al*., 2009; Coutinho and Chapman, 2011; Ayroldi *et al*., 2012). Dexamethasone has many side effects that depend strictly on its used dose and duration. So their immunosuppressive side effect can be exaggerated if the medication is used by supraphysiological doses for long periods, and it should be used carefully in such conditions, bearing in mind their risk versus benefit (Garber *et al*., 1981; Laan et al., 1993). Taking into consideration their dose and duration, which are variable depending on their indication (Yokoyama *et al*., 1992). Short-period use of dexamethasone leads to mild and minimal side effects, contrary to their use for long periods, which leads to serious and undesirable side effects (Buchman, 2001). Dexamethasone at doses of 3, 5, and 7 mg/kg has side effects, as the alteration in the morphology of broiler lymphoid organs (Bursa of Fabricius, thymus, and spleen) leads to severe immunosuppression associated with a high mortality rate (Islam *et al*., 2023). The molecular mechanism of dexamethasone causes immunosuppression through many mechanisms, such as suppressing phagocytic activities and blocking B cells and T cells (Engler and Stefanski, 2003). Furthermore, dexamethasone drugs caused granulocytopenia in humans, lymphopenia and neutrophilia in rats and pigs, and neutrophilic-eosinophilic lymphopenia in cattle (Aengwanich, 2007). After administration of dexamethasone to broilers, a depletion of white blood cells and lower immune organ relative weight were reported (Aengwanich, 2007; Vicuña *et al*., 2015), which is related to the ability of dexamethasone to increase stress and trigger apoptosis in the spleen and thymus tissue (Ayroldi *et al*., 2007). Infectious bronchitis is one of the highly contagious diseases that threaten the poultry industry, causing economic loss. It is caused by the avian coronavirus, the infectious bronchitis virus (IBV) (Jackwood and de Wit, 2013). The disease can be transmitted easily by direct contact with the upper respiratory secretions of diseased birds and/or by indirect pathways through exposure to the faeces of infected birds and even by fomites and vectors (Ignjatović and Sapats, 2000). The active way to prevent the disease was by vaccination, in addition to biosecurity (Guzmán and Hidalgo, 2020). Live attenuated vaccines are considered one of the successful vaccines due to their role, both in humoral and cell-mediated immunity (Guzmán and Hidalgo, 2020). This study was targeted to estimate the effect of the full and half doses of dexamethasone on the immune response resulting after vaccination against IB in the broiler.

MATERIAL AND METHODS

Ethical approvement: All experiments were done according to the ethical approval provided by the Institutional Animal Care and Use Committee/College of Veterinary Medicine, University of Mosul, with ref. No. UM.VET.2023.108

First: chicks

Sixty broiler chicks (60) aged one day old (Rose 308) were purchased from the Baashiqa hatchery and raised in animal houses in the College of Veterinary Medicine/University of Mosul, Iraq, under stick temperature conditions, a light program, and air purification, fed by concentrated pilot feed supplied by Erbil feed No. 1, which was provided until the age of 20 days. Then it was changed to pilot No. 2 until the end of the

experiment and drinking water was continuously supplemented (Al-kalo and Al-Aalim, 2024).

Second:

A- The vaccine

A live attenuated vaccine against infectious bronchitis disease (IB) under the commercial name Bio IB (strain H 120, Meilan Biological Engineering Co. Ltd / China) was used in the study.

B- Dexamethasone:

Bio dexamethasone type (Pharmavet® Company/Turkey) was used in the study. The recommended dose was 2 ml/50 kg body weight, and two concentrations were used: ED100 and ED50, and calculated by up-and-down techniques as follows:

The first dosage of dexamethasone was given at 2 mg/kg, IP. The chicks were assessed using an electro-stimulator (Harvard device, USA) before and after 30 minutes of treatment with the drug. The incidence of pain (as a measurable effect) was indicated by distress calls in the chick's model. The dosage then increased or decreased (depending on the outcome) by 0.5 mg/kg of the initial dosage administered according to the absence and presence of analgesia, respectively, then the ED50 can be calculated from the following equation:

ED50= xf + kd …….(1)

Were xf is the last dose of dexamethasone used; k is the table value of Dixon's Table and d is the amount of dose used for increased or decreased pattern so:

ED50= 1 + (-0.741) (0.5) …….(2)

ED50= 0.63 mg/kg, IP (the dose of dexamethasone producing its effect in 50% of the chicks)

The ED100 (which is the dose required for producing the effect in all animals) can then be estimated as doubling of ED50 as:

ED100=

2(ED50)…….(3)

0.63×2=1.26 mg/g, IP

(Sharma et al., 2023; Abdulhamid and Mousa, 2023)

Optimization of breeding conditions

Each group was placed in a separate room inside the hall, and the floor was spread with sawdust. The lighting and temperature were adjusted appropriately for the age of the chicks by using heaters. The hall was equipped with air extractors to maintain continuous air purification.

Third:

A- Experimental study

Sixty chicks were brought to the house of animals in the Veterinary Medicine College. The 60 chicks were divided into four equal groups of 15 chicks/group to estimate IB antibodies, CD4+, and CD8+ levels, and they were placed in separate rooms after providing suitable temperature and ventilation to the end of the experiment. The four groups were distributed as follows:

First group: They were vaccinated at 7 days of age with IB (strain H 120) via drinking water, and then treated with ED100 of dexamethasone for three days (Mousa *et a*l., 2019; Abdulhamid and Mousa, 2022; Mousa *et a*l., 2022). Five chicks were slaughtered at 14 days to estimate levels of specific antibodies against IB, in addition to CD4+ and CD8+ levels. At 14 days, they were vaccinated with a booster dose of the same vaccine, and then treated secondly with ED100 for another three days. Five chicks were slaughtered at the age of 21 days for the same purpose as above. The remaining 5 chicks were slaughtered at the age of 33 days to follow up on levels of each of the antibodies, CD4+ and CD8+ levels.

Second group: As in the first group, except they were treated with ED50 of dexamethasone (Mousa, 2021; Abdulhamid and Mousa, 2022; Jassim and Mousa, 2021).

Third group: Was used as a positive control group, which they have vaccinated with the IB (strain H 120) vaccine via drinking water as in the first group, without any treatment with dexamethasone during the period of the study.

Fourth group: It was kept as a negative control group that was not vaccinated nor treated with dexamethasone during the period of the study.

B- Blood collection

Blood was collected from the jugular vein, centrifuged, and the serum was separated. The collected sera were stored in deep freeze until use.

Fourth: ELISA test

A specific ELISA kit type was used to estimate specific antibodies against IB Catalog No: abx050271 (Abbexa LTD, UK), in addition to CD4+ and CD8+ T-cell levels estimated by the ELISA kit provided by ELK Biotechnology, USA (Catalog No: ELK7814, ELK7813) according to the manufacturer's instructions.

Statistical analysis

The GraphPad Prism 8 statistical program was used to analyze the result and estimate all significant differences between groups of our study. The two-way ANOVA was used to compare all data means of groups, and all results were given as means ± standard errors (the graphs and significant values were drawn by using the same program).

RESULTS

1- Concentration of antibodies against IB

The results showed that the concentration of antibodies against IB increased significantly as a result of vaccination in each of the ED50, ED100, and positive control groups compared to the negative control group at 7, 14, and 21 days, respectively. While comparing the treated groups themselves, it was found that the concentration of antibodies against IB was increased significantly in the ED50 group compared to each of the ED100 and positive control groups at 7 and 21 days only, while at 14 days the result was somewhat different; the concentration of antibodies against IB was increased significantly in each of the ED50 and positive control groups compared to the ED100 group, as shown in Table (1) and Figure (1).

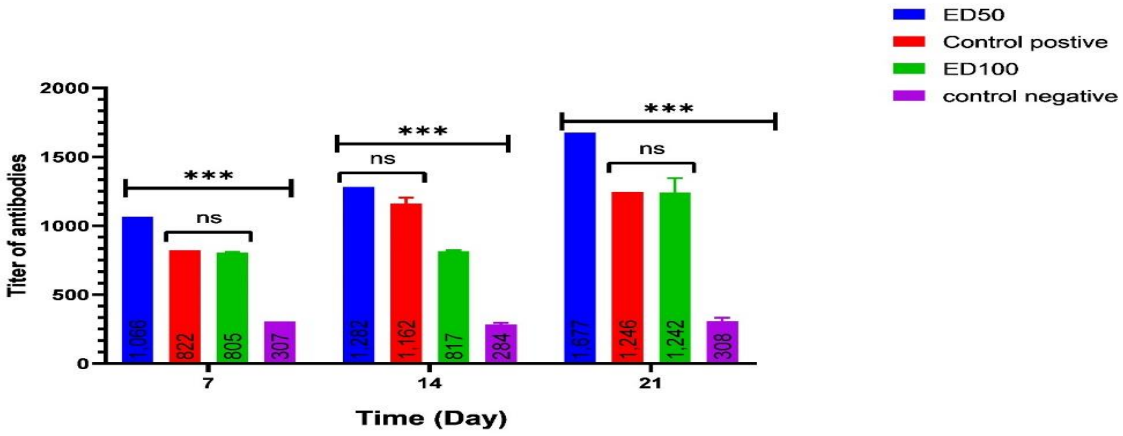

Figure (1): Concentration of antibodies against IB

Table 1: Concentration of antibodies against IB.

Groups/Abs	7 days	14 days	21 days
ED50	1066 c	1282 ac	1677 c
ED100	805.3 ad	816.7 d	1242 ad
Control positive	822.0 ad	1162 aa	1246 ad
Control negative	370.0 b	284 B	307.7 c

The small different letters mean significant differences in the column at P<0.05.

2- Concentration of CD4 cells

The results showed that the concentration of CD4 cells was increased significantly in the ED50, ED100, and positive control groups compared to the negative control group at 7, 14, and 21 days, respectively. While comparing the treated groups themselves, it was found that the concentration of CD4 cells was increased significantly in the ED50 group in comparison to the ED100 and positive control groups at 7, 14, and 21 days, respectively, as shown in Table (2) and Figure (2).

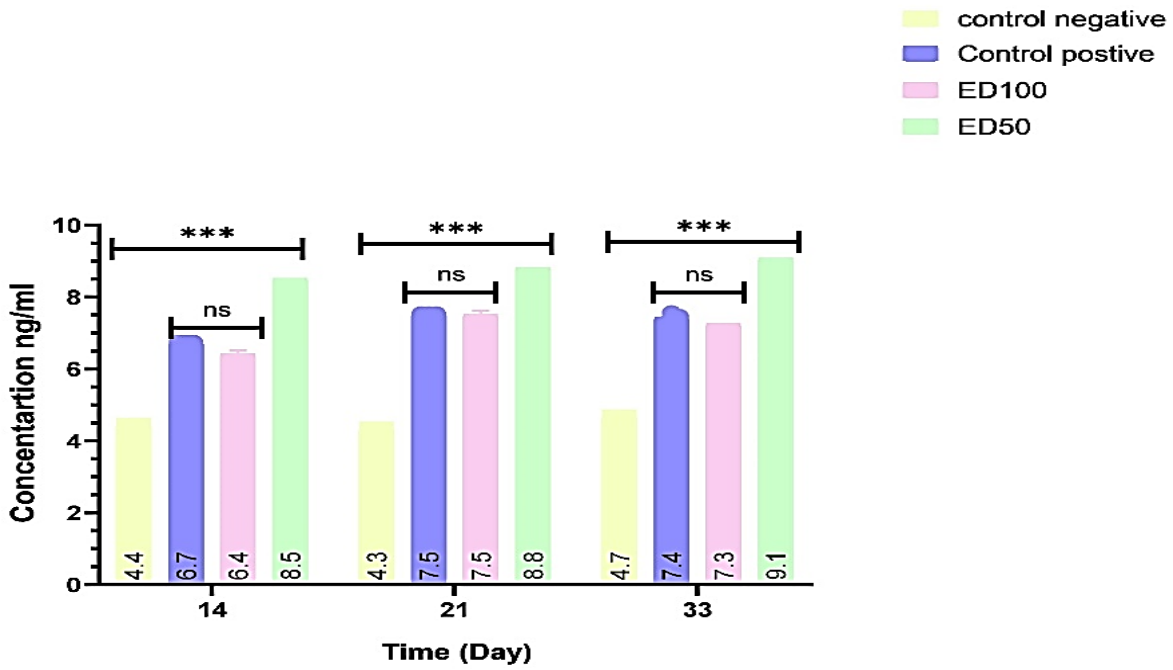

Figure (2): Concentration of CD4 level in ng/ml

Table 2: Concentration of CD4 level in ng/ml

Groups/ CD8+ conc. /days	14 days	21 days	33 days
ED50	8.523 c	8.817 c	9.088 c
ED100	6.409 ad	7.517 ad	7.263 ad
Control positive	6.745 ad	7.537 ad	7.437 ad
Control negative	4.434 b	4.331 b	4.671 b

Small different letters mean the presence of significant differences in the column at $P < 0.05$

3- Concentration of CD8 cells

The concentration of CD8 cells was increased significantly in the ED50, ED100, and positive control groups compared to the negative control group at 7, 14, and 21 days, respectively. While comparing the treated groups themselves, it was found that the concentration of CD8 cells was increased significantly in the ED50 group in comparison to the ED100 and positive control groups at 7, 14, and 21 days, respectively, as shown in Table (3) and Figure (3).

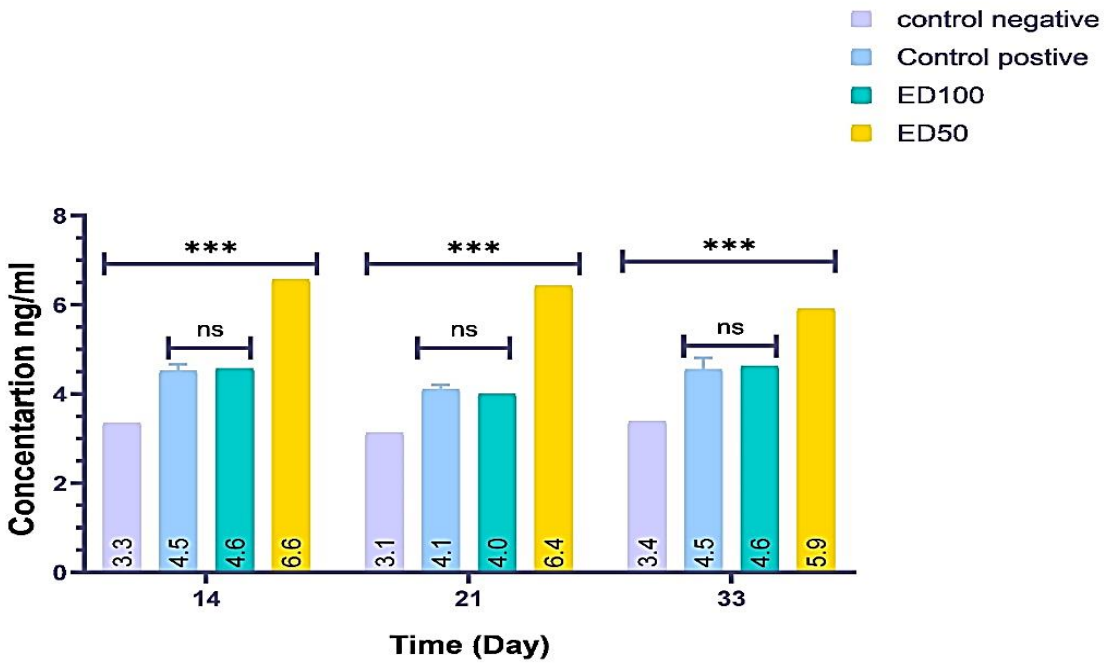

Figure (3): Concentration of CD8 level in ng/ml

Table 3: Concentration of CD8 level in ng/ml

Groups/CD4+ conc. /days	14 days	21 days	33 days
ED50	6.562 c	6.419 c	5.905 c
ED100	4.566 ad	3.997 ad	4.623 ad
Control positive	4.516 ad	4.098 ad	4.546 ad
Control negative	3.345 b	3.124 b	3.380 b

Small different letters mean the presence of significant differences in the column at $P < 0.05$

DISCUSSION

Dexamethasone is one of the potent synthetic corticosteroids. It has multiple uses, such as anti-inflammatory and treatment of asthma, hypersensitivity, and allergy, and others, in addition to their main effect on the immune system (Noreen *et al*., 2021). While numerous studies highlight their immune-suppressive effects, making them a drug of choice for treating certain immunological disorders requiring immune suppression, some research also suggests they may have immune-modulating properties. It is unclear how it acts as an immunosuppressant and has some positive effects on some components of the immune system, simultaneously. A study conducted by Bain *et al*. (2018) revealed their immunosuppression and immune-activating effect at the same time. There are rare studies about their effect on vaccination programs, while using it concomitantly with vaccination, and how it can act if administered in parallel with the vaccine. In our study, we concluded that elevation of specific antibodies against IB with a low dose ED50 of dexamethasone was administered in addition to elevation levels of each of CD4 and CD8 T-cells, indicating their positive effect of low doses in the vaccination program, while using a full dose ED100 gave altered results with subtle changes in the above parameters compared to the ED50 concentration. This conclusion was still vague on how drugs can do this action. However, several effects of dexamethasone, such as minimum dose, anti-inflammatory, innate immunity, and cytokine-releasing

effects, may play an important role in this case. A manuscript published in 2006 by Schuld *et al*. used a low dose of dexamethasone in a study that concluded that sTNF-R p55 was increased while TNF-α and sTNF-R p75 were decreased at the same time, indicating that their modulation of cytokine release and stimulating some elements of the immune system in low doses. A study conducted by Ambwani *et al.* (2023) on chicken splenocyte culture observed a significant increase in IL-10 conc. which plays a key role in the anti-inflammatory effect and elicits an adaptive immune response, in addition to upregulation of NF-κB that elicits both innate and acquired immunity, indicating that it has an immune-modulating effect in parallel to its immune suppression effect. Our findings align with those of Anderson et al. (1999), who observed an increase in CD4+ T-cell levels accompanied by an elevation in B cells, potentially indicating stimulation of the humoral response and antibody production. However, our study also noted a decrease in CD8+ T-cell levels, which could be attributed to the absence of cell-mediated immunity (CMI). Also, another study (Giles *et al*., 2018) concluded that dexamethasone led to an increasing expansion index of CD4+ and CD8+ T-cells, in addition to the upregulation of mRNA of CTLA-4 that prevented some immune suppression effects of dexamethasone. Also, we agreed with Kannan *et al*. (2021), who revealed increasing levels of CD4+ and CD8+ T-cells in COVID-19 patients treated with dexamethasone and remdesivir. Corticosteroids are still controversial medications and need more recent studies to understand their impact in many fields, among them using it with vaccination programs to expand horizons in the future for this effect.

CONCLUSION

It was concluded that a low dose of dexamethasone may have a positive effect on immune response to the vaccination program against IB in poultry, which was used as a model when administered parallel to the IB vaccine. While the full dose ED100 was presented the same results as the positive control group.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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دراسة الاستجابة المناعية في دجاج التسمين المطعمة ضد مرض التهاب الشعب الهوائية المتزامن مع استخدام عقار الديكساميثازون

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ديكساميثازون هو أحد المنشطات الاصطناعية القوية. له تأثير متعدد القدرات على خلايا وأنسجة الجسم، بما في ذلك الجهاز المناعي، حيث أنه يحفز كبت المناعة ولكن في بعض الأحيان يمكن أن يعدل من الاستجابة المناعية، والتي لا تزال غامضة وغير واضحة. على الرغم من ضعف الدراسات حول تأثيرها عند تناولها بالتوازي مع برنامج التطعيم. نحن نهدف إلى استخدام الدواجن كنموذج لدراسة تأثير إعطاء الديكساميثازون بالتوازي مع نظام التطعيم ضد التهاب الشعب الهوائية المعدية. تم تقسيم ستين كتكوتاً بعمر يوم واحد إلى أربع مجموعات؛ عولجت المجموعة الأولى بجرعة كاملة من الديكساميثازون ED100 بلقاح التهاب الشعب الهوائية المعدي، بينما عولجت المجموعة الثانية بجرعة ED50 من الدواء بتطعيم IB، بينما استخدمت المجموعة الثالثة كمجموعة سيطرة موجبة عولجت بلقاح IB، وتم علاج المجموعة الرابعة استخدمت كمجموعة سيطرة سلبية بدون تطعيم. تم استخدام اختبار ELISA للكشف عن الأجسام المضادة ضد التهاب الشعب الهوائية المعدية نتيجة التطعيم ومستويات الخلايا التائية CD4 وCD8 كمؤشرات لتأثير الديكساميثازون على التطعيم. أظهرت النتائج أن الجرعة المنخفضة من الديكساميثازون (ED50) قدمت زيادة معنوية في الأجسام المضادة ضد التهاب الشعب الهوائية المعدية مع زيادة في مستويات CD4 وCD8 مقارنة بالآخرين. نستنتج أن جرعة منخفضة من ديكساميثازون قد تؤثر إيجابيا على الاستجابة المناعية لبرنامج التطعيم عندما تدار مع لقاح التهاب الشعب الهوائية المعدية.