

EVALUATION OF ADDING DIFFERENT CONCENTRATIONS FROM CO-ENZYME Q10 ON IMMUNIZATION WITH CLASSICAL AND RECOMBINANT VACCINE AGAINST INFECTIOUS BURSAL DISEASE IN BROILERS

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ABSTRACT

This study was aimed to detect the effect of co-enzyme Q10 against infectious bursal disease vaccinated with different programs (recombinant HVT-IBD single dose at one day old, classical vaccines D78 at eight days old, and E228 at 16 days old) through the evaluation of levels of interferon-gamma (INF- γ) and tumor necrosis factor-alpha (TNF- α) in serum. Two hundred and ten broilers were divided into seven groups, with 30 chicks in each group. T1, feed Q10 (20 mg/kg diet) and vaccinated by (D78, E288); T2: feed Q10 (20 mg/kg diet) and vaccinated by (rHVT); T3: feed Q10 (40 mg/kg diet) and vaccinated by (D78, E288); T4; feed Q10 (40 mg/kg) and vaccinated by (rHVT); T5; vaccinated (D78, E288) only; T6; vaccinated (rHVT) only; T7; negative control. The results of the titers of interferon-gamma (INF- γ) on day 15 showed that T6 and T5 were significantly ($P \leq 0.05$) higher compared with other groups. In contrast, on days 25 and 33, they showed a decline throughout the experiment. At the same time, tumor necrosis factor-alpha (TNF- α) results indicated that T1 and T7 recorded a lower level than other groups at day 11. In comparison, on days 19 and 25, T1, T2, and T7 had the lowest titers among the different groups. In conclusion, the co-enzyme Q10 (40 mg/kg feed) enhances immunity function by modulating the immune response, especially (INF- γ), and decreasing pro-inflammatory markers (TNF- α).

Keyword: vaccines of Gumboro, immune response, broilers

* Part of M. Sc. Thesis of the 1st author.



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INTRODUCTION

Broiler hens are susceptible to infectious bursal disease (IBD) or Gumboro disease. The illness is both highly contagious and immunosuppressive. A new genetic variation of the virus has emerged, and the world's chicken industry has suffered heavy financial losses (Adino *et al* 2022). The disease is very infectious and spreads quickly; IBD is caused by the IBD virus (IBDV). The characteristic signs are lesions in the fabric of the bursa (FB) and atrophy of the FB; immunosuppression occurs in birds aged three weeks to 3 months (Jumaa *et al*, 2020, Orakpoghenor *et al*, 2020, Ulaiwi, 2015). As IBDV is contagious, the virus is transmissible in many ways, and the

standard way is by contact with ill birds or even with infected fomites (Zhang *et al*, 2022). Decisive biosecurity measures may limit the spread of the illness (Dey *et al*, 2019, Etteradossi & Saif. 2013, Kajal *et al*, 2023). Bursal Disease Virus (IBDV) may be efficiently prevented and managed with attenuated live vaccines, provided they are delivered at the committed intervals. However, most of these live vaccinations may cause the bursa of Fabricius to atrophy during replication, which can cause moderate to severe lesions due to a brief drop in lymphocytes. The possibility that maternally derived antibodies (MDAs) could neutralize the vaccines (Kassab & Muhannad. 2006,

Olesen *et al*,2018, Tsukamoto *et al*,1995, Van den & Meulemans,1991). While MDAs interfere with virus reproduction during immunization, the vaccinated broilers may not be defended from virulent or very virulent IBDV (vvIBDV) strains on the farm. To solve this problem, immunization consisting of strains at different attenuation levels has been enveloped and highly used according to broad MDA levels in different poultry fields. Although these boosters control Gumboro infection, another concern was the possible risk of infection by other pathogens because of the moderate to severe lesions that come with bursa atrophy. Recombinant turkey herpesvirus (rHVT) vaccines are a potential alternative to weakened live IBDV vaccinations. The rHVT-IBD vaccine was born from introducing the VP2 protein from IBDV into the HVT genome. (Bublot *et al*,2007, Prandini, 2009). The VP2 protein from rHVT triggers an antibody response that neutralizes IBDV (Lemiere *et al*,2011). Unlike attenuated IBDV live vaccines, the rHVT-IBD immunization does not damage the bursa, making it a safer alternative; the likelihood that IBDV maternal antibodies will have any effect on this vaccination virus is also low. rHVT-IBD may, therefore, resolve the efficacy and safety issues linked to IBDV vaccinations (Camilotti *et al*,2016, Gelb Jr *et al*,2016, Reddy *et al*,2024, Sedeik *et al*,2019). Coenzyme Q10, or ubiquinone, is a vitamin-like substance mainly found in mitochondria and a lipid-soluble molecule. In contrast, it has a significant role in producing adenosine triphosphate ATP, where it acts as an electron carrier in the electron transport chain (ETC). (Abdulidha *et al*,2020, Lenaz *et al* ,2007, Littarru *et al*,2017, Rauchova *et al*,1995). Coenzyme Q10 (CoQ10) is an exceptional fat-soluble antioxidant that may significantly reduce the damage from oxidative stress by scavenging free radicals (Littarru &Tiano,2007, Masoumi *et al* ,2021, Noh *et al*,2013). Not only does coenzyme Q10 directly neutralize free radicals, but it can also protect antioxidants like vitamin C (ascorbate) and vitamin E (a-tocopherol) (Gvozdjaková *et al*,2015). Coenzyme Q10 is crucial for

mitochondrial immune system maintenance and acts as an immune modulator and non-specific stimulant of the host immunological defense system (El Basuini *et al*,2020). in addition to preventing tumor growth and lowering blood levels of tumor necrosis factor- α (TNF- α) and interleukin-6, taking a CoQ10 supplement enhances the immune response to different viruses (31). Immunological suppression associated with aging and chronic diseases may be curable with CoQ10 treatment and a significant increase in serum immunoglobulin G (IgG) levels (Bliznakov,1978, Kalantar *et al*,2019). The objective of the present study was to investigate the effect of the dietary supplement Coenzyme Q10 as an antioxidant, body performance, and immunomodulatory on different vaccine programs against infectious Bursal disease.

MATERIALS AND METHODS

Ethical approval: The study was done under decree of the College of Veterinary Medicine of Baghdad's animal ethics committee. The Veterinary Medicine College Scientific Committee of the University of Baghdad's Department of Pathology and Poultry Disease reviewed and approved the study's protocol.

Experimental design: Two hundred ten (210) one-day old broiler chicks (Ross-308) multi sexes brought under the optimum condition with feed (ad libitum) and lighting program (24hr) period five weeks (35 days old) in the animal house of the college of veterinary medicine - university of Baghdad. The chickens divide into six random groups as follows :

T1: Thirty chicks supplement with (CoQ10) (UK®) 20mg/kg diet in all periods with Classical vaccinal program (D78 at 8th days old & E 228 at 16th days old / drinking water) (MSD, US) with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at (15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively (Ulaiwi, 2015).

T2: Thirty chicks supplement with (CoQ10) (UK®) 20mg/kg diet in all periods with Recombinant vaccinal program (Poulvac®

Procerta® HVT-IBD at 1st days old single dose s/c in.) (MSD –USA with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at (15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively.

T3: Thirty chicks supplement with (CoQ10) (UK®) 40mg/kg diet in all periods with Classical vaccinal program (D78 at 8th days old & E 228 at 16th days old / drinking water) (MSD, US) with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at (15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively.

T4: Thirty chicks supplement with (CoQ10) (UK®) 40mg/kg diet in all periods with Recombinant vaccinal program (Poulvac® Procerta® HVT-IBD at 1st days old single dose s/c in.) (MSD-USA) with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at (15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively.

T5: Thirty chicks without supplements were given a classical vaccination program (D78 at 8th days old & E 228 at 16th days old / drinking water) (MSD, US) with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at (15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively.

T6: Thirty chicks with no supplement given Recombinant vaccinal program (Poulvac® Procerta® HVT-IBD at 1st days old single dose s/c in.) (MSD-USA) with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at (15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively.

T7: Thirty chicks with no supplement or vaccination (control negative) with measurement of cellular (IFN γ) immune response and pro-inflammatory response (TNF- α) by ELISA Kit (5 serum samples) at

(15th, 25th, and 33rd days old) and (11th, 19th, and 25th days old), respectively.

Statistical analysis

The Statistical Analysis System (SAS, 2018) program was used to detect the effect of different factors on study parameters. The least significant difference (LSD) was used to compare the means (ANOVA/Two-way and ANOVA/One-way) significantly in this study (Cary, 2012).

RESULTS AND DISCUSSION

Determination of interferon-gamma (INF- γ)

level in serum: The titers of Interferon-gamma showed that on the day (15) the levels in T6, T4, and T5 were the highest (18.212, 17.183, and 16.014), respectively, followed by T3, T1, and T2 (13.457, 12.556 and 11.382), While T7 recorded the smaller percentage (9.223) with statically high significance ($P \leq 0.05$). On day 25, The results were the same as on day (15) with not a big difference; T6 and T5 (17.542, 15.551), respectively, were higher than T4, T3, and T1 (12.989, 12.499, and 10.045) with statically high significant ($P \leq 0.05$), whereas T2 and T7 recorded the lowest percentage (9.432 and 9.008). On the other hand, day ,T5 and T6 remained at high levels all over the period (16.991 and 15.814), followed by T4, T2, T3, T1 (9.002, 8.938, 8.782, and 7.313) respectively. At the same time, T7 was lower than other groups (5.619) with statically high significance ($P \leq 0.05$) as in Table (1): These results were accepted by Slah and other researchers, who proved that Q10 has anti-inflammatory properties by reducing the production of cytokines promoting inflammation, such as Interleukin in T4, T3, T2, and T1 (Saleh *et al*, 2017). Additionally, the supplementation with Q10 was determined to be an inflammatory cytokine (18). On the other hand, vaccinated broiler with Recombinant vaccine (HTV- IBD) and classical vaccine (D78 and E228), especially in groups T6 and T5 that had not been treated with co-enzyme Q10, could see that titers were higher than other groups on the same time. This result has been accepted by other researchers who explain that The cell-mediated immune responses partially IFN- γ induction against IBVDV with a peak during

three weeks post-vaccination were observed in broilers vaccinated with both attenuated live vaccines and rHVT-IBD via the subcutaneous

and intraocular route (Ingrao *et al*,2017, Jakka *et al*,2014).

Table 1. Effect of groups and period in Result of INF- γ

Groups	Mean \pm SE of INF- γ (ng/L)		
	15 days	25 days	33 days
T1	12.556 \pm 0.74 C a	10.045 \pm 0.62 C ab	7.313 \pm 0.32 BC b
T2	11.382 \pm 0.57 CD a	9.432 \pm 0.45 C a	8.938 \pm 0.45 BC
T3	13.457 \pm 0.75 BC a	12.499 \pm 0.71 BC ab	8.782 \pm 0.52 BC b
T4	17.183 \pm 0.92 A a	12.989 \pm 0.66 BC b	9.002 \pm 0.58 B c
T5	16.014 \pm 0.87 A a	15.551 \pm 0.80 AB a	15.814 \pm 0.75 A a
T6	18.121 \pm 0.90 A a	17.542 \pm 0.87 A a	16.991 \pm 0.91 A a
T7	9.223 \pm 0.67 D	9.008 \pm 0.75 D	5.619 \pm 0.56 C

LSD: 3.877 *

Means having with the different big letters in same column and small letters in same row differed significantly. * ($P \leq 0.05$).

Determination of TNF alpha level in serum:

Table 2 shows that all groups at (11, 19, and 25) days old recorded highly significant differences at the level ($P \leq 0.05$) of TNF- α titer in serum against Infectious bursal disease Eliza Results of TNF- α at 11 days showed that T6 and T5 (31.538 and 29.104) respectively and followed by T4, T3 and T2 (28.921, 28.096 and 28.095) while T1 and T7 were recorded the lowest percentage (25.614 and 23.848). On day 19, the results were similar to day 11, with a little decline. On day 25, T5 and T6 had a high percentage (28.633 and 28.057) compared with other groups, followed by T3, T4, and T1 (22.376, 21.611, and 21.601), respectively. At the same time, T2 and T7 were recorded as the lowest Titers (20.099 and 19.8760) as in Table (2). These results agreed with researchers who revealed that taking a CoQ10 had several positive effects; some of these effects include preventing tumor growth,

decreasing blood level of tumor necrosis factor- α (TNF- α) and interleukin-6, and enhancing the immunological response to viral infection in different types of animals used for experimental (Liu *et al*,2015, Salary *et al*,2014, Tanner,1992). Beyond that, the defense mechanism of quails CoQ10 has been the subject of several investigations. Research conducted by Rafieian-Naeini *et al*. found that quails reduced the expression of IL-6 and serum TNF- α , in addition to a drop in liver ions (Rafieian-Naeini *et al*,2023,Bayril *et al*,2018) discovered that CoQ10 exhibited anti-inflammatory properties in avians related to cold stress by constraining nitric oxide, tumor necrosis factor-a (TNF-a), IL-1b, and mitogenic protein kinases, which may indicate that CoQ10 might be an effective anti-inflammatory drug (Bayril *et al*,2018, Zahrooni *et al*,2019).

Table 2. Effect of groups and period in Result of TNF alpha

Groups	Mean \pm SE of TNF- α (ng/L)		
	11 days	19 days	25 days
T1	25.614 \pm 1.35 CD a	23.233 \pm 1.29 B a	21.601 \pm 1.27 B a
T2	27.095 \pm 1.52 BCD a	20.247 \pm 1.07 B b	19.876 \pm 0.93 B b
T3	27.096 \pm 1.39 BCD a	24.202 \pm 1.36 B ab	22.376 \pm 1.21 B b
T4	28.921 \pm 2.07 ABC a	24.977 \pm 1.40 B ab	21.611 \pm 1.06 B b
T5	29.104 \pm 2.19 AB a	29.211 \pm 2.54 A a	28.633 \pm 2.15 A a
T6	31.655 \pm 2.64 A a	30.976 \pm 2.60 A a	28.057 \pm 1.86 A a
T7	23.848 \pm 1.24 D a	20.688 \pm 1.06 B a	20.099 \pm 0.97 B a

LSD: 4.518 *

Means having with the different big letters in same column and small letters in same row differed significantly. * ($P \leq 0.05$).

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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The authors declare that they have not received a fund.

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تقييم اضافة تراكيز مختلفة من المرفق الانزيمي Q10 في التحصين باللقاحات الكلاسيكي والمعاد توليفة ضد مرض الجراب

المعدي في فروج اللحم

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المستخلص

هدفت هذه الدراسة إلى تحديد تأثير المساعد الانزيمي - Q10 ضد مرض الجراب الفيروسي المعدي بعد التلقيح ببرامج مختلفة (الجرعة الفردية من اللقاح المعدل وراثياً HVT-IBD في اليوم الأول، واللقاحات الكلاسيكية D78 في اليوم الثامن، وE228 في اليوم السادس عشر) من خلال تقييم مستويات الإنترفيرون-غاما وعامل النخر الورمي-ألفا في المصل. تم تقسيم 210 من افراخ اللحم إلى سبع مجاميع وكل مجموعة تحتوي على 30 فرخاً؛ T1: غذيت Q10 (20 ملغم / كغم علف) ولقحت ب (D78, E288)؛ T2: غذيت ب Q10 (20 ملغم / كغم علف) ولقحت ب (rHVT)؛ T3: غذيت ب Q10 (40 ملغم / كغم علف) ولقحت ب (D78, E288)؛ T4: غذيت ب Q10 (40 ملغم / كغم علف) ولقحت ب (rHVT)؛ T5: لقحت ب (E288, D78) فقط؛ T6: لقحت ب (rHVT) فقط؛ T7: مجموعة السيطرة السالبة. أظهرت نتائج تراكيز الإنترفيرون-غاما في اليوم 15 أن T6 و T5 سجلت مستوى أعلى وبشكل ملحوظ ($P \leq 0.05$) مقارنة بالمجموعات الأخرى. بالمقابل، في الأيام 25 و 33، أظهرتا انخفاضاً على طول التجربة. في الوقت نفسه، أشارت نتائج عامل نخر الورم-ألفا إلى أن T1 و T7 سجلتا مستوى منخفض بالمقارنة بالمجموعات الأخرى في اليوم 11. وبالمقارنة بالأيام 19 و 25 كانت T1 و T2 لديها أقل مستوى بين المجموعات المختلفة. في الختام، يعزز تأثير المساعد الانزيمي Q10 (40 ملغم / كغم) وظيفة المناعة عن طريق تنظيم الاستجابة المناعية وخاصة الإنترفيرون-غاما وتقليل عامل النخر الورمي-ألفا.

الكلمات المفتاحية: لقاحات الكمبورا ، الاستجابة المناعية، فروج اللحم.