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The Effect of Oils Additive on Some Immunological Cytokines Newcastle Disease-Vaccinated Broiler Chickens

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Abstract

THE poultry industry is the most important part of the human food supply because it is a rapid production process that supplies cheap sources of protein in food, so it is considered the most significant sector in the economy, especially in a growing country. This study aimed to investigate the changes that occur in the level of interleukin IL6, IL4, and interferon-γ after using flaxseed and Omega-3 oils in broilers vaccinated with Newcastle disease by using ELISA test as a method. The 240 one-day-old chicks were divided into eight groups and treated either with a Newcastle vaccine or by vaccine and 1% of flaxseed or/and omega 3 oils, blood was collected after 1,3,10,17,24, and 31 days post-vaccine. our results explain a significant increase in all immunological markers (interleukin IL6, IL4, and interferon-γ) especially in groups treated with oils and reaching higher concentration on the 17th and 24th day post vaccination. We conclude that both oils (flax seed oils and omega 3) have augmenter effects on interleukin response and can modulate broiler immunity to provide a good immune state after the vaccine with Newcastle which may give more protection against infection.

Keywords: Broiler, Oils, Interleukin 4 and 6, Interferon- γ, Newcastle vaccine.

Introduction

The Broiler was the most important part of the food industry and economic trade, which used to cover protein demand, especially in the growing country [1]. As many industries found the poultry industry suffers from many productional and management problems, and contentious infection with either bacteria, viruses, or fungi may cause serious financial losses, many efforts to control these infections were made yearly through vaccination and/or application of biosecurity programs in poultry heard, but either broiler or layers poultry farms still suffer from incidence of these diseases [2].

The positive Single strand ribonucleic acid (RNA) Newcastle virus causes Newcastle disease infection is one of the serious infections that affect broiler farms [3]; the infection with Newcastle disease is still considered a serious poultry industrial problem that causes high economic and financial losses around the world [4] due to its rapid infectious rate and increased morbidity and mortality in the infected broiler, the disease caused many respiratory, intestinal or even neurological sings but sometimes high death without any obvious clinical signs; the sings and mortality rates of Newcastle disease differ and related with infectious strain, viral dose, infection method, and finally age and immunological states of broiler [5].

Many strategies were adopted to control the Newcastle infection including improvement of the immune state of birds by vaccination or better management in broiler farms [6], but the virulent virus strain remains causes infection in endemic countries [7].

In many countries, vaccination is considered the first line of defense in preventing Newcastle disease infection and protecting birds from velogenic strain through increased bird humoral and cellular immune defense state [8]; also, the balanced feed was responsible for developing healthier and strong infectious resistance broiler herds [9], any change in feed composition affects the immunity, production, and performance of broilers [10].

Many types of research were done to increase the production and improve the immune state of broiler heard by modulation of feed by adding different types of immune stimulation or enhancer materials, and researchers focus on natural additives especially natural oils such as flax seed oils and omega 3 (fish

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oils), the flax seed oils come from the seed of the flax plant while Omega-3 may be supplemented from fish cod liver oil both of them consider as important sources of unsaturated fatty acid and are considered as important sources of linoleic, α-linoleic acid, and arachidonic acid which has a positive effect on birds' immunity [11]; farther that these oils consider as powerful antibacterial, antifungal and anti-oxidant materials [12,13]; some research approved the effect of using omega 3 in decreasing inflammation in birds [14], improving antibody production [15].

Gutiérrez et al. Proposed that these oils can mechanically modulate immune humoral and cell responses by producing some pro-resolving mediators including prostaglandins, Leukotrienes, protectins, thromboxanes, maresins, and resolvins which affect bird's immunity [16].

Very little study was done to estimate the effect of using natural oils on immunological cytokines so, our study aims to investigate the changes that occur in the level of interleukin IL6, IL4, and interferon-γ after using flax seed and omega 3 oils in broilers vaccinated with Newcastle disease.

Material and Methods

Vaccine:

Two types of Newcastle vaccine were used to immunize broilers first killed Newcastle vaccine which used in 0.1 ml subcutaneous method on day one; and the live attenuation Nobilis® ND Clone 30 Newcastle vaccine which was given with drinking water in different experimental times, each vaccine was supplied by MSD™ Company, Netherland.

Natural oils:

Both flax seed and Omega 3 (fish) oils were used in broiler feed as immune modulation and enhancers, the oils were used either sporadically or in combination with a 1% concentration (1 L/100 kg of feed) of each of them. The flax seed oil is provided by Al-Emad company©, Iraq, while Omega 3 is obtained by Hat vet©, turkey.

Experiment design:

One day old two hundred and forty broiler (Ross 308) chicks were used to explore the effect of oils on cytokines after the Newcastle vaccine. The chicks were divided into eight groups numbers from G1 to G8 Table 1, and each group was raised in separate cages with 24 light programs, with contentious supplying of food and clean water. The experiment was done in the animal house, of the College of Veterinary Medicine, Mosul University. In the period extended from 5/11/2023 to 10/12/2023.

On day one, all chicks received a single dose of 0.1mL subcutaneous from the killed Newcastle vaccine, and then the vaccine schedule proceeded in 7, 14, 21 days.

Collection of Blood samples

All blood samples were collected in 1, 3,10,17,24,31 days after vaccination from all group, each blood sample was put in a gel tube and left to coagulate then the serum was separated by centrifuging for 10 minutes at 3000 round per minute rpm [17,18], and the collected serum was storge in -20℃ until used.

Estimations of IL6, IL4 and Interferon γ level:

The Enzymes linked Immunosorbent assay (ELISA) test supplied by Sunlong©, China was used to detect the changes in the level of interleukin and interferon in serum according to company instructions. The standard curve was generated after dilution of stander with stander dilution provided in each kit and used to calculate the concentration of each parameter test.

Statistical analysis

All results were analyzed with IBM SPSS Version 24, by using a T-test, one-way ANOVA test, and Duncan multiple ranges test to explain significant differences between groups [19].

Results

The results indicate no change in Interferon- γ concentration during the first day in all groups. The concentration of Interferon- γ shows a significant increase during experimental progress between all treated groups. However, there was some non-significant value seen on the 10th day post-vaccinated and vaccinated. A significant increase reaches the maximum in the 17th and 24 days post-vaccinated in G8 (33.18 ± 0.05 pg/ml) and in G7 (31.78 ±0.05 pg/ml), respectively (Fig.1).

Also, interleukin 6 shows no significant difference on day 1, while its concentration shows an increase significantly during the experimental process with some vibration between group responses, and reaches its higher value (27.47 ± 0.05 pg/ml) on day 24 post-vaccination(Fig.2). The interleukin 4 did not differ from interleukin 6 and interferon -γ in day 1; it also recorded no significant value between groups. And it is concentration was increased during the experimental time in all groups to reach the peak in the 17th and 24 days post-vaccination with some non-significant value recorded between some groups. The G4 who received the two oils and were vaccinated recorded the highest interleukin 4 concentration (33.98 ±0.04 pg/ml) in the 24 days (Fig.3).

Discussion

The poultry industry is the most important part of the human food supply because of its rapid production process which supplies cheap sources of

protein in food, so it is considered the most significant sector in the economy, especially in a growing country. Poultry health is considered the piler in the poultry industry, many diseases infect broiler farms and cause a huge economic loss; so many attempts have been made to increase broiler resistance to infection either by vaccination or by improving the immune state by adding many immune stimulants or immune enhancers to their feed. Many attempts were made to support the immunity of broilers by using natural additives especially natural oils such as flax seed oils and omega 3 (fish oils) which provide the ability to improve performance but little manuscript was found in its immunological effect, especially on cytokines.

Our results explain no significant differences were found in all groups treated or vaccinated on day 1 when estimating the serum level of interferon- γ, interleukin 6, and 4. The presence of result of these cytokines on day one may come from the eggs' embryonic life and in our opinion, this may help in the development of protection in the first days of life until the chick's immune system is complete.

Our result showed a significant increase in the interferon- γ serum concentration in all vaccinated groups and this related to immune stimulation occurred by the Newcastle vaccine used [20]. also, interferon- γ control groups which not receive the vaccine show a significant increase in interferon concentration, especially on days 17th and 24th post-treatment this result is related to the inhibitory effect that Newcastle virus has on the immune system which activates negative feedback of interferon production through increasing suppressor of cytokine signaling and any other mechanisms that block the intercellular signals for interferon production [21,22]. All groups treated with oils give highly significant interferon-γ serum concentration which may be explained by the augmenter effect of these oils on the production of interferon-γ which increases T-helper 1 cells and decreases the eicosanoids that are produced from peripheral macrophage associated with increases of polyunsaturated fatty acid-3 that modulate immune cells to increase interferon-γ production [21,23,24].

All groups show an increase in the serum interleukin 6 concentration during the experimental process we agree with [6] that the Newcastle vaccine causes increased production of interleukin 6 messenger ribonucleic acid (mRNA) from immune cells to stimulate inflammatory cells of the immune system; the concentration of interleukin 6 on the 17th-day show increase, especially in groups treated with oils which comes to un-line with result recorded by other researchers [16,25,26]. The changes in polyunsaturated fatty acid found in oil composition affect the production of interleukin, the Simopoulos, 2002 noted that negative increases the production of the pro-inflammatory cytokines especially interleukin 6 when feeding broilers on a diet containing 1:1 percentage of polyunsaturated fatty acid index 6/3 [27]. In regards to interleukin 4 very little research was found dealing with the production of these cytokines either with the Newcastle vaccine or with oils. Our results show an increase in serum interleukin 4 and this increase was higher in the last days of the experiment which revealed the cellular expansion associated with multiple stimulations with the vaccine and its booster doses that led to an increase in T-helper 2 cells [28,29,30].

The increase in the serum interleukin 4 may result from the immunomodulator of oils used as they produce immunoregulatory materials such as protectins, maresins, and resolvins which modulate the conversion of the macrophage from macrophage M1 to macrophage M2 which increase the production of interleukin 4[31,32].

Conclusion

We concluded that both oils (flax seed oils and omega 3) have augmenter effects on interleukin response and can modulate broiler immunity to provide a good immune state after the vaccine with Newcastle which may give more protection against infection.

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Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All Animal handling and all blood collection were done ethically according to procedures recommended by the Institution of Animal Care and Use Committee with certification number UM.VET.2023.068

TABLE 1. Experimental Design Groups

Groups	Treatment live attenuation Clone 30 Newcastle vaccine	1% Flax seed Oil	1% Omega 3
G1	+	-	-
G2	+	+	-
G3	+	-	+
G4	+	+	+
G5	-	-	+
G6	-	+	-
G7	-	-	-
G8	-	+	+

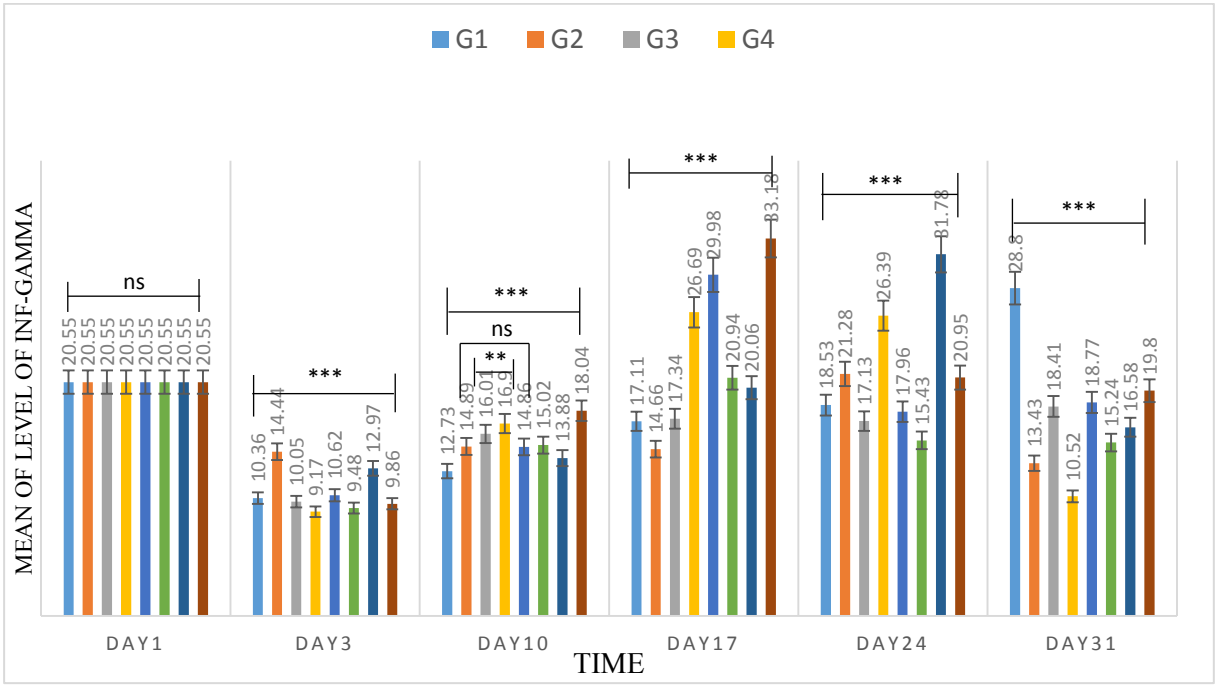

Fig. 1. The concentration of interferon- γ in experimental groups

(*) Indicate a significant difference found at ($P < 0.05$).

(ns) Indicate no significant difference found at ($P < 0.05$).

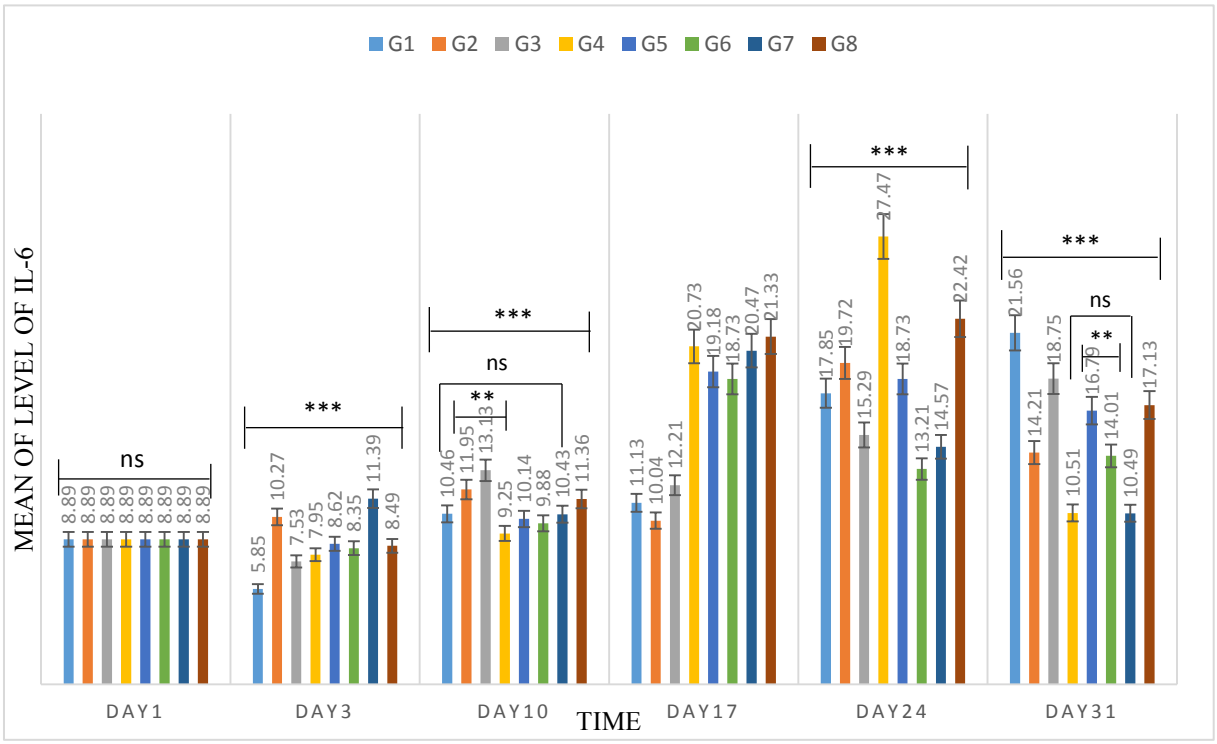

Fig. 2 The concentration of interleukin 6 in experimental groups

(*) Indicate a significant difference found at (P <0.05).

(ns) Indicate no significant difference found at (P <0.05).

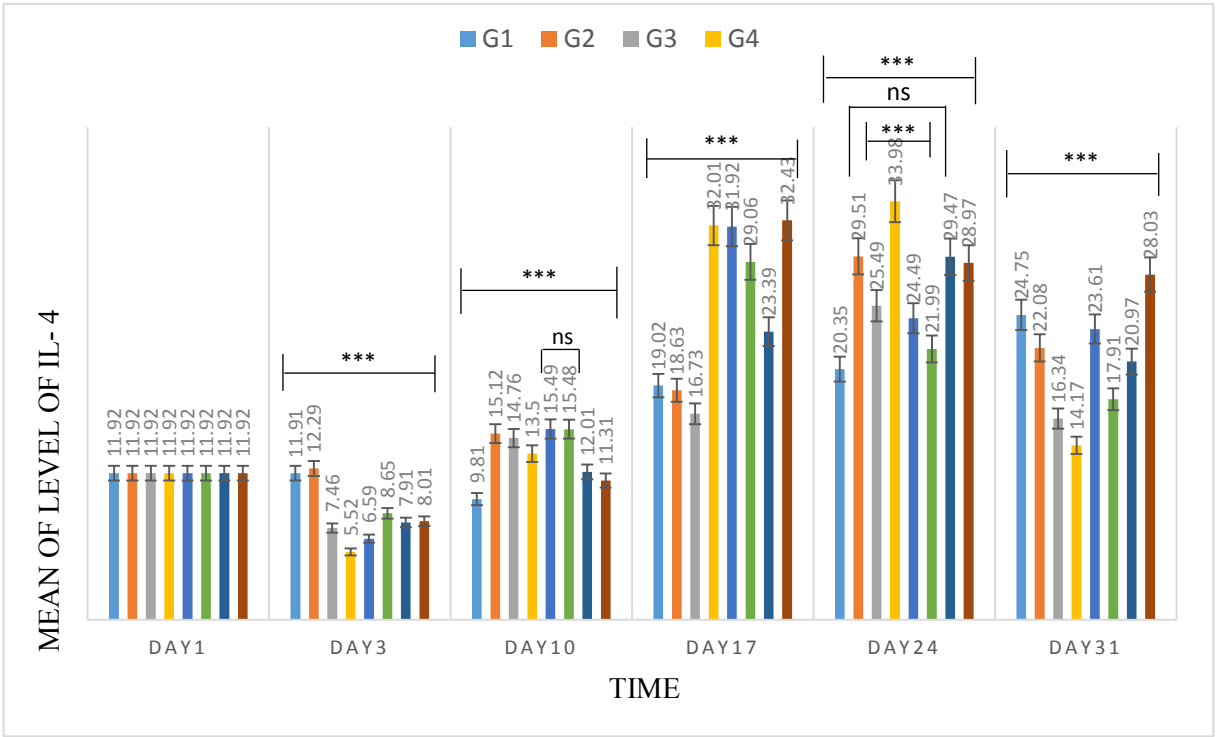

Fig. 3 The concentration of interleukin 4 in experimental groups

(*) indicate a significant difference found at (P <0.05).

(ns) indicate no significant difference found at (P <0.05).

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تأثير إضافة الزيوت على بعض السيتوكينات المناعية في افراخ فروج اللحم الملقحة بلقاح النيوكاسل

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الملخص

تعد صناعة الدواجن أهم جزء من الإمدادات الغذائية للإنسان بسبب عملية الإنتاج السريعة التي توفر مصادر رخيصة للبروتين في الغذاء، لذلك يعد القطاع الأكثر أهمية في الاقتصاد، وخاصة في البلدان النامية . هدفت هذه الدراسة إلى معرفة التغيرات التي تحدث في مستوى الانترلوكين IL6 وIL4 والإنترفيرون γ بعد استخدام زيوت بذور الكتان وأوميجا 3 في فروج اللحم المحصنة ضد مرض النيوكاسل باستخدام اختبار الاليزا حيث استخدم 240 كتكوت بعمر يوم واحد قسمت إلى ثماني مجاميع وعوملت إما بلقاح النيوكاسل لوحده أو بلقاح مع 1% من زيت بذور الكتان مع/أو أوميجا 3، تم جمع الدم بعد 1و3و10و17و24و31 يوم بعد التطعيم. اظهرت نتائجنا زيادة ملحوظة في جميع العلامات المناعية (الانترلوكين IL6، IL4، والإنترفيرون γ) خاصة في المجموعات المعالجة بالزيوت لتصل الى اعلى تركيز في اليومين السابع عشر والرابع والعشرين بعد التطعيم. نستنتج أن كلا الزيتين (زيوت بذور الكتان والأوميجا 3) لهما تأثيرات معززة على استجابة الانترلوكين ويمكنهما تعديل مناعة الدجاج اللاحم لتوفير حالة مناعية جيدة بعد اللقاح بالنيوكاسل مما قد يعطي المزيد من الحماية ضد العدوى.

الكلمات الدالة: دجاج التسمين ، الزيوت، إنترلوكين 4 و 6، إنترفيرون γ، لقاح نيوكاسل.