ENHANCING OF IMMUNE RESPONSE AGAINST NEWCASTLE DISEASE VIRUS FOR BROILER FEEDS ON ZINC-METHIONINE

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ABSTRACT

This study aimed to determine whether zinc methionine supplements have a beneficial effect on the immune response in broilers vaccinated against Newcastle disease (ND). A total of 120 broiler chicks were randomly divided into 6 groups. G1 were fed a basal diet and kept as a negative control G2 and G3 were fed 90 mg/kg of zinc methionine in a basal diet. The two groups were vaccinated with killed and live NDV groups 2 and 3 respectively. Group 3 consisted of chickens vaccinated with live ND vaccine in drinking water, while Group 4 consisted of chickens fed with zinc methionine supplementation 90 mg/kg of basal diet, but not vaccinated (positive control). G5 and G 6 consisting of chickens were vaccinated as mentioned in G2 and G3. The titer of immunoglobulin G (IgG) was measured as a parameter for the immunological status of chickens during the experiment. Results reveal that the titer of IgG against ND was increased in the G2, G3, and G5, in contrast, the titer of IgG against ND was higher in groups of chicken feds on zinc methionine. Antibody titer of IgA against ND was increased in G2 and G5 with significant differences (P<0.05), increase in the titer of IgA was reported in G2 after treatment. The bursal index and spleen index at 25 days have significant results and the G2 and G3 consider the best groups compared with another groups.

Key words: element, amino acid,poultry,vaccine ,bursal index and spleen index.
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تعزيز الاستجابة المناعية ضد فايروس نيوكاسل في اعلاف دجاج اللحم الحاوية زنك –ميثيونين

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المستخلص

هدفت الدراسة الحالية إلى معرفة تأثير مكملات الزنك والميثيونين على إثارة الاستجابة المناعية في دجاج التسمين الملقح ضد مرض فايروس نيوكاسل (ND) . تم تقسيم إجمالي 120 فرخ دجاج تسمين عشوائيًا إلى 6 مجموعات. تم تغذية الدجاج في المجموعة 1 على نظام غذائي أساسي وتم الاحتفاظ به كمجموعة تحكم سلبية. تم تغذية دجاج المجموعتين 2 و3 بجرعة 90 مجم / كجم من ميثيونين الزنك في نظام غذائي أساسي. تم تطعيم المجموعتين بلقاح نيوكاسل الحي والمقتول. تتكون المجموعة 3 من الدجاج الملقح بلقاح نيوكاسل الحي في مياه الشرب، بينما تتكون المجموعة 4 من الدجاج المغذي بمكملات الزنك والميثيونين بجرعة 90 مجم / كجم من النظام الغذائي الأساسي، ولكن لم يتم تطعيمه (مجموعة تحكم إيجابية). تم تطعيم المجموعة 5 والمجموعة 6 المكونة من الدجاج كما هو مذكور في المجموعتين 2 و3. تم قياس عيار الغلوبولين المناعي (IgG) G كمعيار للحالة المناعية للدجاج أثناء التجربة. تكشف النتائج أن عيار IgG ضد ND ارتفع في G2 و G3 و G5 ، في المقابل، كان عيار IgG ضد ND أعلى في مجموعات الدجاج التي تغذت على ميثيونين الزنك. ارتفع عيار الأجسام المضادة IgA ضد ND في G2 و G5 مع اختلافات كبيرة(P <0.05)، تم الإبلاغ عن زيادة فريدة في عيار IgA في G2 بعد العلاج مؤشرالجراب, ومؤشرالطحال في 25 يوما له نتائج مهمة ويعتبر G2 و G3 أفضل المجموعات مقارنة بالمجاميع الأخرى.

الكلمات المفتاحية: عنصر، احماض امينية، دواجن، لقاح، مؤشر الجراب ومؤشر الطحال.

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INTRODUCTION

Newcastle Disease Virus (NDV) is a common poultry disease affecting chickens in Iraq, the disease affects the poultry industry and causes huge losses in poultry farms (5, 7, 14). Vaccines were used to control the disease, most NDV vaccines are commercially available in Iraq and are B1, Lasota (Hitcher-Bio- VAC B1, Fatro, Italy), and Clone 30 (killed vaccines) (Nobilis, MSD, Netherlands) (8, 15, 39). NDV strains have been isolated and diagnosed in several studies in Iraq (19) . Previously, the common NDV vaccine used in Iraq was the Abu Ghraib 68 strain (AG68), vaccine strain displayed satisfactory and higher protection than those of other lentogenic strains, probably due to the close antigenic relationship between the local vaccine strain and the circulating NDV infection strains in Iraq (4,33). In the early 1990s, the AG68 strain disappeared for unknown reasons, and vaccine failure occurred from time to time among the flocks vaccinated with imported vaccines, resulting in substantial economic losses to the poultry industry (16). In Iraq, there is very little information regarding the differences between the invading NDV strains and the traditionally used vaccine strains Zinc-methionine (Zn-Met) is an organo-amino acid zinc complex that differs from inorganic zinc, zinc proteinates, and zinc polysaccharide complexes (17). The zinc-methionine supplement to diets fed to broiler breeders has promoted cellular immunity in the progeny (12). Studies improve the benefit of Zinc-methionine added to the diet of poultry, which may provoke the immune system function against infectious diseases (13). Substituting Zinc-methionine in poultry rations is one such effort to reduce the costs with the enhancement of the nutritional quality of poultry meat (1). Zinc-methionine could be advantageously incorporated in broilers' diets at lower levels compared to inorganic zinc for apprehending higher zinc bioavailability and lower excretion of zinc to the environment (45). Zinc methionine (Zn–Met), as an organic zinc source, is better than inorganic zinc in improving immunity and anti-stress (25,42). Saleh *et.al* (42) reported that dietary Zn–Met enhances the humoral immune response and antioxidant enzyme activity of heat-stressed broilers. On the other hand, Ahmed (6) reported that supplemented with Zn–Met posed to antidiarrhea and growth promotion by enhancing average daily gain (ADG) and reducing diarrhea rate and serum D-lactate content. The current study aimed to determine whether zinc methionine supplements have a beneficial effect on enhancing the immune response against Newcastle disease Virus (NDV) in broilers immunized with killed and live NDV vaccines.

MATERIALS AND METHODS

Ethical approva: This study does not require ethical approval. However, all broilers in the current work were treated humanely following the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) (46).

Broiler chickens, diet, and Management:

A total of 120 one-day-old broiler chickens (ROSS-308 broiler chicks) were purchased from a commercial hatchery in Baghdad. The chickens were housed in the animal house/Department of Pathology/ College of Veterinary Medicine/University of Baghdad /Iraq. The experiment was conducted between May 18th to June 22nd, 2023. The chicks were kept in separate cages. Feed and water were ad libitium available to all broilers. Standard management procedures were used, and chicks were reared on the floor with a litter of wood shavings. Each group was equipped with a feeding tray 38 cm in diameter and fountains of 4 liters for three weeks. The fountains and feeding trays were at optimum levels which helped the broilers to get their water and feed easily. The environmental temperature was fixed at 33°C in the first week of life and decreased up to 2°C in the first week till 21°C at the end of the experiment and the room was equipped with a mercurial thermometer. A starter (crumbles) feed was used for 1–14 days, a grower (pellets) feed was used for 15–28 days, and a finisher feed was used for 29–35 days.

Experiment design: The 120 broiler's chicks were divided into 6 groups: Group 1 (G1): control negative–broiler chickens were fed on a basal diet. Group 2 (G2): Chickens were fed with basal diet plus 90 mg/kg zinc methionine and vaccinated with killed ND vaccine at one day old and ND eye drop vaccine at 14 days

old. Group 3 (G3): Chickens were fed with basal diet plus 90 mg/kg zinc methionine and vaccinated with live ND vaccine at 1, 7, and 21 days old in drinking water. Group 4 (G4): positive control- Chickens were fed with basal diet plus 90 mg/kg zinc Group 5 (G5): Broiler chickens were fed on basal diet without adding the zinc methionine and vaccinated as in group 2. Group 6 (G6): Broiler chickens were fed on a basal diet without adding the zinc methionine and vaccinated as in group 3.

Vaccines protocol: There are two types of Newcastle (ND) commercially vaccines available that were used in the study including killed and live ND vaccines. Killed ND vaccine (clone 30) trade name (Nobilis, MSD, Netherlands) was injected S\C at 1 day old. Two types of live vaccine B1 (Hitcher (BIO-VAC B1, Fatro, Italy), and Lasota (BIO-VAC B1, Fatro, Italy). The killed vaccine was injected S\C at 1 day old (G2+G5), while live vaccine type B1 was administered at eye drop at 1 day old and 14 days. Lasota Live vaccine is administered by drinking water at 7 and 21 days. Vaccination procedures were manifested according to published studies (28,38,40, 41).

Blood samples: Before vaccination, 1-2 ml of blood were obtained from the wing vein, and after vaccination blood was collected from chickens at days 12,21 and 28. Serum samples were separated by centrifugation at 1500 rpm for 15 minutes and stored in appropriately labeled tubes at – 20°C for subsequent processing. Newcastle virus antibody levels in the chickens were determined by Enzyme-Linked immunosorbent assay (ELISA) (9), using a kit from Bio-Rad (Germany)

Determination of maternal immunity:

The blood was collected from 20 chicks from the jugular vein at 4 days old of the control group (control negative) to determine the maternal immunity for NDV. Blood was also collected from other groups at 12,21, and 28 days to determine the titer of Immunoglobulin (IgG and IgA) for NDV (14,33,36).

Statistical analysis

To determine the influence of different elements in the present study parameters, the Statistical Analysis System- SAS (SAS Institute, 2000, version 1) application was employed. In this study, the least significant difference –LSD test (ANOVA) was utilized to make a meaningful comparison between all the means (P value ≤ 0.05).

RESULTS AND DISCUSSION

Determination of maternal immunity:

The maternal immunity (MatAbs) for NDV of maternal IgG antibodies titer in twenty chicks at four days was 5842±1.05. MatAbs are antibodies that are passed from a hens to her offspring, typically breeder hens to the progeny through the egg; MatAbs play a crucial role in protecting progeny, including chicks, from various infectious diseases during the early stages of life when their immune systems are still developing and facing microbe or vaccination processes (9,48). The transfer of MatAbs from the hen to the chick occurs primarily through the egg yolk. The hen produces maternal antibodies in response to exposure to pathogens or vaccines, and they are then transmission into the developing eggs. Chicks absorb these antibodies from the yolk sac into their bloodstream during the last stages of embryonic development(29,48).

Immunity against NDV

The immunoglobulin (IgG) titer in experimental broilers at 12, 21, and 28 was determined. Table 1 shows the results of the experiment, the IgG was increased at 12 days of age in groups 2, 3, and 5 of chickens, but the titer of IgG at 21 days old shows high titer in groups 2 and 5. The titer of IgG antibody continuing increased at age 28 days old in groups 2 and 3 indicating the benefits of Zinc-methionine adding to the diet that enhances the immunity against NDV. This study agreed with the results obtained by Al-Garib *et al.,* (10) and Dzogbema *et al.,* (30) who illustrated that the antibody specificity of the captured immunoglobulin (Ig) was confirmed by binding of NDV, after inoculation with live virus, antibodies of the IgG and IgM classes were mainly found in serum. The IgG antibodies provide passive immunity to the chicks, meaning that they offer immediate protection against specific pathogens without the chicks having to produce their antibodies when antibody transfers can recognize and neutralize pathogens that the mother has encountered, helping to prevent or reduce the severity of Newcastle disease virus infections in the chicks (36).

Table 1. Immunoglobulin g (IgG) titer in Zinc-Methionine groups for Broiler Chicks immunized with Newcastle Disease vaccines

Age	G1	G2	G3	G4	G5	G6	LSD
12 days	3057±153.8 A c	6950±61.7 C a	6034±282.7 B a	3140±165.7 A c	6632±109.8 C a	5900±277.3 B b	2103.4
21 days	595±80.3 B d	7110±51.2 B a	5310±89.9 C ab	610±71.4 B c	6822±128.2 B a	3765±176.4 C b	2791.2
28 days	0 ± 0 D c	7267±67.6 AB a	7060±121.7 A ab	0 ± 0 C c	6947±75.5 AB b	6827±139.5 A b	244.7
LSD	1013.4	159.6	629.9	1754.1	128.7	629.33	-

***Capital litter represented the vertical comparison of data and small litter represented a horizontal comparison of data; a significant difference was P value ≤ 0.05**

The current study reveals that the titer of IgG against NDV was decreased in the control group versus the treated groups. However, it's important to note that maternal antibodies have a limited lifespan in the chick's body due to the chicks growing, their immune systems begin to mature, and they start producing their antibodies (23). At the same time, the maternal antibodies they received gradually decline in concentration, and this period is known as the "window of susceptibility" or "immunological gap" because the chicks become vulnerable to infections once the levels of maternal antibodies drop below a protective threshold, but before their immune systems are fully functional (24). The present study reported an increase in the titers of IgG antibodies in the serum of chicken in groups G2 and G3 feeding with zinc-methionine and vaccinated, poultry producers need to consider the presence of maternal antibodies when designing vaccination programs for chicks (20). Vaccination timing is crucial to ensure that the chicks receive active immunization at a point when maternal antibody levels have declined sufficiently to allow the chicks' immune systems to respond to the vaccine (37). Vaccination alone too early can result in interference by maternal antibodies, reducing the effectiveness of the vaccine, while vaccinating too late can leave the chicks susceptible to infections during the immunological gap. The best results were obtained when the vaccinated chickens were fed on basal diet supplement with zinc methionine to maintain the concentration of IgG in two groups G2 and G3 28 days as (7630±99.6) and (7344±144.4) respectively. Khare *et al.*, (29) reported that the concentration of IgG at age 15 days was more prevalent than other ages of chicken.

Antibody titer (IgA) against NDV:

Table (2) shows an increase of immunoglobulin type A in groups G2 and G5 at 12 days (3455±43.9 and 3353±40.6) these groups were vaccinated by eye drop vaccine with significant differences (P<0.05) with other groups, on the other hand there are increase the titer of IgA at the 21 and 28 days in the group G2 (4041±68.3, 4567±96.6 respectively) compared with group G5 used the same methods of vaccination for NDV, the same results found in the group G3 compared with group G6, but groups G1 and G4 showed sever decrease in IgA antibody.

Table 2 .Immunoglobulin A titer in Zinc-Methionine groups for Broiler Chicks immunized with Newcastle Disease virus.

Age	G1	G2	G3	G4	G5	G6	LSD
12 days	0 ± 0 B c	3455±43.9 C a	2953±142.4 B b	0 ± 0 B c	3353±40.6 B a	2802±133 B b	423.9
21 days	0 ± 0 B c	4041±68.3 B a	2476±141.2 C b	0 ± 0 B c	3596±120.7 B a	2263±162.8 C b	466.8
28 days	0 ± 0 D c A d	4567±96.6 A a A a	3807±143.7 A b A b	0 ± 0 B d A d	3985±91.45 Ab Ab	3294±166.9 A c A c	581.7
LSD	12.8	433.6	602.5	13.7	326.4	310.2	-

***Capital litter represented the vertical comparison of data and small litter represented a horizontal comparison of data; a significant difference was P value ≤ 0.05**

The increase in the antibody titer (IgA) after 20 and 30 days was reported by others (37,40) suggesting that the immune system of chickens is maturing during this period, the findings contribute to the understanding of the development of the mucosal immune response, specifically the production of IgA antibodies, which play a crucial role in defending against pathogens at mucosal surfaces (40). Titer of IgA is an important tool in the immune system, particularly in mucosal surfaces, such as the respiratory, gastrointestinal, and urogenital tracts (50). It plays a significant role in defense against pathogens and provides localized immunity (22).Titer of IgA is the predominant immunoglobulin found in secretions, including saliva, tears, and mucus, it plays a crucial role in protecting mucosal surfaces from invading pathogens by preventing their attachment and entry into the body(43). Table 2. Also shows that the titer of IgA in groups 1 and 4 decreased to zero because Chicken IgA can neutralize pathogens by blocking their attachment to host cells and inhibiting their entry and colonization. It can also prevent the establishment of infection by excluding pathogens from entering the body through mucosal surfaces (3).

Measurement of Bursa index in chickens in all groups at 25 days: Table (3) shows the Bursal index at 25 days of G4 (0.231) has significant results compared with G1 (0.2082) due to the effects of methionine and zinc on activation of the bursa, The G2 and G3 (0.2658 and 0.2514) respectively consider the best groups compared with G1, G4, G5, and G6 (0.2082, 0.231, 0.2396 and 0.2378) respectively because this groups (G2 and G3) was feeding methionine and zinc in the same time it is vaccinated against NDV compared with G5 and G6 was only vaccinated groups and G4 was only taken methionine and zinc and G1 was controlled negative (not vaccinated and did not taken methionine and zinc).

Table 4. Measurement of bursal index at the age of 25 days in all groups.

	G1	G2	G3	G4	G5	G6
Bursal index	**0.208± 0.023 d***	**0.2658± 0.015 a**	**0.2514± 0.022 b**	**0.231± 0.024 c**	**0.2396± 0.018 c**	**0.2378± 0.056 c**
	LSD (0.0092)					

***Small litter represented a horizontal comparison of data; a significant difference was the P value ≤ 0.05**

The study agreed with Sultan *et al.* (44) who observed an interesting finding regarding the bursal index. The bursal index in the group receiving MOS was twice as high as that of the control group, indicating increased bursal size,this observation suggests that the MOS supplementation had an impact on the development or size of the bursa of Fabricius, a critical organ for the immune system in poultry, and decided the potential immunoprotection role of an infectious bursal disease (IBD) vaccine in immunopotentiator chickens challenged with velogenic viscerotropic Newcastle disease virus (VVNDV)(44). On the other hand, the current study disagreed with Fan *et al.* (32), and Ulaiwi (47) who observed that the commercial broilers showed 27 to 40 % positive for maternal antibodies against IBDV before infection with the SHG19 strain. At 4 days' post infection the SHG19 strain induced severe atrophy of the bursa with a BBIX of 0.44 ± 0.16. The vaccination programs in the poultry industry often include vaccines that target the Bursa of Fabricius to prevent or control certain diseases. However, there is no specific "Bursa Index" vaccine for chickens (27). Vaccination against ND helps prevent the spread of the virus and protects the Bursa of Fabricius from damage that can occur during infection (1,28,47). The bursa of Fabricius of all unvaccinated chickens appeared atrophy and had a gelatinous yellowish transudate covering the mucosal surface. Chickens immunized with the live rLaS-VIIF/HN-VP2 did not show obvious gross lesions (13,18). Administration of NDV vaccines should be done in consultation with a poultry veterinarian or an expert in poultry health. They can assess the specific disease risks in a particular region and recommend the appropriate vaccination program to protect the Bursa of Fabricius and overall flock health (41).

Measurement of spleen index in chickens in all groups at 25 days: The spleen index of chicks at 25 days found a significant

difference (LSD= 0.0085; P< 0.05) between the six groups, the study found an increase in spleen index in weight and dimension in the G2 and G3 (0.1618 and 0.1514) respectively, rather than in groups G1, G4, G5 and G6 (0.107, 0.1354, 0.1342 and 0.1216) respectively, because G2 and G3 were vaccinated and feeding methionine and zinc. Group 4 (G4) (0.1354) has significant results in spleen index compared with G1 (0.107) and this evidence for two groups (G2 and G3) feeding zinc minerals and methionine responsible to increase the activity of vaccine against NDV with activation of immunity system and spleen in poultry consider immunological organ (table 5). The study in disagreement with the finding reported by Al-Mola (11) and (16), who found that the immune organ indexes defined as organ weight/body weight ratio were measured at 4, 7, 14, 21, 24, 28, and 35 days of age. On days 28 and 35 of the experimental period, unvaccinated birds had significantly ($P \leq 0.05$) higher spleen index than vaccinated birds. The spleen is an essential organ in the immune system of chickens and plays a role in filtering blood, removing old or damaged red blood cells, and producing immune cells. The spleen's size indicates the condition, and overall health and immune status of chicks. However, chickens have no specific "spleen index" vaccine (7,34). In poultry health, the evaluation of the spleen is often performed during post-mortem examinations or through non-invasive imaging techniques (35). The spleen size and appearance can provide insights into the presence of diseases or conditions that may affect the immune system. Table 4 reveals an enlarged spleen (splenomegaly), which may indicate a systemic infection, inflammation, or certain diseases affecting the immune system in both groups G1 and G4 of chickens. Zinc and methionine with virus vaccine in the G2 and G3 in chicken providing a balanced and nutritious diet is essential for supporting the immune system with adequate intake of vitamins, minerals, and other important nutrients helps maintain the health and function of the spleen and other immune organs (2,8,45). Several researchers support the addition of Zinc to broilers' diet, De Grande *et al*. (27), and Cui *et al*. (26) use dietary zinc as a source of impacts on intestinal morphology and oxidative stress in young broilers, also the different sources of zinc enhanced intestinal epithelial barrier and Jejunal mucosal of Newborn Calves (49) and broilers with Escherichia coli. On other occasions, the supplementation of broilers' diets with useful sources affected the condition of physiological and anatomical performance of broilers (21,31).

Table 5. measurement of Spleen index at the age of 25 days in all groups.

	G1	G2	G3	G4	G5	G6
Spleen index	**0.107± 13.67 e***	**0.1618± 17.82 a**	**0.1514± 17.34 b**	**0.1354± 14.89 c**	**0.1342± 16.64 c**	**0.1216± 16.17 d**
P	**LSD: (0.0085)**					

***Small litter represented a horizontal comparison of data; a significant difference was the P value ≤ 0.05**.

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