# Using T cell lymphokines to enhance the immune response against Newcastle disease in vaccinated broiler chickens fed naturally contaminated diet with different mycotoxins

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**Abstract**

The current study aimed to reduce the toxic effect of different mycotoxins and enhance the immunity against ND virus in broiler chickens by using lymphokines from hyperimmunized birds with *Salmonella* typhymurium. The study included three stages, the first stage included isolating *Salmonella typhymurium*. The second stage was immunized chicks with *Salmonella typhymurium*. The final stage of the study was accomplished by treating 250 broiler chicks (divided into 5 groups, 50 chicks /each) with the following treatments; G1: 0.5 ml lymphokines was injected I/P at day one old with live ND vaccine (la Sota strain) after 30 minutes in drinking water, the process repeated after 10 days; G2: the same as in G1 but inactivated killed vaccine was used s/c,no repetition was carried out at 10 days ; G3: a combination of G1 and G2 with revaccination of live La Sota vaccines only at 10 days; G4 : only vaccinated with live La Sota vaccine repeated at 10 days; G5: no treatments (negative control). All groups were challenge with local isolate of NDV ($100ELD_{50}$ $10^5$) at 25 days, all groups except the fifth group were fed on contaminated diet with mycotoxin. The results of the present study showed a significant increase ($P < 0.05$) in antibodies titre against ND in the third group, followed by the first and second groups Measured by ELISA and hemagglutination (HI) test, A significant decrease ($P > 0.05$) in the oxidation status ($H_2O_2$, MDA and LPO) and significant increase in the antioxidant defense (GSH-PX) in the liver and spleen samples. We conclude from the current study that the Salmonella immune lymphokines (SIL) helps in enhancement the level of immunity against Newcastle disease and n reduction the side effects of which mycotoxin.

**Key words**: ND, SIL, Mycotoxin, T cell, ELSA, HI

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# استعمال اللمفوكاينز للخلايا التائية لتعزيز الاستجابة المناعية ضد مرض النيوكاسل في دجاج اللاحم الملقح المستهلكة لعلائق ملوثة طبيعيا بسموم فطرية

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**الخلاصة**

هدفت الدراسة الحالية الى تقليل التأثيرات السمية للسموم الفطرية وتعزيز المناعة ضد مرض النيوكاسل في الدجاج اللاحم باستخدام اللمفوكاين من الطيور الممنعة بثلاث جرع من *Salmonella typhymurium*. تضمنت الدراسة ثلاث مراحل، المرحلة الأولى شملت عزل *Salmonella typhymurium*، أما المرحلة الثانية تضمنت تمنيع الافراخ بالسالمونيلا تايفيموريم، أما المرحلة الثالثة والأخيرة من الدراسة تضمنت اخذ ٢٥٠ فرخة اللاحم بعمر يوم واحد، وقسمت الى خمس مجاميع كل مجوعة ٥٠ فرخة، حقنت المجموعة الأولى بعمر يوم واحد ٠,٥ مل بالتجويف البريتوني Lymphokines وبعد ٣٠ دقيقة لقحت بلقاح النيوكاسل (La Sota strain) عن طريق ماء الشرب، واعيدت الحقن

واللقاح بعمر ١٠ يوم. اما المجوعة الثانية حقنت بعمر يوم واحد ٠,٥ مل بالتجويف البريتوني Lymphokines وبعد ٣٠ دقيقة لقحت فقط باللقاح الزيتي للنيوكاسل (La Sota strain) حقن تحت الجلد بعمر يوم واحد. وحقنت المجموعة الثالثة بعمر يوم واحد ٠,٥ مل بالتجويف البريتوني Lymphokines وبعد ٣٠ دقيقة لقحت المجوعة الثالثة باللقاحين لمرض النيوكاسل بنفس العترة (الزيتي تحت الجلد واللقاح الحي عن طريق ماء الشرب بعمر يوم واحد) وأعيد التلقيح فقط باللقاح الحي بعمر ١٠ مسبوق Lymphokines. أما المجموعة الرابعة لقحت بلقاح النيوكاسل (La Sota strain) عن طريق ماء الشرب (١ و ١٠) يوم ولم تحقن Lymphokines واعتبرت مجموعة سيطرة موجبة. أما المجموعة الخامسة لم تلقح ولم تحقن Lymphokines واعتبرت مجموعة سيطرة سالبة. جرعة عن طريق الفم جميع المجاميع بالعزلة المحلية لمرض النيوكاسل بجرعة ($100ELD_{50}$ $10^5$) بعمر ٢٥ يوم، كل المجاميع غذيت على عليقة ملوثة بسموم الفطرية ما عدا المجموعة الخامسة. أظهرت نتائج الدراسة الحالية وجود زيادة معنوية بمستوى ($P<0.05$) بمعيار الأجسام المضادة لمرض النيوكاسل في المجوعة الثالثة تلتها المجموعة الأولى ومن ثم الثانية بفحص الاليزا وفحص أنباط التلازن الدموي، وانخفاض معنوي بمستوى ($P<0.05$) في معدلات إنزيمات الحالة التأكسدية (MDA and LPO،H2O2 ) وارتفاع معنوي بأنزيم المضاد للتأكسد (GSH-PX) في الكبد والطحال. نستنتج من الدراسة الحالية أن استخدام (Salmonella immune lymphokines SIL) يساعد على تعزيز المناعة اللقاحية ضد مرض النيوكاسل وتقليل التأثيرات الجانبية للسموم الفطرية.

## Introduction

Mycotoxins are diverse group of harmful particles to animal and human, which are the secondary metabolites of different toxigenic molds, especially aspergillus, penicillium and fusarium species, mycotoxins are produced in the grains as well as in the pre- and post-harvest feed in different environmental conditions and due to the large diversity of their toxic effects and their synergistic properties therefore, mycotoxins consider more risky to the consumers of food and contaminated feed (1). The consumption of low concentrations of ycotoxins for long periods exposes cells of the intestinal mucosa to damage, however the intestinal mucosa possesses both natural and specific immune components (2). Bouhet and Oswald (3) the main function of physical barrier of the intestinal mucosa is the transfer the electrical resistance through the epithelial layer, which found in the monolayer cells. Mclaughlin *et al*. (4) show that mycotoxins damage the proteins found in cell junctions and thus reduce the transmission of electrical impulses which is the transfer of cytokines between immune cells. Metabolic products of innate toxins affect the immune system of the intestinal mucous, which in turn significantly affects the performance and productivity of the animal, because the stimulation of mucous immunity is important in protecting against many of the germs that invade these surfaces and enter the body and cause diseases (5). It is known that oral vaccination is one of the most practical and economical methods in poultry However, the presence of mycotoxins in diet interferes with the immune response by affecting the mucous membranes and thus reduces the level of local immunity resulted after vaccination, making birds vulnerable to infectious and rapidly spreading diseases (6). Newcastle disease is a contagious, endemic and rapidly spreading disease that causes significant economic losses to the poultry industry. The pathogen of this disease is *Paramyxovirus*, Genus *Avulavirus*, family *Paramyxoviridiae* (7). Although many vaccine programs are used to ND control, the disease is endemic in many countries (8). Some research has confirmed that immune response is enhanced by many herbal and vitamins, but immune response is still low due to mycotoxins (9). Rashad and Mushtaq (10) confirmed that the use of lymphokines from *Salmonella Typhmurium* (*Slmonella* immune lymphokines SILK), enhanced the immune response against the ND challenge without using the vaccine by stimulating many immune cytokines, which are regulatory proteins produced by some Immune cells in to protect the body against infections. Mushtaq *et al*. (11) confirm use of SILK in the early days stimulated the immune response to protect the chicks from infectious bronchitis disease after the challenge of local isolation of the disease. Mushtaq, (12) also showed the role of SILk in promoting the maternal immunity at one day-old against avian influenza infection. Therefore, we expect that the addition of SILK to mycotoxins -contaminated diets could reduce the effects of mycotoxin during oxidative reduction and increased antioxidant defense systems with high response against ND.

## Materials and methods

### Detection of *Mycotoxin* in diet

Mycotoxin was detected using the ELISA test. A 20-gram feed sample is mixed with methanol (Fisher, Pittsburgh, PA, USA). Mixed with water (30/70 vlv) and shaking for 3 minutes. Then the mixture filtrated with Whatman filter (Whatman Clifton, NJ, USA), collects the supernatant and determined the concentration of toxins using ELISA kit (Agra Quantum mycotoxin assay, Romer, Singapore). Starter and finisher diet (A1) for (G1, G2, G3 and G4), while starter and finisher diet (B1) for G5 (Table 1).

### Preparation of lymphokines

*Salmonella typhymurium* was isolated from *Salmonella typhymurium*-infected birds. Salmonella was grown on nutrient broth and peptone water. The dishes were incubated

at 37°C for 24 hours until sedimentation occurred with small white deposits. The bacteria were then planted on selected medium such as MacConkey agar and SS agar. After the growth of bacteria on the growth media and the appearance of black spots, the bacteria were identified biochemically using API20. After determining the type of bacteria *Salmonella typhymurium*, the bacterial infected dose was determined by injecting of chicks by $1 \times 10^8$ cfu. Based on spectrophotometric, the required concentration of the bacteria was determined at a standard level of 625 nm in length. Two groups of chicks were included, each group included 20-day-old chicks. The first group had three oral doses (ages 7, 14, 21). Other group did not give anything and considered as control group. At 35-day, T cell cells collected from spleen of infected birds, after crushing of infected spleens, T- cells were harvested by centrifugation at 1500 rpm. T-cells were then cultivated on RBM dishes with the addition of Co- A for stimulation of lymphokines secretion. Lymphokines were collected by centrifugation of filtrate at 3000 rpm according to (13).

Table 1: Residual of Aflatoxin, Ochratoxin and T2 contamination of feed collected feed mills estimated by ELISA test

| Groups | | Aflatoxin ppb | Ochratoxin ppb | T2 toxin ppb |
|---|---|---|---|---|
| Starter | A1 | 46.768 | 3.232 | 25.528 |
| | B1 | 2.565 | 0.323 | 0 |
| Finisher | A2 | 48.661 | 4.765 | 30.636 |
| | B2 | 1.221 | 0.435 | 0 |

ppb: part per billion.

**Preparation of viral inoculum**

Newcastle disease isolate was taken from the Department of Pathology and Poultry Diseases, college of Veterinary Medicine, University of Baghdad. $ELD_{50}$ was identified as $10^5$ according to (13), and the sample was kept at deep freezing (-80).

**Experimental design**

The experiment was carried out using 250 chickens of one-day broiler, divided into five groups each group 50 chicks, treated as follow: the first group was injected 0.5 ml intraperitoneal with Lymphokines at one day, after 30 minutes vaccinated with Newcastle (ND) vaccine (La Sota strain) through drinking water, the injection and the vaccine was repeated at 10 days. The second group was injected 0.5 ml intraperitoneal with Lymphokines at one day, after 30 minutes was vaccinated only with killed ND vaccine at 1 day subcutaneously. The third group injected 0.5 ml intraperitoneal with Lymphokines, after 30 minutes the third group vaccinated Newcastle disease with the same strain kill and live vaccine through subcutaneous and drinking water respectively and was vaccinated only with the live vaccine at 10 days preceded by lymphokines. The fourth group was vaccinated with ND vaccine by drinking water at 1 and 10 days with not treated with Lymphokines and considered as positive control group. The fifth group was not vaccinated and not treated with Lymphokines considered as negative control group. All groups were challenge with local isolate of NDV ($100ELD_{50}$ $10^5$) at 25 days, where is the group which consume mycotoxins only.

**Samples**

Blood samples from the jugular vein were collected at different period 1, 7, 14, 21, 28, 35 and 42 to determine the antibody titer against Newcastle disease using ELISA and haemaaglutantination inhibition test after separation of the serum.

**Oxidative status and antioxidant defense in liver and spleen**

A total of five tissue samples of liver and spleen were collected from each group at 42 days after 12 hours of fasting. One gram was taken from each sample (liver or spleen), Placed in ice-cold and mixed with saline buffer (1: 9 wt/v) then crushed with ultra-turrax (T8, IKA-Labortechnik, Staufen, Germany). The concentration of the Mixture becomes 0.1 g/mL for analysis. The mixture was separated by a centrifuge (1000 rpm) for 10 minutes at a temperature of 4°C. It is then kept at -70°C until it is used to determine concentrations of (H2O2, MDA, LPO and GSH-PX) using the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, china).

**ELISA examination**

For determination the level of titers against ND virus vaccination in birds' protocol was performed according to ProFLOK NDV direct ELISA kit (Synbiotics-USA).

**Examination of hemagglutination inhabitation**

Hemagglutination inhabitation is one of the most specific tests for detection of antibodies against Newcastle disease in serum of infected or vaccinated birds the procedure has applied according to (14).

**Challenge test**

At 25-day, challenge test was performed using local isolate of Newcastle disease at ($100$ $ELD_{50}$ $10^5$) according to method of (13). The chicks were monitored for 10 days, and the morbidity and mortality were recorded for each group.

**Statistical analysis**

The final results were statistically analyzed using statistical analysis system (SAS). The means were distinguished according to the least significant differences and at a significant level ($P < 0.05$).

## Results

### Immunity against Newcastle

Ten chicks were slaughtered before dividing chicks in groups in order to determine ND maternal immunity was accomplished by using ELISA and HI techniques. Titer WERE 7340.9±202.7 and 204.8±22.1 respectively. The study identified the role of lymphokines in improving the immune response to ND under effect of mycotoxins by giving 30 minute before vaccination. Table 2 shows significant differences at level (P<0.05) in Ab titre against ND in all periods. A significant increase at level (P <0.05) in Ab in the G3 followed by the (G1 and G2) groups respectively in (14, 21, 28, 35) days compared with G4 which recorded a significant decrease in Ab against ND, while G5 which is resemble the maternal immunity, it was recorded a decrease reached to zero in 14 days and continuous to 35 days. After challenge with local isolate of NDV all groups were recorded significant increase in Ab at 42 days, but G5 was recorded highly increase in Ab reach to 15792.2 ± 903.9.

Table 3 showed HI results of Abs against ND were identical to ELISA results. There were significant differences at level (P <0.05) in Ab titre against ND for all periods. There was A significant increase at level (P <0.05) in Ab in the G3 followed by the (G1 and G2) groups respectively in (14, 21, 28, 35) days compared with G4 which recorded a significant decrease in Ab against ND, while G5 which is resemble the maternal immunity, it was recorded a decrease reached to zero in 14 days and continuous to 35 days. After challenge with local isolate of NDV all groups were recorded significant increase in Ab at 42 days, but G5 was recorded highly increase in Ab reach to 415.4 ± 15.5.

### The oxidative status and antioxidant defense

Table 4 showed the oxidative status and antioxidant defense in chicks vaccinated against ND and treated with SILK under effect of mycotoxin reassembly by (H2O2, MDA, LPO and GSH-PX) in liver tissues, at 42 days after challenge with local isolate NDV. The third group showed a significantly decrease (P>0.05) in levels of oxidative enzymes followed by the (G1 and G2) groups respectively, compared to (G4 and G5) groups, which recorded a significant increase (P< 0.05) in levels of oxidative enzymes, however a significant increase (P< 0.05) in levels of antioxidant enzymes (GSH-PX) in G3 followed by (G1 and G2) respectively, compared to (G4 and G5) groups were recorded a significant decrease at level (P >0.05).

Table 5 showed the oxidative status and antioxidant defense of the spleen tissue. The third group showed a significantly decrease (P>0.05) in levels of oxidative enzymes followed by the (G1 and G2) groups respectively, compared to (G4 and G5) groups, which recorded a significant increase (P< 0.05) in levels of oxidative enzymes, however a significant increase (P< 0.05) in levels of antioxidant enzymes (GSH-PX) in G3 followed by (G1 and G2) respectively, compared to (G4 and G5) groups were recorded a significant decrease at level (P >0.05).

Table 2: Effect of SILK on antibody titer against Newcastle disease in different periods by ELISA test

| Periods | ND antibody titre Means ± Stander error | | | | |
|---|---|---|---|---|---|
| | 14 days | 21 days | 28 days | 35 days | 42 days |
| G1 | 2406.1±147.8 B | 3043.4±166.5B | 3607.4±231.3B | 4577.7±169B | 6158±279.1C |
| G2 | 1728.6±139.1C | 2245.9±109.7C | 3039.4±73.4C | 3676.2±109.3C | 4422.4±136.4D |
| G3 | 3269.9±169.4A | 4233.1±209.9A | 5936.7±214.9A | 7379.2±268.2A | 9516.2±233.2B |
| G4 | 890.3±41.8D | 1733.7±123.7D | 2112.2±185D | 2357.1±157.9D | 2112.2±185E |
| G5 | 0±0E | 0±0E | 709.4±69.9E | 1633.7±135.6E | 15792.2±903.9A |
| LSD | 340.77 | 400.88 | 483.46 | 518.15 | 1274.4 |

Number of samples: 10; Different letters mean significant difference at level of (P<0.05); LSD: less significant differences.

Table 3: Effect of SILK on antibody titer against Newcastle disease in different periods by HI test

| Periods | ND antibody titer Means ± Stander error | | | | |
|---|---|---|---|---|---|
| | 14 days | 21 days | 28 days | 35 days | 42 days |
| G1 | 61.2±2.9B | 77.8±3.1B | 93.2±4B | 120.2±8.1B | 158.4±9.7C |
| G2 | 43.4±3.47C | 58±4C | 78.6±3.2C | 93.8±2.5C | 114.6±5.9D |
| G3 | 83.8±3.9A | 108±4.9A | 155.4±9.4A | 202.2±9.8A | 248.6±15B |
| G4 | 23.6±1.9D | 43±5D | 53.4±6.2D | 61.6±5.2D | 54.2±4.16E |
| G5 | 0±0E | 0±0E | 22.4±2.77E | 41.8±5.7E | 415.4±15.5A |
| LSD | 11.785 | 16.294 | 23.863 | 21.321 | 46.229 |

Number of samples: 5; Different letters mean significant difference at level of (P<0.05); LSD: less significant differences.

Table 6 showed the clinical signs and mortality in groups vaccinated against ND and treated with SILK, after challenge with virulent local isolate of NDV ($100ELD_{50}$ $10^5$) at 25 days, there was a significant decrease (P> 0.05) in rates of morbidity and mortality in treated groups with SILK (G3, G1 and G2) groups, while not treated groups (G5 and G4) showed a highly significant increase (P< 0.05) in morbidity and mortality rates.

Table 4: Effect of SILK on oxidative status and antioxidant defense in liver exposed to mycotoxins

| Periods | Means ± Stander error | | | |
|---|---|---|---|---|
| | $H_2O_2$ (mmol/g pro) | MDA (nmol/g pro) | LPO (µmol/g pro) | GSH-px (U) |
| G1 | 14.55±0.29BC | 2.99±0.05D | 1.11±0.03BC | 37.44±0.3A |
| G2 | 15.75±0.26B | 3.44±0.13C | 1.26±0.03B | 34.31±0.26B |
| G3 | 13.57±0.32C | 2.63±0.08D | 0.97±0.01C | 39.08±0.55A |
| G4 | 18.28±0.38A | 4.97±0.05B | 2.08±0.04A | 30.26±0.5C |
| G5 | 18.08±0.32A | 4.26±0.13A | 1.84±0.11A | 32.04±0.38C |
| LSD | 1.33 | 0.41 | 0.24 | 1.79 |

Number of samples: 5. Different letters mean significant difference at level of (P<0.05); GSH-Px: glutathione peroxidase; MDA: malondialdehyde; H2O2: hydrogen peroxide; LPO: lipid peroxidation. LSD: less significant differences.

Table 5 Effect of SILK on oxidative status and antioxidant defense in spleen exposed to mycotoxins

| Periods | Means ± Stander error | | | |
|---|---|---|---|---|
| | $H_2O_2$ (mmol/g pro) | MDA (nmol/g pro) | LPO (µmol/g pro) | GSH-px (U) |
| G1 | 15.35±0.27BC | 2.19±0.22BC | 4.06±0.04BC | 42.31±0.37B |
| G2 | 16.15±0.27B | 2.64±0.31BC | 4.88±0.16AB | 41.22±0.51B |
| G3 | 14.37±0.22C | 1.83±0.16C | 3.77±0.13C | 44.48±0.5A |
| G4 | 19.28±0.35A | 3.97±0.32A | 5.31±0.14A | 37.06±0.39C |
| G5 | 18.68±0.34A | 3.26±0.29AB | 5.11±0.36A | 38.66±0.48C |
| LSD | 1.24 | 1.13 | 0.84 | 1.91 |

Number of samples: 5. Different letters mean significant difference at level of (P<0.05); GSH-Px: glutathione peroxidase; MDA: malondialdehyde; H2O2: hydrogen peroxide; LPO: lipid peroxidation. LSD: less significant differences.

Table 6: The morbidity and mortalities through 10 days after challenge with local NDV isolate at 25 days

| Periods | Means ± Stander error | |
|---|---|---|
| | Morbidity % | Mortality % |
| G1 | 40% (8) d | 10% (2) d |
| G2 | 60% (12) c | 25% (5) c |
| G3 | 25% (5) e | 0% (0) d |
| G4 | 80% (16) b | 55% (11) b |
| G5 | 100% (20) a | 100% (20) a |

Number of chicks for each group: 20. Different letters mean significant difference at level of (P<0.05).

**Discussion**

The results of the present study showed a significant increase in the Abs against ND in G3 compared with other groups, this return to the role of SILK enhancing the immune response after vaccination with live vaccines through drinking water in the first periods of chick life, also increasing response after oil vaccination these results agree with (15), whom demonstrated the role of SILK in increasing the cytokines which are natural stimulators and mediators of immune cells towards infectious organism thus increasing special Abs against antigens (Ag) and have an effective and influential role in stimulating and differentiation of immune cells in natural and acquired immunity. Rashad and Mushtaq, (10), demonstrated the role of SILK in protection the chicks from ND infection after challenge with virulent local isolate of ND in the first day. Immune response in G1 was better than G2 the results were consistent with (10), who demonstrated that the immunogenicity of the live vaccine was a cellular immunity primarily dependent on Cytokines and its role in stimulating many defense cells against infectious antigens, While the vaccination with killed vaccines stimulating Humoral immunity, which relies on the stimulation of B cells that produce Ab according to amount of Ag in the killed vaccine without reliance on other defense cells, which explains the slow immunity available from the killed vaccines. The reason of increase in immune response in G5 after challenge virulent local isolate of ND, return to not vaccinated against ND to protect the chicks from infection, These results agreed with (16), who proved that the chicks were exposed to NDV infection in the third week

after decline the maternal immunity and highly increase of Abs with high mortality in chicks due to acute infection. But the low Ab titre in G4 return to toxic effect of mycotoxin these results agree with (4) show that mycotoxins damage the proteins found in cell junctions and thus reduce the transmission of electrical impulses, that transfer of cytokines between immune cells. Also metabolic products of innate toxins affect the immune system of the intestinal mucous, which in turn significantly affects the performance and productivity of the animal, because the stimulation of mucous immunity is important in protecting against many of the antigens that invade these surfaces and enter the body and cause diseases (5).

G3 show significant decrease in oxidative status like (H2O2, MDA and LPO) in hepatic and splenic tissues at 42 days after challenge with virulent local isolate of ND at 25 days, return to role of SILK in enhancement the immune response against NDV by protected the hepatic and splenic cell from infection with NDV these results agree with (17), whom demonstrated the role of acquired immunity from live vaccines in stimulated the immune cells forwards to antigens by increasing the cytokines thus prevented the Ags to damage the vital organs like (liver and spleen). Also agree with results of (18), whom proved decrease in oxidative status and increase in antioxidant defense in bird vaccinated with live vaccines of ND and challenge with virulent local isolate of ND. While G5 which showed opposite results because birds were not vaccinated and not protected against NDV these results go in the line with (16). A Group 4 shows opposite results because of the toxic mycotoxins effects which have been closely associated with ROS generation mainly include hydroxyl (OH-), O2 and hydrogen peroxide (19). Excessive increase of ROS promotes lipid peroxidation, which inhibits membrane functions by reducing membrane solubility and large changes in all enzymes and membrane receptors (20). In addition, MDA is formed at the end of fat peroxide and reflects the final grade of lipid oxidation in the body. Free radicals and lipid peroxides are controlled by the antioxidant defense system, which contains enzymatic compounds such as GR and GSH-PX (21).

Group 5 which showed opposite results because birds were not vaccinated and not protected against NDV and these results went with (16). Also Group 4 shows opposite results Due to the mycotoxins toxic effects, which is in agreement with (10).

## Conclusion

From all above-mentioned results, it could be concluded that SILK before vaccination helps protect the chicks from infection with NDV by enhancement and stimulate the immune cells, also make them ready to defend against any ages.

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## Conflict of interest

There is no difference or contradiction with the scientific and practical interest, but rather the attribution of scientific reality with new scientific efforts and ideas.

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