

EFFECT OF GARLIC EXTRACT AND ASCORBIC ACID SUPPLEMENTATIONS ON IMMUNE RESPONSE OF ND VACCINATED CHICKENS

BY

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ABSTRACT

This study was conducted to evaluate the effect of supplemental garlic extract and ascorbic acid on growth performance and immune response of ND vaccinated chickens. A total number of 120, one day old, male Cobb broiler chicks were divided into 4 groups, G1 birds kept non treated non vaccinated (-ve control), G2 birds vaccinated with NDV (2 doses Hitchner B1 and LaSota at 7th and 19th day of chicken age), G3 birds administered garlic extract (0.5 gm/kg B.wt) orally at 6 day old till 20 day old and vaccinated with NDV (2 doses Hitchner B1 and Lasota), G4 birds administered ascorbic acid (1gm/ Liter of drinking water) orally at 6 day old till 20 day old and vaccinated with NDV (2 doses Hitchner B1 and LaSota). All birds were monitored for the humeral immune response then challenged with virulent NDV at 40 days of age. HI titration of antibodies for NDV in serum was used to determine the humeral immune response of the chicks at 26, 33 and 40 days of chicken age. Average daily feed intake (ADFI), body weight gain (BWG) and feed conversion rate (FCR) were also determined. The obtained result showed that the protective immunity induced in the vaccinated groups was varied. A 90% protection was obtained in chickens vaccinated and supplemented ascorbic acid (G4), while ND only vaccinated group (G2) showed 80% protection, the obtained protection rates against challenge with virulent ND virus observed to be parallel to the results of HI- test. Garlic extract (G3) and ascorbic acid (G4) significantly improve the performance, BWG and FCR when compared with vaccinated non treated group (G2). Finally, the overall finding of this study revealed that vaccination programs should be directed toward eliciting and maintaining a high immunity level to ND vaccinated birds,

moreover, the use of garlic extract and ascorbic acid supplementation have an immunomodulatory effect during the ND vaccination program in chickens as well as a beneficial effect on the chicken performance.

Key words: Chickens, Garlic extract, Ascorbic acid, NDV.

INTRODUCTION

One of the most active research areas in recent years has focused on finding of new feed additives that stimulate immune reaction and improving performance of animal (**Majewska, 2001**).

Garlic supplement in broiler chickens has been recognized for its strong stimulating effect on the immune system in addition to its positive effect on digestion in birds due to the very rich aromatic oil content of garlic (**Demir et al., 2005**). Feeding dietary garlic reduced cholesterol levels of broiler meat without altering growth of the chickens or feed efficiency (**Konjufca et al., 1997**). Also **Lewis et al., (2003)** reported increased weight gain due to the garlic supplemented feed in broilers. It had earlier been reported that the mixtures of garlic and ginger significantly promoted growth of broiler chicks by enhancing digestion of food nutrients and probably improved food absorption through the wall of gastrointestinal tract (**Ademola et al., 2007**).

Poultry require supplementary dietary vitamins since common feed ingredients used in poultry production do not provide adequate quantities to meet the minimum requirements (**Leeson and Summers, 2001**). Poultry have the ability to synthesize ascorbic acid, or vitamin C (VC), in their body (**McDowell, 2000**). However, environmental and pathological stressors are known to alter VC use or synthesis or both in fowl (**Pardue and Thaxton, 1986**). Several researchers observed a significant improvement in growth of chicks by the addition of VC under high temperature. Broilers fed diets containing VC were less stressed due to having reduced body temperature and respiratory rates and showed higher feed intake than control birds (**Mckee, and Harrison, 1995**). Substantial available reports showed that under field conditions, feeding VC enhanced productivity, immune response, disease resistance and survivability under stressful conditions (**Zulkifli et al., 1996**).

The goal of this study was conducted to evaluate the effect of garlic extract and ascorbic acid supplementations on growth performance and immune response of ND vaccinated chickens.

MATERIALS AND METHODS

MATERIALS

I) **Drug**

1-a: Ascorbic acid (Hy-Mix- Vit C – 200) 20% :was obtained from Kiro vest company, Cairo, Egypt.

Dose: 1 gm/ Liter drinking water.

1-b: Garlic and aqueous extract preparation

Extract preparation: Fresh bulbs of garlic (*Allium sativum* L.) were purchased from local market, and then extracted according to **Iwalokun et al., (2004)**.

Dose: 0.5 gm\ kg B.wt (**Yassin, 2005**).

II) **Virus strains (Virulent strain of Newcastle disease virus).**

Virulent strain of Newcastle disease virus was kindly supplied by Newcastle Disease Vaccine Department, Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo. It was a local velogenic viscerotropic Newcastle disease virus (vvNDV) strain, isolated and identified by **Sheble and Reda (1976)** and used as a reference challenge virus.

III) **Vaccines (Vaccinal strain of Newcastle disease)**

1- Izovac - B1 Hitchner vaccine: Lentogenic strain against Newcastle disease (IZO S.P.via A Bianchi 9-25124 Brescia, Italy and Batch no. 0437 F) was titrated in 9-11 days old embryonated chicken eggs and its titer was calculated according to **Reed and Muench (1938)**. The vaccinal titer was $10^{9.5}$ EID₅₀/vial. It was used for vaccination of chickens at 7th day of chicken age. **Nobilis -LaSota vaccine:** Lentogenic strain against Newcastle disease (produced by Intervet International B.V.- Boxmeer-Holland and Batch no. 11650 BJ 0l) was titrated in 9-11 days old embryonated chicken eggs and its titer was calculated according to **Reed and Muench (1938)**. The vaccinal titer was $10^{9.5}$ EID₅₀/vial. It was used for vaccination of chickens at 19th days of chicken age.

IV) Chickens:

A total number of 120, one day old, male Cobb broiler chicks (40-45 gm) were purchased from local hatchery. All birds were reared in cages, kept in strictly isolated room and were provided with a commercial starter feed (Cairo Poultry Company).

METHODS

1- Washed chicken erythrocytes:

Chicken blood was collected in 4% sodium citrate from adult apparently healthy chicken. Washed RBCs were used as 1% concentration in saline for plate haemagglutination and haemagglutination inhibition test (OIE, 2004).

2- Plate Haemagglutination test:

The titration of the haemagglutination activity of the ND antigen was done according to the procedure described by **Beard (1989)**. The HA titer was calculated as the reciprocal of the highest dilution of virus giving a complete HA pattern.

3- Virus Re-isolation:

Liver, spleen, lung and brain samples were collected aseptically from dead and sacrificed birds post-challenge by vvNDV. Samples were prepared for virus reisolation according to **Anon (1971)**.

4- Grouping and Experimental design (Table, 1)

A total number of 120, one day old, male Cobb broiler chicks (40-45 gm) randomly distributed in 4-wire pens birds were divided equally into 4 groups. G1 birds kept non treated non vaccinated (-ve control), G2 birds vaccinated with NDV (2 doses Hitchner B1 and LaSota), (G3) birds administered garlic extract (0.5 gm/ kg B.wt) orally at 6 days old till 20 days old and vaccinated with NDV (2 doses Hitchner B1 and Lasota), G4 birds administered ascorbic acid (1 gm/Liter of drinking water) orally at 6 days old till 20 day old and vaccinated with NDV (2 doses Hitchner B1 and LaSota).

Blood samples were taken from 5 birds of each group pre vaccination (1 and 7 day) (6 day) and 19, 26, 33 and 40 days old. Blood was collected without anticoagulant and allowed to coagulate sera were collected for determination of humeral immunity and antibody titer.

The body weight (BW) of chickens in all groups and feed intake were recorded during the experiment.

5- Challenge test:

At the end of the experiment (40 day), 10 chickens from each group were challenged intramuscular with 0.2 ml suspension containing 10^6 EID₅₀ of NDV Velogenic strain.

6- Haemagglutination (HA) and Haemagglutination-inhibition test (HI):

HA and HI (beta- procedure) tests were carried out for assessment of humeral immune response following the techniques given by **Beard (1989)**.

7- Evaluation of growth performance:**7- a. Body weight and body weight gain**

Five chicks of each group were weighted at 26, 33 and 40 days of chicken's age and calculate the average body weight. Then the average body weight gain per chick was calculated by the difference between the value of body weight at particular age from the corresponding value of the next age.

7-b. Feed intake and feed utilization

The ration was provided regularly at the same time every day, feed intake per group was calculated through the difference between the weight of the offered ration and the remained part, and then divided by the number of the chickens of each group to obtain the average feed intake per chick. The feed utilization was expressed as feed conversion rate (FCR) which calculated according to **Ensminger, (1980)**.

8- Statistical analysis: The obtained data in the present study were statistically analyzed for analysis of variance (ANOVA) and least significant difference (LSD) at $p < 0.05$ as described by **Snedecor and Cochran (1981)** using computerized SPSS.

RESULTS AND DISCUSSION

Newcastle disease (ND) is an economically one of the important viral diseases of poultry (**Parede and Young, 1990**). The severity of the disease produced by a virulent strain of NDV has been found to be greatly influenced by the immune status of the host. It occurs in a variety of avian species and may cause respiratory distress, diarrhea, cessation of egg production, nervous signs, and high mortality (**Alexander, 1999**). The cellular and humeral response have been suggested to play important roles in the hosts defense against NDV infection, cell mediated immunity (CMI) has been reported as the first immunological

response, being detected as early as 2-3 days after ND vaccination (**Andereason and Latimer, 1989**).

In the present study, a trial was carried out to investigate the effect of garlic extract and ascorbic acid supplementations on growth performance and immune status of ND vaccinated chicks.

Effect of garlic extract and ascorbic acid supplementations on humeral immune response of ND vaccinated chicks.

HI (Log_2) titers of pre- and post-intraocular vaccination against Newcastle disease (Hitchner on day 7th & LaSota on day 19th of chicken life) in chicks were measured. HI (Log_2) titers of ND-maternally derived antibodies (Table, 2) were 5 ± 0.17 and 4 ± 0.11 on 1st and 7th day of chicken life. The titers decreased gradually to 3.2 ± 0.13 and 3.1 ± 0.09 on day 19th of chicken life in untreated unvaccinated (UUV) & vaccinated (ND only) groups, respectively. Also, the levels decreased gradually to 2.8 ± 0.19 and 3.8 ± 0.76 on day 26th of chicken life in G1 control -ve group & vaccinated group (ND only), respectively. Similar findings were obtained by **Saeed et al., (1988)** who reported that ND maternally derived antibody level declined to zero at day 25. The chicken treated with garlic extract had non significant changes on antibody titers of the vaccinated chickens. These findings were agreed with those recorded by **Pourali et al., (2010)** who stated that serum antibody titer levels against NDV were not significantly affected ($P < 0.05$) by dietary garlic supplementation. While, G4 (ascorbic acid treated group) revealed a significant increase of antibodies ($P < 0.05$) when compared with those of G1 negative control (UUV) & G2 (ND only) vaccinated groups which indicating it's immunostimulatory effect. These results were parallel to those of **Nada et al. (2009)** who concluded that VC enhance the immune response in chicken through increased macrophage, phagocytosis, total antioxidant capacity as well as increase protection rate against challenge with velogenic strain NDV.

Concerning the protection rate test, the result showed that the protective immunity induced in the vaccinated groups was varied. A 90% protection was obtained in chickens vaccinated and supplemented ascorbic acid (G4), while ND only vaccinated group (G2) showed 80% protection, the obtained protection rates against challenge with virulent ND virus observed to be parallel to the results of HI- test (Table, 3). The obtained protection rates against challenge with virulent ND virus observed to be parallel to the results of HI- test.

Effect of garlic extract and ascorbic acid on growth performance of ND vaccinated chicks:

The effect of tested medications on average body weight, body weight gain (BWG) and feed conversion rate (FCR) were summarized in Tables (4&5&6).

Dietary garlic extract supplementations to chicks had a significant increase in average body weight at 26, 33 and 40 days of chicken life as well as in body weight gain. These results were in agreement with those observed of **Kumar et al., (2005)**. Similarly, **Demir et al., (2003)** reported an improvement in BWG and FCR in broiler chickens fed in low concentrations of commercial garlic products. Moreover, **Lewis et al., (2003)** mentioned that garlic contains allicin, which promotes the performance of the intestinal flora, therapy improving digestion and enhancing the utilization of energy leading to improved growth.

Bampidis et al., (2005) stated that garlic has a beneficial effect on growth, digestibility and carcass traits leading to increased weight gain, feed efficiency, protein efficiency ratio (PER) and specific growth rate. In the same manner, **Lee et al., (2012)** concluded that garlic extract offered the greatest benefits of containing high levels of allicin, which due to its fermentation in the colon by beneficial bacteria, enhancing the gastrointestinal system and immune system. In addition, allicin increased the available energy for growth and improve the performance. Moreover, **Ademola et al., (2012)** found that dietary garlic and ginger mixtures significantly improved laying performance in terms of hen day production, egg weight, feed conversion, weight gain, final live weights of pullet growers and laying hens.

Vitamin C (VC) supplementation showed a significant increase in average body weight in 26, 33 and 40 days of chicken life as well as in their body weight gain. Similar results were approved by **Kassim and Norziha (1995)** who recorded an improved growth and feed efficiency in broiler chicks supplemented with VC, but he did not find improvement in feed conversion. Moreover, **Lohakare et al., (2005)** recorded that VC supplementation at higher levels (200 ppm) is beneficial for broilers during heat stress and improve the performance and immunity.

In our study the improvement in growth performance in both garlic extract and ascorbic acid medicated groups, is clear, which supports the findings of the above-mentioned researchers.

Table (1): Grouping and Experimental design:

Groups		Number of birds	Vaccination		Challenge 3 weeks post vaccination to 10 (40 day old chicks)
			Hitchner at 7 day age	LaSota at 19 day age	
G1	Negative control (UUV)	30	-	-	+
G2	Positive control (ND only)	30	+	+	+
G3	Garlic extract +ND	30	+	+	+
G4	Ascorbic acid +ND	30	+	+	+

UUV = Untreated-Unvaccinated

Table (2): Haemagglutination Inhibition (HI) (Log₂) titers of pre-and post-intraocular vaccination against Newcastle disease (Hitchner on day 7th & LaSota on day 19th of chicken life) in chickens (n=5).

Groups		pre-Hitchner vaccination (day)		(HI) (Log ₂) titers post-LaSota vaccination			
		1 day	7 day	0 week	1 st week	2 nd week	3 rd week
				19 day	26 day	33day	40 day
G1	Negative control (UUV)	5 ± 0.17	4 ± 0.11	3.2 ± 0.13 ^a	2.8 ± 0.19 ^c	2.5 ± 0.12 ^c	2.0 ± 0.10 ^c
G2	Positive control (ND only)			3.1 ± 0.09 ^a	3.8 ± 0.76 ^b	4.1 ± 0.23 ^b	5.3 ± 0.36 ^b
G3	Garlic extract +ND			3.9 ± 0.24 ^a	3.9 ± 0.33 ^b	4.3 ± 0.03 ^b	5.2 ± 0.14 ^b
G4	Ascorbic acid +ND			3.6 ± 0.13 ^a	4.5 ± 0.06 ^a	6.2 ± 0.17 ^a	8.3 ± 0.54 ^a

UUV = Untreated -Unvaccinated

The different letters at the same column mean that there was a significant change at P<0.05.

Table (3): Protected percentage of the challenge with VvNDV at 3rd week post LaSota vaccination

Group		No of birds	Live birds	Dead birds	Protective %
G1	Negative control (UUV)	10	0	10	0%
G2	Positive control (ND only)	10	8	2	80%
G3	Garlic extract +ND	10	8	2	80%
G4	Ascorbic acid +ND	10	9	1	90%

UUV = Untreated -Unvaccinated

Table (4): Effect of garlic extract and ascorbic acid on average body weight (gm) of ND vaccinated chicks (n=5).

Group	Average body weight (gm)			
	at 0 week post vaccination (19)	at 1 st week post vaccination (26 day)	at 2 nd week post vaccination (33 day)	at 3 rd week post vaccination (40 day)
G1 Negative control (UUV)	419.9±53.49 ^b	709.9±33.44 ^b	1232.9±22.19 ^b	1527.9±34.19 ^b
G2 Positive control (ND only)	415.9±43.30 ^b	792.9±23.59 ^b	1219.2±13.09 ^b	1520.9±52.53 ^b
G3 Garlic extract +ND	520.1±19.01 ^a	883.7±43.29 ^a	1272.2±63.39 ^a	1663.1±45.31 ^a
G4 Ascorbic cid +ND	516.9±53.30 ^a	873.7±29.40 ^a	1299.2±44.06 ^a	1686.9±32.37 ^a

UUV = Untreated -Unvaccinated

The different letters at the same column mean that there was a significant change at P<0.05.

Table (5): Effect of garlic extract and ascorbic acid supplementations on average body weight gain (gm) and of of ND vaccinated chicks.(n=5).

Group	Average body weight gain (gm)		
	at 1 st week post vaccination (26 day)	at 2 nd week post vaccination (33 day)	at 3 rd week post vaccination (40 day)
G1 Negative control (UUV)	290.00 ± 19.60 ^b	523.00 ± 11.21 ^b	1108±34.19 ^b
G2 Positive control(ND only)	307.6± 45.70 ^b	496.3 ± 07.00 ^b	1105±31.11 ^b
G3 Garlic extract+ND	363.00 ± 12.31 ^a	588.2 ± 09.68 ^a	1142 ±23.31 ^a
G4 Ascorbic cid+ND	356 ± 05.82 ^a	525.5 ± 15.438 ^a	1170 ±37.14 ^a

UUV = Untreated -Unvaccinated

The different letters at the same column mean that there was a significant change at P<0.05.

Table (6): Effect of garlic extract and ascorbic acid supplementations on feed conversion rate % of ND vaccinated chickens

Group	Feed conversion rate %		
	at 1 st week post vaccination (26 day)	at 2 nd week post vaccination (33 day)	at 3 rd week post vaccination (40 day)
G1 Negative control (UUV)	1.430±12.17 ^b	1.588±45.10 ^b	1.440±14.10 ^b
G2 Positive control(ND only)	1.950±04.10 ^a	1.850±11.90 ^a	1.917±31.11 ^a
G3 Garlic extract+ND	1.547±64.07 ^b	1.470±36.95 ^b	1.481±22.19 ^b
G4 Ascorbic cid+ND	1.540±43.10 ^b	1.540±56.82 ^b	1.430 ±24.21 ^b

UUV = Untreated -Unvaccinated

The different letters at the same column mean that there was a significant change at P<0.05.

CONCLUSION

By the results of the present study, we can support the concept that humeral immunity to NDV is a key component in the protection against ND. Therefore, vaccination programs should be directed toward eliciting and maintaining a high antibody level to NDV in flocks of birds by using immunostimulant feed additives like ascorbic acid during the ND vaccination program in chickens. Moreover, garlic extract can be used in chicken flocks to improve its performance.

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المخلص العربي

تأثير مستخلص الثوم وحمض الأسكوربيك علي الإستجابة المناعية

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تم إجراء هذه الدراسة لمعرفة التأثير الفارماكولوجي المناعي لمستخلص الثوم وحمض الأسكوربيك علي المناعه المصلية في الدجاج المحصن ضد مرض النيوكاسل ، كما تم دراسة تأثير تلك المواد علي معدل الزيادة في وزن الجسم للدجاج وكذلك الوزن الحي وعلي معدل التحول الغذائي . وقد اجريت هذه الدراسة علي عدد ١٢٠ كتكوت والتي قسمت بالتساوي الي أربع مجموعات .المجموعة الأولى (٣٠ كتكوت) تركت ضابطه غير محصنة وغير معالجة، المجموعة الثانية (٣٠ كتكوت) ضابطه ايجابييه وتم تحصينها بلقاح الهيتشينو (بالتقطير العيني) في اليوم السابع من عمر الدجاج ولقاح لاسوتا (بالتقطير العيني) عند عمر تسعة عشر يوما. المجموعة الثالثة (٣٠ كتكوت) تم تحصينها بلقاح النيوكاسل (الهيتشينو واللاسوتا) وعولجت بمستخلص الثوم (٥، جم /كجم وزن حي) عن طريق الفم قبل التحصين الأول بيوم (عمر ستة ايام) ولدة اسبوعين متتالين حتي عمر عشرون يوما . المجموعة الرابعة (٣٠ كتكوت) محصنة بلقاح النيوكاسل (الهيتشينو واللاسوتا) ومعالجة بحمض الأسكوربيك (١ جم /لتر) عن طريق ماء الشرب قبل التحصين الأول بيوم (عمر ستة ايام) ولدة اسبوعين متتالين (عمر عشرون يوم). تم أخذ عينات الدم (٥ من كل مجموعة) لمدة ثلاثة اسابيع متتالية بدون مضاد التخثر ثم فصل المصل لقياس مستوى الأجسام المناعية لمرض النيوكاسل. كما تم قياس أوزان الدجاج لمدة ثلاثة اسابيع متتالية وحساب معدل الزيادة في وزن الجسم للدجاج وكذلك الوزن الحي ومعدل التحول الغذائي . وقد اسفرت النتائج عن إرتفاعا معنويا في الأجسام المناعية لمرض النيوكاسل في المجموعة المعالجة بحمض الأسكوربيك عن المجموعات الأخرى. بالنسبة للتأثير علي الوزن الحي لوحظ زيادة في أوزان الدجاج في المجموعات المعالجة بمستخلص الثوم وحمض الأسكوربيك. كما ادي استخدام هذه العقاقير الي حدوث تحسن في التحول الغذائي بالمقارنة بالمجموعة الضابطة الايجابية.

الخلاصة: ينصح باستخدام حمض الأسكوربيك كمادة رافعة للمناعة اثناء تحصين الدجاج ضد مرض النيوكاسل خاصة تلك التي تتعرض للإجهاد كما ان مستخلص الثوم قد أحدث تحسنا ملحوظا في اوزان الدجاج وكذلك معدل التحول الغذائي للدجاج .