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Effect of wheat germ oil and ascorbic acid supplementation on the immune response of broiler chicks to ND vaccines

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SUMMARY

In the present study, the effect of wheat germ oil as source of vitamin E and ascorbic acid as source of vitamin C on the immune response of broiler chicks to ND vaccine was determined. Four groups of chicks were used. Group 1 was vaccinated with 2 doses of ND vaccines (Hitchner then Lasota) and simultaneously supplemented with wheat germ oil via oral administration for 15 days. Group 2 was vaccinated with 2 doses of live ND vaccines (Hitchner then Lasota strains) and simultaneously supplemented with ascorbic acid via oral administration for 15 days. Group 3 was vaccinated with 2 doses of ND vaccines (Hitchner then Lasota strains). Group 4 was kept non-vaccinated as control chicks. All birds were monitored weekly for the humeral and cellular immune response then challenged with the virulent NDV at 35 days of age. HI test for titration of antibodies for NDV, phagocytic activity, Nitric oxide, Lysozyme activity and total antioxidant in serum were used to determine the immune response of the chicks. Protective immunity induced in the vaccinated groups was varied. 80 and 60 % protection were obtained in chicks vaccinated and supplemented with wheat germ (group 1) and ascorbic acid (group 2); respectively. Live ND vaccinated group showed 60% protection. The present study reports the effect of supplementation of wheat germ (vitamin E) as immunostimulant in augmentation of the immune response ND vaccines.

INTRODUCTION

Wheat germ oil contains more than 60% of polyunsaturated fatty acids of omega3, omega6 family and also considered as a source of vitamin E. Vitamin E is an essential

antioxidant present in body tissues and is considered the first line of defence against lipid peroxidation by protection of cell membrane from free radical injury (Sealey and Gatlin, 2002). Interestingly, in chicks dietary ascorbic acid could be necessary to achieve best performance under specific conditions, especially in environmental stress (Chen et al., 2003). Although chicken possess the innate ability to synthesize ascorbic acid, diet supplementation of amino acid may be beneficial (Pardue et al, 1985).

Vitamins C and E are among the most important nutrients influencing the immune system and the supply of vitamins can reduce mortality, improve performance and increasing specific and non specific immune responses (Shiau and Hsu, 2002 and Puangkaew et al., 2004).

Dietary ascorbic acid was shown to increase antibody response to sheep red blood cells (Pardue et al., 1985), increase the resistance to a combined Newcastle disease virus, Mycoplasma gallisepticum infection and to a secondary E.coli infection. Ascorbic acid is thought to be important in optimizing the function of the immune system through enhancement of neutrophil production and also through protection against free radical damage (Bendich et al., 1986). Ascorbic acid is found in high concentration in leukocytes and being utilized at higher rate during infection and phagocytosis (Thomes and Holt, 1978). Both vitamins are potent antioxidants that offer protection against damage to various tissues (Adham et al., 2000), prevent immunosuppression (Belo et al., 2005), improve respiratory burst of phagocytes (Ortuno et al., 2001) and enhance phagocytic activity (Puangkaew et al., 2004). Furthermore, ascorbic acid supplementations enhance interferon production and increase T lymphocyte response to conconavalin A in mice (Siegal, 1974 and 1975).

The purpose of this study is to determine the immunological effect of wheat germ oil and vitamin C supplementation on chickens vaccinated with NDV vaccines.

MATERIAL AND METHODS

Experimental birds: A total of 120 one-day-old chicks were obtained from poultry production company, Egypt. They were floor reared, fed on a commercial balanced Poultry ration, and kept under good hygienic conditions throughout the experiment.

Ascorbic acid (Vitamine C): It was obtained from Memphis Co. for pharm & chemical. Industry Cairo - Egypt.

Wheat germ oil (Vitamin E): It was obtained from Sedico, Pharmaceutical Company Cairo, Egypt.

Viruses:

A. Challenge NDV strains: Velogenic viscerotropic strain of NDV was obtained from Serum and Vaccine production. Institute, Abbasia, Egypt.

B. NDV vaccine: The lentogenic strains of NDV (Lasota strain, Hitchner strain) were purchased from Intervet local agency, Cairo, Egypt. They were used for vaccination of chickens at 7 days of age (Hitchner strain) and 21 days of age (Lasota strain).

Candida albicans: It was kindly supplied by the Dept. of Mycology, Animal Health Research Institute, Dokki-Giza. Twenty four hours old subculture of candida albicans was used as antigen for evaluation of macrophages phagocytic activity.

Media, reagents and chemicals: RPMI 1640, Ficoll-hypaque, fetal calf serum, Giemsa strain, heparin preservative free (500 IU/ ml) were obtained from Sermend Lab., Germany.

Micrococcus lysodeikticus: Sigma chemical Co., St.louis, USA

Griess reagent: Sulphanimide, Naphthyl ethaylene diamine-di-hydrochloride, H3PO4 (it was freshly prepared).

Total antioxidant capacity kit: It was obtained from Biodiagnostic Company. Cat. No TA2513. Egypt.

Measurement of phagocytic activity of peripheral blood monocytes using candida albicans:

Separation of peripheral blood mononuclear cells using ficoll hypaque density gradient was carried out as described by Boyum, (1968). Mononuclear cell layer was collected, washed and resuspended in RPMI-1640 supplemented with 10% fetal calf serum and viability was done (Hanks and Wallace, 1985). The test was performed according to procedure described by Anthony et al., (1985) and Chu and Dietert, (1989).

Phagocytic percentage and index were calculated as follows:

$$\text{Phagocytic \%} = \frac{\text{No. of macrophages ingesting candida}}{\text{Total No. of Macrophages}} \times 100$$

$$\text{Phagocytic index} = \frac{\text{No. of macrophages ingesting more than 3 blastospores}}{\text{Total No. of macrophages with ingested blastospores}}$$

Haemagglutination inhibition test (HI): It was carried out as described by Beard, (1989).

Measurement of Lysozyme activity: It was carried out by agarose gel lyses assay, according to the method described by Schultz, (1987). Briefly, lysoplates were prepared by dissolving 1% agarose in 0.06 M PBS at pH

6.3. Five hundreds mg of uniform suspension of Micrococcus lysodeikticus in 5ml saline were added to 1 liter of agarose. Plates were poured, then, 25ul of serum samples and standard lysozyme were added in each wells. After 18 hours, the cleared zones diameter was measured to both standard lysozyme and serum sample then the concentration was estimated.

Determination of Nitric oxide: It was carried out according to Green et al., (1982). Briefly 100ul of serum sample was transferred into flat-bottom 96-well ELISA plate and 100ul of Griess reagent were added to each well. The optical density was determined at 570nm with an ELISA plate reader. Absorbent of test samples was converted to 10um of nitrite by comparison with absorbent values of sodium nitrite standard curve within linear curve fit.

Total antioxidant capacity: It was determined according to Koracevic et al., (2001).

Total antioxidant concentration (m/L) was calculated as follows: $x = A_B - A_{SA} \times 3.33$, where A_B = absorbance of blank; A_{SA} = absorbance of sample

Experimental design:

One hundred and Twenty of one-day old commercial chicks were used in this study and were divided into 4 groups 30 chicks each:

Group (1): birds administered with 14.2 mg/Kg wheat germ oil orally at 6 day old till 22day old and vaccinated with NDV (2 doses Hitchner B1 and Lasota).

Group (2): birds administered with 1gm vitamin C/ liter of drinking water at 6 day old till 22 day old and vaccinated with NDV (2 doses Hitchner B1 and Lasota).

Group (3): birds vaccinated with NDV (2 doses Hitchner B1 and Lasota).

Group (4): birds non - vaccinated and non-treated.

Two blood samples were taken from 5 birds of each group weekly intervals for 5 successive weeks via heart puncture. One sample was taken in sterilized plastic centrifuge tube containing heparin for separation of mononuclear cells used in phagocytic activity. Whereas the other sample was taken without anticoagulant for serum separation and used for detection of Heamagglutination titer (HI), lysozyme activity, Nitric oxide and total antioxidant capacity.

At the end of experiment, 10 chickens from each group were challenged intramuscular with 0.2 ml suspension containing 10^6 EID50 of NDV Velogenic strain (challenge test). Birds were kept under observation for 2 weeks with daily recording of symptoms and deaths.

Statistical analysis:

Data obtained were statistically analyzed using analysis of variance and comparing between groups were performed

using least significant difference (LSD) at P< 0.05 according to Petrie and Watson, (1999) and computerized using SPSS.

RESULTS

The effect of wheat germ oil and vit.C on phagocytic activity of macrophage in experimental groups of chickens is presented in Table (1). Chickens vaccinated, treated with germ oil (group 1) revealed significant increase in phagocytic % and index at 2nd , 3rd ,4th and 5th weeks compared to only vaccinated (group 3) or non vaccinated non treated (group 4). On the other hand, chickens treated with vit.C showed significant increase in phagocytic% at 3rd and 5th weeks, also phagocytic index at 2nd ,3rd ,4th and 5th weeks compared to group 3 or group 4 (Table 1). Data of control chicks (group 4) showed the wanning of maternal antibodies. Both chicken treated with wheat germ oil and vit C (group 1 and 2) showed high antibody titers at 2nd and 5th week of age in comparison to those vaccinated and non treated chickens as well as control group (group 3 and 4). Higher antibody titers were noticed in chicken treated with vit C at 2nd week of age among groups. After challenge, HI titers in both treated groups were lower than the vaccinated non treated group (Table 2). The effect of wheat germ oil and vit.C on total serum antioxidant, lysozyme and nitric oxide are presented in Table (3). Chickens vaccinated, treated with wheat germ oil (group 1) revealed significant increase in total antioxidant capacity at 2nd and 3rd weeks compared to vaccinated non treated chickens (group 3). However, chickens vaccinated, treated with vit.C (group 2) showed significant increase in total antioxidant capacity at 2nd week of the experiment compared to (group 3). Concerning the lysozyme activity, both treated groups (1 and 2) showed significant increase in serum lysozyme activity between 1-2 weeks through the experiment. In contrast, nitric oxide was decreases in birds of group 1 at 3rd week compared to birds of group 3 (Table 3).

The challenge of birds in all groups revealed different level of protection among groups ranging from 80%. (group 1); 60% (group 2 and 3) and 0% in the control group 4 (Table 4).

Table (1) Phagocytic % and index of chicken macrophages treated with wheat germ oil and Ascorbic Acid and/ or vaccinated with NDV vaccines

Group / Time	2 rd week		3 rd week		4 th week		5 th week	
	Phagocytic %	Phagocytic index	Phagocytic %	Phagocytic index	Phagocytic %	Phagocytic index	Phagocytic %	Phagocytic index
1	a B 62 ± 1.2	a B 0.22 ± 0.01	a B 64 ±2.80	a B 0.21±0.01	a B 66 ± 1.8	a B 0.19 ± 0.09	a B 54 ±0.8	a B 0.18 ± 0.01
2	C 61 ± 1.3	a C 0.22 ± 0.03	a C 64 ± 3.7	a C 0.17±0.02	C 63 ± 1.8	a C 0.20 + 0. 03	a b C 47 ± 1.2	a C 0.16± 0.09
3	A 58 ± 1.4	A 0.11 ± 0.01	A 57 ± 1.1	A 0.10±0.05	A 59 ± 0.8	A 0.10 ± 0.05	A 42 ± 1.8	A 0.09 ±0.005
4	a b C 51 ± 0.8	b C 0.08 ±0.005	b C 54 ± 4.1	b C 0.08+0.008	a b C 47 ± 3.7	b C 0.08 + 0.008	a b C 37 ± 1.6	b C 0.08 ±0.008

Aa.Bb significantly different between two comparison groups in the same column against capital letter at $P \leq 0.05$ using least significant difference (LSD)

Table (2): Geometric mean NDV titer in serum of the chickens of the treated and non treated groups as measured by Heamagglutination inhibition test.

Group / Age	Group1	Group2	Group3	Group4
1st week of age	2.8	3.2	4	3
2nd week of age	8	16	4.9	2.5
3rd week of age	3	4	4	0
4th week of age	2.3	2.6	3.5	0
5th week of age	9.8	9.8	3	0
9th week of age (2 weeks post challenge)	35.3	25.6	48.5	-

Group (1): vaccinated chickens and treated with wheat germ oil
Group (2): vaccinated chickens and treated with ascorbic acid
Group (3): vaccinated chickens and non treated
Group (4): control non vaccinated non treated chickens

Table (3): Serum Total antioxidant (mM/L), Serum lysozyme (ug/ml) and nitric oxide (uM/ml) in chickens treated with wheat germ oil and vit.C.

Group / Time	2 nd week			3rd week			4 th week			5 th week		
	Anti-oxidant	lysozyme	Nitric oxide	Anti-oxidant	lysozyme	Nitric oxide	Anti-oxidant	lysosyme	Nitric oxide	Anti-oxidant	lysozyme	Nitric oxide
1	A b 1.4 ± .06	24± 4.3	14± 1	A 1.1±0.15	A 24±4.3	A 14±1.0	1.3±0.17	24±4.3	21±2.7	A 1.9±0.2	32±2.2	23±1.4
2	B 1.2 ±0.15	a 28± 3.7	16±0.75	B 1.2±0.14	B 28±3.7	16±0.5	1.5±0.33	30±8.7	21±1.1	1.5+02	24±4.3	25±3.6
3	ab 0.7±0.13	a 17± 0.5	15±0.25	0.9±0.02	ab 14±2.3	18±1.4	a 1.2±0.16	28±3.S7	20±1	1.5±0.12	28±3.7	23±1.3
4	ab 0.6±0.21	20± 3.7	16±0.64	a b 0.7±0.14	20± 3.7	16±0.3	0.9±0.16	24±4.3	18±1.6	a 1.0±0.08	24±4.3	19±2.1

Aa.Bb significantly different between two comparison groups in the same column against capital letter at $P \leq 0.05$ using least significant difference (LSD)

Table (4): Protection rate of different groups of chickens against challenge with vvNDV

Group No.	Total no. of birds	Challenge test at 35 days of age		
		No.of dead birds	No. of survival	Protection %
Group (1)	10	2	8	80
Group (2)	10	4	6	60
Group (3)	10	6	4	40
Group (4)	10	10	-	0

Group (1): NDV vaccinated chickens and treated with wheat germ oil
Group (2): NDV vaccinated chickens and treated with ascorbic acid
Group (3): NDV vaccinated chickens and non treated
Group (4): control non vaccinated non treated chickens

DISCUSSION

In the present study, a trail was carried out to investigate the effect of oral administration of wheat germ oil and vit.C on immune status of chickens and vaccination effectiveness to NDV vaccine. The chickens treated with wheat germ oil or vitamin C revealed significant increase of antibodies at 2nd and 5th of age among groups and indicating their immunostimulatory effect. This observation is consistent with results obtained by Wu et al., (2000) who found that increased number of IgG antibody secreting cells in the spleen, increase number of IgM cells in the bursa in ascorbic acid fed chicken. Also, Gross, (1992) stated that feeding ascorbic acid to chickens may improve humoral immune responses to pathogens. Supplimentation of vitamins to weaner diet increased concentration of IgM throughout the weaner period in piglets (Lauridsen and Jensen 2005). In addition, Montero et al., (2001) and Ortuno et al., (2003) reported the supplementation of vit.C and/or E in the diet of fish has shown greater efficiency on the production of bacterial fighting antibodies. Meydani et al., (1988) found that vit E supplementation in mice increase lymphocyte proliferation. As suggested by Navarre and Halver (1989), the increased production of antibodies may be related to increased activity of lymphoid cells. Waagbo et al., (1993) reported high level of specific antibodies after vaccination in fish fed vit C. Moreover, Hossain et al., (1998) demonstrated that vit E supplemented in diet of broiler breeders or vit E injection in eggs results significant increase in antibody titers to killed Newcastle disease vaccine. On the other hand, Prinz et al., (1977) recorded long term treatment of vit C increased serum IgA and IgM but not IgG levels in human.

A significant increase in phagocytic percentage and phagocytic index of macrophages in both groups treated with wheat germ oil and treated with vit.C was determined when compared with control. These results agree with Mulero et al., (1998) who reported an increase of leukocyte phagocytosis, migration and respiratory burst of fish treated in vitro with vit.C and/or vit.E for 48hours. Tengerdy (1989) indicated that bactericidal activity of mammalian phagocytes increased when vit E was provided at moderate dosages. Furthermore, Hamada et al., (2002) found that intragastric administration of vit E into rats showed increased phagocytosis of alveolar macrophage through activating macrophage-activating factor (MAF) in bronchoalveolar lavage fluid of lungs. Also, Ai et al., (2006) recorded significant

increase in phagocytosis percentage and respiratory burst in fish fed diets supplemented with vit.C for 8 weeks. Furthermore, Hung et al., (2007) found that tilabia fed diet containing singular or combination of vitamins A, C, and E increased macrophage proliferation, maintained macrophage viability and protective activity. Moreover, Tewary and Patra (2008) recorded fish fed vitamin C supplemented diet showed higher phagocytic activity, phagocytic index and burst activity. In contrast, Hogan et al., (1992) have shown that neutrophils from cows treated with vit.E exerted a greater intracellular kill of bacteria in comparison with control but their phagocytic index and percentage of phagocytic cells remained unchanged. In the present study, results of the effect of vit.C and wheat germ oil in lysozyme activity indicated that in chickens vaccinated and treated with vit.C (group 2) showed significant increase in serum lysozyme activity at 2nd and 3rd weeks. Whereas, chicken vaccinated, treated with wheat germ oil (group 1) revealed significant increase at 2nd week only compared to control group. In a previous study by Kiron et al., (2004) such significant increase in serum lysozyme activity in fish fed diet containing vit.E was noted. Also, Ai et al., (2006) observed significant increase in lysozyme activity in fish fed diet supplemented with vit.C. Furthermore, Pen et al., (2007) found that fish diets containing ascorbic acid revealed higher lysozyme activity of mucous and serum than control.

Nitric oxide assay on the tested samples indicate that chicken treated with wheat germ oil showed significant decrease in serum nitric oxide. Nitric oxide (NO) is a potent effector's molecule, synthesized from L-arginine through the action of Nitric oxide synthase (Moncada et al., 1991).

Macrophage-derived Nitric oxide plays an important role in host defense in response to infection, contributing to the elimination of the offending microbes (Malawistal et al., 1992). The same observation was reported by Beharka et al., (1997) who found that peritoneal macrophage from old mice fed diet supplemented with vit.E produced significantly lower level of nitric oxide than old mice fed control diet. The mechanism by which vit.E reduces (NO) production is still unclear. Vit.E could act as a pure anti-oxidant, reducing (NO) levels as it is produced, or alternatively, Vit.E could inhibit the induction of inducible nitric oxide synthase by preventing of its gene via inhibition of nuclear transcription factor. (NFKB). The total antioxidant capacity was higher in chicken treated with wheat germ

oil at 2nd week. These results were in accordance with Kiron et al., (2004) who recorded high level of plasma antioxidant capacity in fish fed vit E. Vitamin C and vitamin E are important to animal health and act as natural antioxidants to remove harmful free radicals produced through normal cellular activity and from environmental stressors. In addition, they may regulate cellular events. Paranich et al., (2000) confirmed that wheat germ oil is a source of easily assailable vit E, when rat administrated orally wheat germ oil lead to rapid increase in the content of vit E in the brain, liver, heart, lung, kidneys and spleen and lipid peroxidation process was inhibited.

On the level of protection against velogenic Newcastle disease virus, chicken treated with wheat germ oil showed the highest protection 80%, while chicken treated with vit C showed 60% protection. The results are coincide with those obtained by Okishima et al., (1996) who examined the effect of vit E supplementation on the decrease of cellular immune function following the development of murine AIDS. They reported high vit E (500 or 2500 Iu/Kg diet) suppressed the enlargement of spleen, increased production of gamma interferon and suppressed production of tumor necrosing factor α. Similar results confirm the protection factor of vitamins. Mortiguchi et al., (1996) reported vit E supplementation to rats for 4 weeks accerelate recovery of x-ray irradiation-induced decrease in cellular immunity. Also, Ai et al., (2006) found that fish fed with vit C for 8 weeks and challenge with (vibrio harveyi) showed that lower cummulative mortality compared to control group. Furthermore, Tewary and Patra (2008) recorded fish fed vit C revealed high protection level against challenge with Aeromonas hydrophilia.

In conclusion, wheat germ oil seems to be more active than vitamin C on macrophage phagocytosis and protection test. The mode of action of wheat germ oil upon phagocytosis seems involve the protection of membrane unsaturated lipids, which are essential for maintain membrane fluidity against possible oxidation (Blazer and Wolke 1984).

Interpretation of these results indicate that wheat germ oil and vitamin C may be sufficient to enhance immune response in chicken through increased macrophage phagocytosis, total antioxidant capacity, serum lysozyme production while decreasing (NO) production was observed in vit E group. As well as increase protection rate against challenge with velogenic strain NDV.

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تأثير زيت جنين القمح وحمض الأسكوربيك على الاستجابة المناعية لكتاكيت التسمين المحصنة بلقاح النيوكاسل

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تم دراسة تأثير زيت جنين القمح كمصدر لفيتامين هـ وحمض الأسكوربيك كمصدر لفيتامين ج على الاستجابة المناعية لدجاج التسمين المحصن بالنيوكاسل.استخدمت أربع مجموعات للدراسة:

المجموعة الأولى تم تحصينها بلقاح النيوكاسل وفي نفس الوقت تم تجريعها بزيت جنين القمح عن طريق الفم لمدة 15يوم.

المجموعة الثانية تم تحصينها بالنيوكاسل وفي نفس الوقت تم تجريعها بحمض الأسكوربيك لمدة 15 يوم.

المجموعة الثالثة تم تحصينها فقط بلقاح النيوكاسل.

المجموعة الرابعة مجموعة ضابطة للتجربة لم تحصن ولم تجرع.

جميع المجموعات تم تقيمها أسبوعياً لقياس الاستجابة المناعية السائلة والخلوية وعند عمر 35يوم تم عمل اختبار التحدي بالنيوكاسل شديد الضراوة وتم قياس الأجسام المناعية للقاح النيوكاسل وكفاءة خلايا الماكروفاج الابتلاعية (Phagocytosis) ومستوى أكسيد النيتريك والليسوزيم وكفاءة مجموعة مضادات الأكسدة في السيرم.أظهرت النتائج عن نسبة حماية 80% ضد فيروس النيوكاسل في المجموعة المجرعة بزيت جنين القمح بينما كلا من المجموعة المجرعة بحمض الأسكوربيك والمجموعة المحصنة فقط بلقاح النيوكاسل أظهرت 60% وأوضحت النتائج إن زيت جنين القمح أعطى مناعة وصد لفيروس النيوكاسل بمستوى أعلى من حمض الأسكوربيك.