

IMMUNOLOGICAL AND BIOCHEMICAL EFFECTS OF NIGELLA SATIVA AND ULTRA NATURAL PLUS ON TURKEYS VACCINATED WITH NEWCASTLE AND TURKEY RHINOTRACHEITIS VIRUS VACCINES

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SUMMARY

The experiment was designed to study the immunological and biochemical effects of Nigella Sativa (NS) and Ultra-Natural plus (UP) on turkey poult vaccinated against Newcastle disease (ND) and Turkey Rhinotracheitis (TRT) virus vaccines. The results indicated the immunostimulant effect of both NS and UP but the former was more superior. Results at 4 weeks of age proved that Nigella Sativa gives ND HIGM titer, Optical density for TRT, Total serum protein and gamma globulin were 8.6, 0.943, 2.88 and 1.02 versus of 7.4, 0.922, 2.55 and 0.85 in Ultra- Natural plus; respectively. Nigella Sativa challenged groups using NDV and TRTv showed 93.34% and

100%; respectively while Ultra-Natural plus challenged groups showed 86.67% and 93.34%; respectively.

INTRODUCTION

Nowaday, plant extracts are assuming a position of prime importance in poultry industry. Most poultry men and researchers have been advocating both growth-promoting and immunostimulating effects of number of plant origin ingredients to improve physical performance. Exposure of birds to certain pathogens leads to reduction in their immune response to infection or vaccination with other pathogens (Springer et al., 1983). The application of immunostimulant is not only to

raise the resistance of the birds but also to improve the immune response to vaccination (Asify, 1990 and Awwad et al., 2003). The routine use of drugs to modify or modulate an animal immune competence as a part of therapeutic management of specific clinical conditions is still at a very preliminary stage in veterinary medicine (Brander et al 1991). *Nigella Sativa* is a herbaceous plant which is a member of Ranunculacea family containing 38.7% crude fat (75.2% of which oleic and linoleic acid) and 21% protein (so it is considered as a good source of protein), in addition to potassium, phosphorus, magnesium and sodium. *Nigella Sativa* oil was found therefore to increase the immunity and maintained good health (Abd-El Aal and Attia, 1993). Ultra-Natural plus is biostimulating agent for live stock and poultry as it contains natural ingredients of *Yucca Schidigera* extract, seaweed extract and many enzymes. The use of *Yucca Schidigera* extract in poultry feed is good alternative to maintain metabolic and environmental ammonia levels within acceptable limits to improve the productivity parameters. Seaweed extract (*Ascophyllum nodosum*) is a marine kelp, an excellent source of over sixty minerals that are naturally chelated by the plant, plus twenty two amino acids, twelve vitamins and polysaccharides. It is also an excellent source of protein bound iodine which is especially important for stimulation of immunity and endocrine function (Al-Bar et al 1993 and Adel Esa and Sahar Abd- Elhamied 2003). This work aims to study the immunopotential effects of both

Nigella Sativa and Ultra-Natural plus plant on turkey pouls vaccinated with both ND and TRT virus vaccines.

MATERIAL AND METHODS

1- Experimental birds:- A total number of 160 one day old turkey pouls were obtained from commercial non vaccinated flocks whose their sera were free from TRTv antibodies by AGPT with low levels of NDv antibodies by HI test.

2- Specific pathogen free (SPF) eggs :- were obtained from Koum-Oshim SPF producing farm and was used for preparation of NDV HA antigen and virus titration as well as virus reisolation from challenged turkey pouls (Anon 1970).

3- Viral vaccinal Strains:-

- Turkey Rhinotracheitis: live attenuated TRTv vaccine of titer $10^{2.5}$ TCID₅₀/dose was kindly obtained from Intervet international B.V.- Holland.
- Newcastle disease live virus vaccines:- Both Hitchner B1 strain vaccine of a titer $10^{9.5}$ EID₅₀ / ml ($10^{6.5}$ EID₅₀ /) and La Sota strain of a titer $10^{9.5}$ EID₅₀/ ml ($10^{6.5}$ EID₅₀/ dose) was obtained from IZO SPA via A. Bianchi, 9, 25124 Brescia (Italy).
- Virulent Turkey Rhinotracheitis virus strains for challenge test:- Locally isolated virulent strain of TRTv of titer 10^6 TCID₅₀/ml was obtained from the Animal Health Research Institute, Immunity Unit, Dokki, Cairo.

- A velogenic vesicotropic Newcastle disease virus strain having a titer of 10^8 EID₅₀ was obtained from veterinary Serum Research Institute, NewCastle disease unit, Abbasia, Cairo.
- Positive sera against NDv and TRTv were obtained from virology Department, Fac. of Vet. Med. Beni-Suef University and used as positive control.
- immunomodulators:-**
- Nigella Sativa seed: was purchased from Horticulture Research Institute and grinded to be added in feed as 1% (Khodary et al., 1996).
 - Ultra-Natural plus:- Biostimulant agent produced by Ultra Bio-logics Inc. Canada, Batch No VUNP0302 was added also 0.2 ml/ liter to the drinking water successfully for the first 5 days and repeated at 2nd week for another 5 days.
 - Enzyme Linked immunosorbent assay (ELISA), commercial ELISA kits was obtained from pathosure Avian Rhinotracheitis Cambridge Veterinary Sciences LTd, UK and used for detection of TRTv antibodies in the collected serum samples (Cook, 2000).
 - **Ration:-** Balanced ration was obtained from commercial source without any food additives.
 - **Vaccination of turkey poults:** - turkey poults were vaccinated at 1 and 14 days old with TRTv vaccine and also vaccinated at 7 and 18 days old with Hitchner B1 and La Sota; respectively.
- 9- Evaluation of the immune response.**
- Haemagglutination Inhibition test (HI test):- was used to evaluate NDv antibodies in the collected serum samples according to the procedure described by Majujabe and Hiehner, 1977 and the geometric mean (GM) titer was calculated according to Anon, 1971.
 - 10- Analysis of serum biochemical parameters:** Total serum proteins were measured according to Weichsealbaum, 1946. Serum protein fractions was determined according to Ritzmann and Daniels, 1979 and the statistical analysis were made using two way of variance according to Berly and Lindgren, 1990.
 - 11- TRTv Challenge test:-** 15 turkey poults from each group were subjected to challenge with TRTv at 28 days of age. Each bird was installed by occulonasal route with 100µl of TRTv of a titer 10^6 TCID₅₀/ml according to Cook et al, 1991.
 - 12- NDv challenge test:-** 15 turkey poults from each group were challenged orally at 28 days of age with virulent NDv strain of a titer 10⁸ EID₅₀/ ml by a dose of 0.1 ml according to Abou-ElKhair et al., 1998.
 - 12- Virus reisolation:-** TRTv and NDv from challenged turkey poults was done according to Stone et al., 1978.
 - 13- Statistical analysis:** It was done according to Snedecor and Cochran, 1976 to determine statistically significant differences between means.
- The Experimental design:**
- A total number of 160; 1- day old turkey poults

were divided into 4 equal groups of 40 poult for each. Each group was kept in a separate, clean, disinfected pens and fed on a commercial balanced ration and treated as follows:

Birds in groups 1 - 3 were received both TRT and ND vaccines while those in group 4 were kept as non vaccinated negative control. Birds in group 1 were kept as vaccinated +ve control group while group 2 was fed a ration mixed with 1% crushed Nigella Sativa seed and group 3 was treated with Ultra-Natural plus in drinking water.

Poults in all groups were kept under daily observation from which serum samples were weekly collected for 4 successive weeks to evaluate the immune response for NDv and TRTV as well as the biochemical analysis.

At 28 days of age 30 poults from each group were taken and subdivided into 2 equal subgroups of 15 poults for each. The first subgroup was challenged with NDv while the other was challenged with TRTV. Challenged poults were kept under observation for clinical symptoms, PM lesion and virus reisolation for 2 weeks post challenge.

RESULTS AND DISCUSSION

There are large number of immunomodulating agents that are capable of stimulating the immune response of birds to face the problem of

immunosuppression and vaccination failure which constitute a challenge to poultry industry in Egypt and all over the world. In this investigation we used the Nigella Sativa seed as food additive and Ultra-Natural plus as immunostimulating agent in turkey poults which has been vaccinated with TRT and ND virus vaccines. The data presented in table (1) showed that the average HIGM titer in NDv vaccinated turkey poults significantly higher in group (3) that drink Ultra-Natural plus (7.4) and in group (2) that feed Nigella Sativa ration (8.6) at the 4th weeks of age than those in vaccinal control group (1) 6 HIGM titer. On the other hand, the prevalence of TRTV antibodies in vaccinated turkey poults as measured by ELISA (Table 2) indicated high antibody titer in group (3) that drink Ultra-Natural plus (0.922) and in group (2) that feed Nigella Sativa ration (0.943) versus 0.892 in control vaccinated non treated group the at 4th week of age. The data presented in table (3) showed significantly higher total serum protein concentration in group (2) and group (3) (5.1 and 4.99 respectively at 4th week of age) than in the 1st control non treated group (4.04). The increase in total serum protein concentration was due to the increase in α globulin fraction as presented in table (4). In this table the electrophoretic pattern of serum proteins in group (2) that fedon Nigella Sativa seed showed significantly higher levels of α globulin fraction (1.62) than (0.77) in group (3) that drink Ultra-Natural plus and (0.65) in group (1) at 4th week of age.

non specific immunostimulating effect of Ultra-Natural plus and Nigella Sativa was indicated the high HIGM titer of NDV antibodies in table (1) and the high ELISA optical density for TRTV antibodies in table (2) as well as by increase in total serum protein (Table 3) and increase in α globulin in table (4). These findings are supported by, Al-Bar et al., 1993 and Adel and Sahar Abd Elhamied 2003 who repeated that the biostimulant Ultra-Natural plus induced increase in leukocytic and lymphocytic counts associated with an increase in gamma globulin and total globulin. They also indicated that the biostimulant agent, markedly stimulated lymphocytes proliferation, enhance induction of T-lymphocytes and stimulated immunoglobulins production by B. cells.

On the other hand, Elliot et al., (1989), Oster and Schulz (1991) and Afrozul Hag et al., (1995) reported that Nigella Sativa increased interleukin 3, therefore it has an effect on macrophage. These results comes in agreement with the results previously obtained by Afrozul Hag et al., (1995) the 1st international conference of Scientific miracles of Quran and Sunnah held in Islam

Abad, Pakistan. These workers showed that black seed (Nigella Sativa) enhanced by 55% the ratio between T. helper cells (CD4) and the suppressor T. cells (CD8) and 30% average enhancement of the Natural killer cell activity. It is of interest to know that Hedaya (1995) studied the effect of Nigella Sativa seed extract on haematological and biochemical parameters in rat and reported that total serum proteins and globulin fraction were significantly increased by low doses of Nigella Sativa seed extract. Data presented in table (5 & 6) showed the result of challenge test for both NDV and TRTV where higher percentage of protection in group (2) that fed on Nigella Sativa ration than in other groups for both challenged viruses.

From all these results we could concluded that both Nigella Sativa and Ultra-Natural plus were potent immunostimulant substances and the former was more superior than the later and that the use of these immunostimulants are of great value to enhance immune response to vaccination for facing the hazard of immunosuppressive state and vaccination failure problem which cause great economic losses in poultry industry.

Table (1): Average HIGM titer in sera of NDV vaccinated treated and non treated turkey poults

Group No.	Vaccines TRTV & NDV	Treatment	HIGM titer/ weeks post vaccination				
			0	1st	2nd	3rd	4th
1	+	-	2.6	0.2	5.8	5.1	6
2	+	NS	2.6	0.2	6.2	5.8	8.6
3	+	UP	2.6	0.2	6.1	5.8	7.4
4	-	-	2.6	0.2	0.2	0.00	0.00

UP =Ultra-Natural plus

NS = Nigella sativa

HIGM = Haemagglutination Inhibition Geometric Mean

Table (2): Prevalence of TRTV antibodies in vaccinated turkey poults as measured by ELISA.

Group No.	Vaccines TRTV & NDV	Treatment	ELISA Optical densities/ weeks post vaccination				
			0	1st	2nd	3rd	4th
1	+	-	0.225	0.653	0.779	0.836	0.892
2	+	NS	0.225	0.776	0.890	0.925	0.943
3	+	UP	0.225	0.725	0.814	0.881	0.922
4	-	-	0.225	0.235	0.220	0.211	0.201

UP=Ultra-Natural plus

NS= Nigella sativa

Table (3): Total serum protein concentration in different turkey poult groups.

Week	Total serum proteins (g/dl)				Albumin (g/dl)				Total globulins (g/dl)			
Group No.	G ₁	G ₂	G ₃	G ₄	G ₁	G ₂	G ₃	G ₄	G ₁	G ₂	G ₃	G ₄
zero day	3.46±0.09	3.46± 0.09	3.46± 0.09	3.46± 0.09	1.76± 0.04	1.76±0.04	1.76±0.04	1.76±0.04	1.68±0.0 7	1.68±0.0 7	1.68±0.07	1.68±0.07
1 st week	4.04± 0.04	3.98± 0.10	3.13± 0.03	3.64± 0.07	1.57±0.0 2	1.86±0.05	1.77±0.03	1.82±0.02	2.00±0.0 6	2.47±0.0 5	2.12±0.03	1.80±0.03
2 nd week	3.98± 0.08	4.46± 0.08	4.67± 0.08	3.94± 0.04	1.69±0.0 3	1.76±0.05	2.09±0.09	2.06±0.03	2.27±0.0 3	2.69±0.0 5	2.39±0.02	1.89±0.05
3 rd week	4.17± 0.06	4.95± 0.09	4.76± 0.20	4.33± 0.03	1.86±0.0 3	1.96±0.08	2.36±0.03	2.36±0.02	2.32±0.0 4	2.99±0.0 4	2.42±0.04	1.98±0.02
4 th week	4.04± 0.09	5.10± 0.06	4.99± 0.08	4.43± 0.04	1.78±0.0 5	2.14±0.03	2.50±0.05	2.31±0.03	2.25±0.0 5	2.88±0.0 3	2.55±0.10	1.89±0.05

Table (4): Electrophoretic serum protein patterns (g/dl) in different turkey poult groups.

Week Group No.	α -globulins (g/dl)				β -globulins (g/dl)				γ -globulins (g/dl)			
	G ₁	G ₂	G ₃	G ₄	G ₁	G ₂	G ₃	G ₄	G ₁	G ₂	G ₃	G ₄
Zero day	0.96±0.00 9	0.96±0.0 09	0.96±0 .009	0.96±0. 009	0.35±0.0 2	0.35±0.02	0.35±0.02	0.35±0.02	0.40±0.0 2	0.40±0.0 2	0.40±0.02	0.40±0.02
1 st week	1.15±0.00 5	0.95±0.0 4	0.86±0 .03	0.91±0. 02	0.31±0.0 9	0.72±0.02	0.57±0.05	0.40±0.03	0.56±0.0 2	0.78±0.0 5	0.67±0.04	0.44±0.04
2 nd week	1.00±0.03	1.20±0.0 3	1.24±0 .03	1.02±0. 02	0.60±0.0 6	0.58±0.02	0.60±0.03	0.50±0.03	0.66±0.0 4	0.90±0.0 3	0.72±0.02	0.37±0.02
3 rd week	1.03±0.00 6	1.19±0.0 2	1.05±0 .04	0.88±0. 02	0.62±0.0 5	0.63±0.01	0.72±0.02	0.66±0.02	0.67±0.0 09	1.115±0. 03	0.95±0.01	0.45±0.02
4 th week	1.08±0.00 7	1.14±0.0 3	1.00±0 .06	1.05±0. 03	0.68±0.0 3	0.71±0.03	0.77±0.03	0.55±0.02	0.65±0.0 2	1.02±0.0 3	0.85±0.04	0.51±0.03

Table (5): The protection percent against mortality rate in different turkey poult groups after oral challenge with virulent NDV at 28 days of age.

Subgroup No.	No. of birds	Mortality rate	Protection %	Virus Reisolation
1	15	5/15	66.7	+
2	15	1/15	93.34	+
3	15	2/15	86.67	+
4	15	15/15	0.0	+

Table (6): The protection percent against mortality rate in different turkey poult groups after ocular challenge with TRT v at 28 days of age.

Subgroup No.	No. of birds	Mortality rate	Protection %	Virus Reisolation
1	15	3/15	80	+
2	15	0/15	100	-
3	15	1/15	93.34	+
4	15	9/15	40	+

REFERENCES

- Aal, S. and Attia, R. S. (1993): Characterization of chick cumin seeds. 1- Chemical composition and lipids Exch. 14 (4): 46 - 481.
- Elkhair, M.; Afaf, H. A.; Abouzaid, A. A. and Abd ki, M. H. (1998): Trial for preparation of combined ivate oil emulsion vaccine against NewCastle and virus disease 4th Vet. Med. Zag. Congress. 173 - 155.
- Abdel, Eisa and Sahar, S. Abd El Hamied (2003): Clinicopathological studies on bio-stimulant agent in broiler chickens. Kafr El Sheikh Vet. Med. J. Vol. 1 No. (1): 631 -644.
- Afify, M. A. (1990): Studies on the role of some immunostimulants in using poultry vaccines. Ph. D. Vet. Thesis. Cairo Univ.
- Afrozul Hag; Mohamed Abdullatif; Peter, I. L.; Kalid, S. A. and Sultan, T. (1995): Nigella Sativa effect on human lymphocytes and polymorphnuclear leukocytes phagocytic activity. Immunopharmacology. 30: 147 - 155.

- Al-Bar, A.; Ishamil, P. R. and Nakur, H. S. (1993): Effect of dietary yucca *Schidigera* extract of environmental ammonia and growth performance of chicken and rabbits. *J. Animal Sci.* 71 (1) P. 114.
- Anon (1970): Methods of examining poultry biologics and for identifying and quantifying avian pathogen. *Nat. Acad. Sci. Washington, D. C.*
- Award, M. H.; Kukul, M. A. and El Shobaki, F. A. (2000): Immunopotential of Infectious Bursal Disease (IBD) vaccination. *Vet. Med. J. Giza Vol. 48 (1): 27 - 33.*
- Berly, D. A. and Lindgren, B. W. (1990): Statistics, theory and methods. Books. Gale Publishing Company California pp. 93 - 121.
- Brandt, G. C.; Papp, D. M.; Bywater, R. J. and Jenkins, W. L. (1991): *Veterinary Applied Pharmacology and Therapeutics*. 5th Ed. Baillier Tindall 24 - 28 Oval Road London. P. 401.
- Cook, J. K.A. (2000): *Avian Rhinotracheitis*. *Rev. Sci. Tech. Off. Int. Epiz.* 19: 602 - 613.
- Cook, J. K. A.; Ellis, M. M. and Huggins, N. B. (1991): The pathogenesis of turkey Rhinotracheitis virus in turkeys poultis inoculated with the virus alone or together with two strains of bacteria. *Avian Pathology* 119: 181 - 185.
- Elliott, M.; Vadas, M. A.; Englington, J. M.; Park, L. S.; Clark, S. C. and Lopez, A. F. (1989): Recombinant human interleukin 3 and granulocyte macrophage colony stimulating factor shows common biological effects and binding characteristic on human monocytes. *Blood* 74: 2349 - 2359.
- Hedaya, S. A. (1995): Effect of *Nigella Sativa* seeds extract on some hematological and biochemical parameters in rats. *Alex. J. Vet. Sci.* 11 (2): 95 - 99.
- Khodary, R. M.; El-Azzawy, M. H. and Hamdy, I. R. (1996): Effect of *Nigella Sativa* on egg production, hatchability percentage and some biochemical values in laying hens with Ref. to fertility *Vet. Infocokrets* 7th Sci. Cong., 17 - 19 Nov., *Med. Ass. Egypt*, Pp. 91 - 101.
- Majumbe, K. A. and Hichner, S. B. (1977): Antibody response to strain combination of NewCastle disease virus as measured by Haemagglutination Inhibition test. *Avian Dis.* 12: 576 - 584.
- Oster, W. and Schulz (1991): *Inturukin-3*. Biological and Clinical effects. *Int. J. Cell Cloning* 9: 5 - 23.
- Rizman, S. E. and Daniels, J. C. (1979): Diagnostic proteology: separation and quantitative assays in *Laboratory Medicine* Harper and Row Inc., Hagerstown.
- Snedcor, G. W. and Cochran, W. G. (1976): *Statistical methods* 6th Ed. Iowa, State University Press Ames. Iowa.
- Springer, W. T.; Olson, N. O.; Kerr, K. M. and Fabacher, C. J. (1983): Responses of specific pathogen free chicks to Reo virus (WUV-2937) and Infectious Bursal Disease virus. *Avian Dis.* 27: 911.
- Stone, H. D.; Brugh, M.; Hopkins, S. R.; Yoder, H. W. and Beard, C. W. (1978): Preparation of inactivated oil emulsion vaccines with avian viral or mycoplasma antigens. *Avian Dis.* 22 (4): 666 - 674.
- Weichselbaum, T. C. (1946): An accurate and rapid method for determination of proteins in small amount of blood serum and plasma. *Am. Chem. Path.* 10: 40 - 48.

التأثير المناعي والكيميائي الحيوي لحبة البركة والأترا ناتشرال بلس في الرومي المحصن بلقاضي النيوكاسيل والتهاب الأنف والقصبه الهوائية

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تم تصميم التجربة لدراسة التأثير المناعي والكيميائي الحيوي لحبة البركة والأترا ناتشرال بلس في الرومي المحصن بلقاضي النيوكاسيل والتهاب الأنف والقصبه الهوائية.

وأظهرت النتائج أن كل من حبة البركة والأترا ناتشرال بلس لهما تنشيط مناعى ولكن تفوق الأول فيهما.

عند عمر ٤ أسابيع أعطت حبة البركة أجساماً مناعية للنيوكاسيل والتهاب الأنف والقصبه الهوائية وبرتين كلى وجاما جلوبيولين ٨.٦ و ٩٤٢.٠٠ و ٢.٨٨ و ١.٠٧ فى مقابل ٧.٥ و ٩٢٢.٠٠ و ٢.٥٥ و ٠.٨٥ بالنسبة لأتترا ناتشرال بلس على التوالي.

وفى إختبار التحدى لمجموعة حبة البركة بالنسبة للنيوكاسيل والتهاب الأنف والقصبه الهوائية كانت نسبة الصد ٩٣.٢٤ و ١٠٠٪ على التوالي بينما كانت ٨٦.٦٧ و ٩٣.٢٤٪ على التوالي فى مجموعة الأتترا ناتشرال بلس.