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IMMUNOMODULATION OF NIGELLA SATIVA AND NUTRILAC ON EXPERIMENTALLY IMMUNOSUPPRESED CHICKS BY REO-VIRUS INFECTION

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SUMMARY

This work was designed to study the experimentally immunosuppressive effect of Reovirus infection and the immunomodulating effect of either Nigella sativa seed or the acidifier nutrilac on the immune system of one- day old chicks against NewCastle disease virus inactivated vaccine and the results was measured by HIGM titer where it showed that Reovirus infection is functionally alliterate the immune system of one- day old chicks by immunosuppresion giving HIGM titer 2 comparing with the control (non infected chicks) which gave HIGM titer 4.8 at 21 days post vaccination with inactivated NewCastle disease virus vaccine. On the other hands both nigella sativa and acidifiers nutrilac were a potent immunostimmulant and can counter the immunosuppresion effect induced by Reovirus infection. But Nigella sativa seed was superior in its immunostimulant activity than the acidifier nutrilac where it gave HIGM titer 8.6 comparing with acidifier nutrilac which gave 7.4 at 21 days post vaccination with inactivated Newcastle disease virus vaccine.

INTRODUCTION

Immunosuppressive state in poultry farms constitute a major problem facing the rapidly expanding poultry industry in Egypt and allover the world. There are several infectious agents that have been incriminated as causes of this condition. Viral agents were found to play the major role as causative agents for the immunosuppressive state. Avian Reovirus was one of these viral agents.

Reovirus infection resulted in lymphopenia marked lymphocytic hyperplasia in spleen and lymphoid cell degeneration in the bursa (Page et

al., 1982). Locally isolated strain of Reovirus found to alliterating the functional activity of T. cell response to NewCastle disease virus vaccination (Hegazi et al., 1989).

Potentiation of the immune response in poultry can occur by using some additives. However the routine use of drugs to modify or modulate animal immunocompetence as a part of therapeutic management of specific clinical conditions is still at a very preliminary stage in veterinary medicine (Brander et al. 1991).

Awaad et al (1999 a) proved the immunopotentiation of weak organic acid preparation known as Nutrilac produced by NUTRI-AD (Belgium) for chickens using both immuno and bioassays as criteria. Moreover there is a herbaceous plant known as Nigella sativa which is a member of Ranunculaces family that have the ability to increase the immunity and mentain a good healthy condition (Abd- El-Aal and Attia, 1993).

The aim of this work is to study the immunomodulating effect of both Nigella sativa seed and acidifiers Nutrilac on immunosuppressed chicks by Reovirus infection against NewCastle disease inactivated vaccine. The immune response was measured by detection of haemagglutinating (HI) antibodies as well as challenge test.

MATERIAL AND METHOD

MATERIALS:-

1- Virus:

a- Standard avian Reovirus strain of a titer 10^6 $TCID_{50}$ was kindly supplied by the Animal Health Research Institute Immunity Unite, Dokki, Cairo.

b- A Velogenic Viscerotropic New Castle disease virus of a titer 10^8 EID_{50} was obtained from Veterinary Serum Research Institute, NewCastle Disease Unite, Abbasia, Cairo and was used as challenge virus after it's titeration.

c- Lasota virus: Lasota live virus vaccine was passaged in ECE and the allantoic fluid of inoculated eggs were collected and used as HA- antigen for HI test.

2-Virus vaccine:-

Combined ND and Infectious Bursal Disease (IBD) inactivated virus vaccine was kindly supplied by poultry and Rabbit Disease Department, Fac. of Vet. Med., Beni- Suef. The vaccinal dose was used according to the vaccine producer.

3-Experimental birds:

A total of 120; one day- old balady chicks obtained from a private hatchery were used. Collected sera from these birds proved to be free from Reovirus antibodies by A.G.P.Test.

.- Sera:

Serum samples were collected weekly from each group and subjected for HI test to detect NDV antibodies.

5- Agar gel medium for precipitation test:

It was prepared as described by (Crawle, 1961).

6- Immunomodulatores:

a- Nigella sativa seed were commercially purchased and grinded to be added by 1% to the ration.

b- Acidifiers Nutrilac: Nutrilac liquid produced by NUTRI- AD International, Belgium, Lot No. NLL9803 was used 3ml/ liter for 1st 5 days repeated at 3rd week for 3 days.

7- Reovirus precipitating antigen:

A precipitating antigen against Reovirus was kindly supplied by the Animal Health Research Institute, Immunity Unite, Dokki, Cairo and the antigen was used in AGPT to prove that the used one day old chicks were free from Reovirus antibodies.

8- Hyper- immune serum:

Hyperimmune serum specific to both NDV and Reovirus were kindly obtained from Virology Department, Fac. of Vet. Med. Beni- Suef. These sera were used as positive control test in both HI and AGP test.

9- Fertile Chicken Eggs:

Nine day old fertile chicken eggs were obtained from commercial farm and used for preparation of antigen (HA). Titration of used viruses as well as virus reisolation from dead challenged birds was carried out.

10- Ration:

The used ration was obtained from a commercial source without any food additives.

METHODS:-

1- Reovirus infection: 0.1ml of Reovirus containing 10^4 $TCID_{50}$, was inoculated via foot pad route at first day of experimented chicks according to Bekhit (1988).

2- Vaccine administration: The vaccinal dose was 0.3 ml/ chicks intramuscularly according to the vaccine producer.

3- Haemagglutination Inhibition Test (HI): was carried out according to the standard procedure described by Majujab and Hitchner (1977) and geometric mean (GM) titer was calculated according to Brugh (1977).

4- AGPT : was applied as described by Rossler & Rosenberger (1989).

5- Challenge test: was applied according to Abo-El-Khair et al (1998). Birds were challenged orally at 21 days post- vaccination with

virulent NDV of a titer 10^6 EID_{50} by a dose 0.1ml/ bird and kept under observation for 15 days post challenge, dead birds and those showing symptoms during these period were subjected for P.M. examination.

6- **Virus reisolation:** reisolation of NDV from challenge birds was done according to Stone et al (1980).

7- **Statistical analysis** was applied according to Snedecor and Cochran (1976) to determine statically significant differences between means.

Experimental design:

The used chicks (120) were divided into six equal groups : (group 1- 6) each of them contain 20 chicks. These groups were treated as following:-

- Birds of group 1, 3 and 5 were infected with Reovirus; by inoculation of each with 0.1ml containing 10^6 $TCID_{50}$; via foot pad route at 1st day of life.
- Birds of groups 1 and 2 were feed from 1st da of experiment on ration containing 1% Nigell sativa.
- Birds of groups 3 and 4 were given nutrilac vi drinking water also from the 1st day of age.
- Birds of group 5 were infected with Reoviru and kept as non treated birds.
- Birds of group 6 were kept as non-infected non-treated (negative) control gorup.

All chicken gorups were kept under daily observation for clinical signs and mortalities with the collection of 5 random blood samples for sera from each gorup at weekly intervals for detection of maternal HI-antibodies. At 21 days of age (where maternal HI antibodies were negligible), birds of all gorups were received oil vaccine by intramuscular inoculation of 0.3 ml/ bird. The vaccinated birds were observed for 3 weeks with collection of 5 blood samples from each group weekly for detection of HI-antibody response.

- Birds of all groups were callengeds at 21 days post vaccination according to Abo- ElKhair et al (1998).

Table (1). Geometric means of maternal HI- antibodies in used chicken groups (Treated by Nigella sativa and Nutrilac)

Group No.	Treatment			HI geometric mean titer			
				Time of sampling			
	Reo-virus infect	N.S *	Nut *	Zero day	7 days	14 days	21 days
1	+	+	-	6.2 ± 0.50	5 ± 0.50	3.4 ± 0.20	0.3
2	-	+	-	6.2 ± 0.50	4.6 ± 0.70	2.6 ± 0.10	0.2
3	+	-	+	6.2 ± 0.50	4.8 ± 0.70	3.4 ± 0.20	0.3
4	-	-	+	6.2 ± 0.50	5 ± 0.50	3.6 ± 0.20	0.4
5	+	-	-	6.2 ± 0.50	4.4 ± 0.70	3.6 ± 0.20	zero
6	-	-	-	6.2 ± 0.50	4.4 ± 0.70	3 ± 0.10	zero

+ = Treated. N. S.= Nigella Sativa

- = Non treated. Nut. = Nutrilac

RESULTS

From Table (1) and Fig. (1) it was shown that maternal antibody for NDV declined in different groups at 21 days of age without any significant difference between means.

*** Clinical Symptoms:** Experimentally infected chicks with Reovirus showed lameness in 30% and foot pad swelling at site of inoculation in 50% after 3 days post inoculation then disappear and became apparently normal.

From Table (2) and Fig (2) it was clear that group 2 (feed ration containing 1% Nigella sativa and non infected with Reovirus) and group 4 (non infected and treated with Nutrilac in drinking water) showed significant higher HIGM titer for NDV than control group 6 (non infected and non treated). While group 5 (infected with Reovirus and non-treated) showed significant lower HIGM titer for NDV than control non-infected group No. 6.

From Table (3) is clearly shown that only group 5 (which infected with Reovirus and non treated) was affected with challenge and gave clinical symptoms.

Table (2) Geometric means of HI antibody response to NDV vaccination in treated and non- treated different chicken groups.

Group No.	Treatment			HI geometric mean titer for NDV at different intervals post- vaccination		
				Time of sampling		
	Reo-virus infect	N.S •	Nut •	7 days post-vaccination	14 days post-vaccination	21 days post-vaccination
1	+	+	-	0.2	2.6 ± 0.10	4.6 ± 0.70
2	-	+	-	0.4	4.4 ± 0.70	8.6 ± 1.2
3	+	-	+	0.2	2.4 ± 0.10	4.4± 0.70
4	-	-	+	0.2	4.2 ± 0.70	7.4 ± 1.2
5	+	-	-	0.0	1.4 ± 0.10	2.0 ± 0.10
6	-	-	-	0.2	2.6 ± 0.10	4.8 ± 0.70

+ = Treated. N. S.= Nigella Sativa
- = Non treated. Nut. = Nutrilac

Table (3): Results of oral challenge of vaccinated chicks with virulent ND virus strain.

Group No.	Clincal symptoms and mortalities	P.M. examination	Virus reisolation
1-4	apparently normal with no mortalities	Apparently normal	- ve
5	All birds showed marked depression, conjunctivitis, some birds showed nasal discharge and 2 birds were died at 5 th days post challenge	Severe catarrhal inflammation of m.m. of respiratory and digestive tract mainly in gizzard and proventriculus with minute peticheal haemorrhage	+ ve
6	Apparently normal with no mortalities	Apparently normal	- ve

Fig. (1): Geometric means of maternal HI antibodies in different treated and non treated chicken gorups.

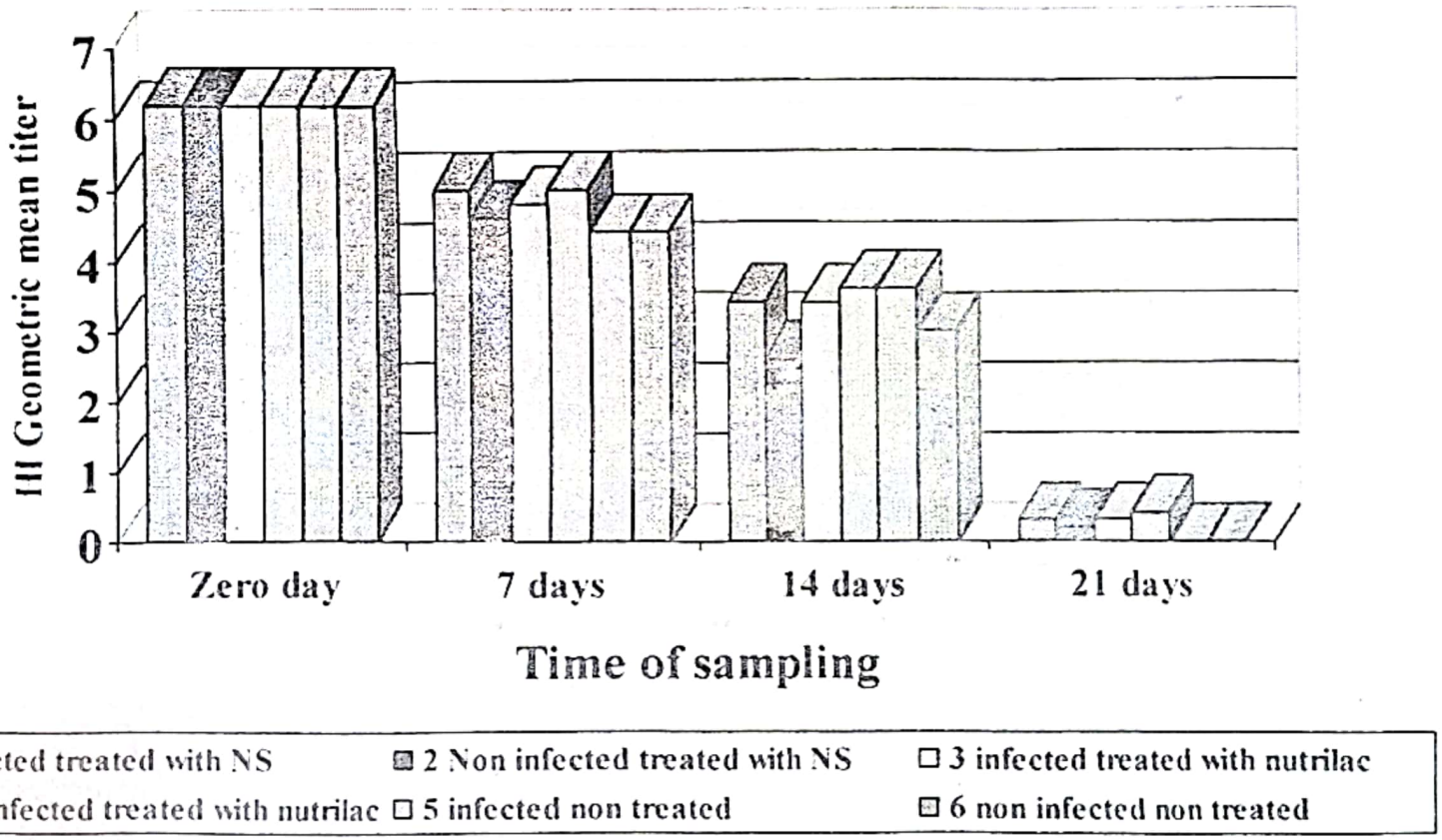

Fig. (2): Geometric means of maternal HI antibodies response to NDV vaccination in different treated and non treated chicken groups

DISCUSSION

Commercial poultry production in Egypt have been developed during the last years. Many years ago, the problem of immunosuppression and vaccination failure constituted a challenge to this industry. Viral diseases have been incriminated as the main cause for this condition, one of the most important viral agents is the avian Reovirus infection. In the present study chicks were experimentally infected with Reovirus by foot pad inoculation to study the effect of Reovirus infection on the immune response of chicks to inactivated NDV vaccination.

There are large number of immunomodulatory agents that are capable of stimulating the immune response of chickens, in the present investigation we studied the use of natural plant Nigella sativa seed as food additive and a synthetic liquid product known as Nutrilac in drinking water.

For studying the effect of Reovirus infection on the immune response of chicks to inactivated NDV vaccine we allowed to maternal antibody of chicks to wane before vaccination. Data presented in Table (1) and Fig. (1) showed the result of HIGM titer for NDV maternal antibody where it decline in all groups at 21 days old without any significant differences between means.

For assaying the immunomodulatory effect of the studied immunostimulating substances on humoral immune response of immunosuppresed chicks. Data presented in Table (2) and Fig.(2) showed the results of HIGM titer for NDV vaccination where Reovirus infection to one day old chicks resulted in significant reduction in HIGM titer to NDV at 14 and 21 days post vaccination (1.4 and 2) as compared with control non infected group (2.6 and 4.8) and this might be attributed to the effect of Reovirus on the immune system of the chicks represented by structural atrophy of the bursa and lymphoid cell degeneration, this speculation is supported by Page et al (1982), Montogamery et al., (1986), Rifuliadi, (1986) and Bekhit (1988) who reported that birds infected with Reovirus showed lymphopenia, marked lymphocytic hyperplasia in spleen and lymphoid cell degeneration in atrophied bursa.

On the other hand, group 1 of chicks infected with Reovirus and treated by 1% Nigella sativa in the ration showed no alteration on immune response to vaccination with NDV and gave HIGM titer nearly the same of control non infected group at 21 days post- vaccination (4.4 and 4.6 respectively) without any significant difference between them. Protection of chicks from the immunosuppressive effect of Reovirus is attributed to the immunostimulant Nigella sativa, this speculation is supported by the result of NDV antibody titer of group 2 which was not infected with Reovirus and feed ration containing 1% Nigella sativa which showed HIGM titer for NDV about two fold in front of control non- treated group (8.6 and 4.8

respectively) at 21 days post- vaccination.

The immunostimulant effect of Nigella sativa is supported by Elliot et al., (1989) and Afrozal Hag et al., (1995) who reported that Nigella sativa increase inturlukin1B (IL- 1B) and IL-3 indicating stimulatory effect on macrophage.

On the other hand, group of birds number 3 which infected with Reovirus and treated with Nutrilac in drinking water showed no alteration in immune system by Reovirus infection and HIGM titer for this group showed no significant difference with those of control non- infected group No. 6 at 21 days post- vaccination (4.4 and 4.8 respectively). The protection of this group of chicks from the immunosuppressive effect of Reovirus infection might be attributed to the immunostimulant action of Nutrilac which is supported by the results of group 4 (non- infected with Reovirus) and received Nutrilac in drinking water and gave HIGM titer one and half fold in front of control non-treated groups, number 6 (7.4 and 4.8 respectively) at 21 days post- vaccination this speculation is supported by Awaad et al., (1999a & 1999b) who concluded that Nutrilac has stimulatory effect on both humoral and cell mediated immunity.

Data presented in Table (3) showed that the results of challenge of vaccinated chicks with virulent NDV, where all groups were apparently protected except group number 5 which infected with reo-virus and not received any of the immunostimulant used, they show clinical signs and P.M. lesions for NDV infection, with virus reisolation from infected birds.

From these results we could concluded that:
Reovirus infection is functionally affect the immune system of the birds by immunosuppression. Both Nigella sativa and Nutrilac are potent immunostimulant and can compensate the immunosuppressive state induced by Reovirus infection but Nigella sativa is superior to Nutrilac in its immunostimulant activity where it give two fold increase in HIGM titer for NDV vaccination infront of one and half fold for Nutrilac than control group.

Uses of these immunostimulant is of great value for facing the hazard of immunosuppressive state which cause serious economic losses in poultry industry.

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