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EFFECT OF IMMUNOMODULATOR AND DIFFERENT ROUTES OF VACCINATION ON ANTIBODY TITERS AGAINST NEWCASTLE DISEASE IN UNIGOLD BIRDS

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ABSTRACT

The study was conducted to evaluate the effect of Lisovit supplementation and compare efficacy of different routes of vaccination on immunity against ND in Uni Gold birds. 180 UniGold adult birds (36th to 40th week of age) were randomly divided into 12 groups having 15 birds in each group (6 groups of male and female birds). Group A, C, E, G, I and L were given lisovit @ 200mg/kg of body weight. Group C, D, J and J were vaccinated through oral route (drinking water). Group E, F, K and L were vaccinated through ocular route (eye drop). One day before before vaccination (0 day) geometric mean titer in group A, B, C, D, E, F, G, H, I, J, K and L, was 9.12, 18.19, 95.49, 18.19, 47.86, 41.68, 23.98, 31.62, 165.95, 36.3, 18.19 and 36.3 respectively. At 7th day GMT in these groups was 18.19, 12.02, 165.95, 144.54, 72.44, 47.86, 41.68, 23.98, 144.54, 125.89, 380.18 and 218.77 respectively. At 30th day GMT in these groups was 36.3, 20.89, 288.4, 218.77, 218.77, 23.98, 83.17, 47.86, 125.89, 436.51, 190.54 and 144.54, respectively. At 45th day GMT in these group was 5.24, 12.02, 7.94, 31.62, 41.68, 13.8, 20.89, 54.95, 95.49, 54.95, 125.89 and 72.44, respectively. At 0 day the ELISA mean titer value in group B, E, H and L was 1378.4, 6083.4, 7127.6 and 6910.0 respectively. At 7th day ELISA titer in these group was 1981.4, 6346.5, 1285.8 and 10051.6 respectively. At 30th day ELISA titer in these groups was 1650.2, 4155.2, 266.4 and 7487.6 respectively. At 45th day ELISA titers in these groups were 654.0, 2838.2, 1990.8 and 4597.4, respectively.

Keywords: antibody, immune-modulator, newcastle disease, routes, unigold, vaccination

INTRODUCTION

Domestic poultry farming plays a significant role in the nation's overall egg and meat output in emerging nations (Sadiq, 2004). Out of total productin from poultry sector in the year 2022-23 the share of rural poultry in egg and meat was 19 and 6%, respectively in Pakistan (GoP, 2023). Poultry production is a cost-effective and revenue generating enterprise in rural regions across all emerging nations. The local farming of indigenous poultry has been largely neglected as a result of the shift in focus from local to commercial poultry farming to close the gap between supply and demand caused by the increased requirements for proteins. The primary sources of protein that fulfil demands are meat and chicken eggs. Poultry is typically raised and held by women and children in rural regions; they can be utilized as a source of income when cash is hard to come by or as a bartering mechanism (Guèye, 2000).

Traditional poultry systems yield very little in the way of eggs produced and weight growth per hen annually, but this is because very little is provided in the way of management, disease control, housing, nutrition, and other inputs. Poverty will be reduced and food security will be enhanced by any economically viable strategy that may boost bird output (Alders and Spradbrow, 2001).

ND is the leading cause of economic distress for rural poultry due to mortality. It is a remarkable disease with a high morbidity rate of up to 80% (Nwanta *et al.,* 2006). It is impossible to rule out the possibility that ND, more than any

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other animal viral or bacterial infectious illness, may have a greater and more lasting impact on the global economy (Alexander *et al.,* 2008). The ND virus can spread from recently acquired or introduced birds through the selling or purchase of sick birds, exposure to the excretions and feces of infected birds, and extended contact with contaminated people, equipment, feed, water, and clothing (Hossain and Mozaffor, 2010).

Vaccines are frequently employed as a potent weapon and instrument to manage, stop, and restrict viral diseases in poultry birds. Therefore, to eliminate and reduce the occurrence of fatal clinical illnesses. At the farm, field or even household level, a suitable vaccination plan and guide must be followed and accepted (Marangon and Busani, 2006). In order to combat the ongoing threat that ND poses, the majority of countries have applied and developed a plan that emphasizes and implements widespread vaccination efforts in order to prevent deadly epizootics worldwide (Alexander *et al.,* 2008).

In order to prevent infectious diseases that are contagious and deadly, such as ND and IBD, birds and flocks should be vaccinated against these highly dangerous and devastating diseases using appropriate vaccination schedules. Following vaccination, observation and monitoring of the immune responses is equally as crucial and delicate as vaccination itself in order to accurately measure the immune-logical status of birds and flocks (Muhammad *et al.,* 1996).

The University of Agriculture, Faisalabad, has developed a new breed of egg-laying animal called UniGold. There is currently no information available regarding this breed's immune status or response to an immunomodulator or ND vaccine. Therefore, after administering various therapies, it is necessary to measure and compare the antibody titers. Therefore, the purpose of the current study was to assess the impact of Lisovit on antibody titers against ND using two distinct vaccination routes (drinking water and eye drop), as well as to compare the antibody titers against ND in UniGold breed male and female birds.

MATERIALS AND METHODS

The University of Agriculture, Faisalabad's Poultry Research Centre served as the project's study area. A total of 180 UniGold birds, aged between 36 and 40 weeks, were randomly assigned to 12 groups, with 15 birds per group. Male birds made up half of the groupings, while female birds made up the other half. Every bird was housed in a battery system. The birds were kept in feeding troughs with a battery system and fed layer feed once a day in the morning. The birds' water troughs were guaranteed to have clean water in them all day long. In a 24-hour period, the water troughs were cleaned twice.

A specific set of birds received an eye drop or drinking water vaccination against the live LaSota strain of NDV. These birds were vaccinated against NDV alone because the purpose of this research experiment was to assess the antibody titers of ND in male and female UniGold birds. There was no further vaccination against any other bacterial or viral illness. There was no usage of growth boosters or antibiotics. ND vaccinations were administered as eye drops or through drinking water. The control groups received no vaccinations. For three days following vaccination, 200 mg/kg of body weight of Lisovit (Biomin, Austria) (Lysozyme 22% and Vitamin E 0.5%) was given as an immune-modulator. Lisovit was given in drinking water at a dose of 200 mg/kg body weight. The titers against NDV were measured using the HI test. In one third of the groups, ELISA was also employed to evaluate titer estimation methods (Table 1). ELISA testing was done on groups B, E, H, and L. ELISA optical density (OD) measurements were transformed into titer values.

Table 1. Allocation of treatments to experimental group

Sex	Group	No. of birds	Route of ND Vaccine	Lisovit	HI test	ELISA test
Male	A	15	-	√	√	-
	B	15	-	-	√	√
	C	15	DW	√	√	-
	D	15	DW	-	√	-
	E	15	ED	√	√	√
	F	15	ED	-	√	-
Female	G	15	-	√	√	-
	H	15	-	-	√	√
	I	15	DW	√	√	-
	J	15	DW	-	√	-
	K	15	ED	-	√	-
	L	15	ED	√	√	√

DW = Drinking Water; ED = Eye drop

Blood samples were taken one day prior to vaccination, seven days following vaccination, thirty days following vaccination, and forty-five days following vaccination. Five specimens were obtained from every group. At each sampling, a total of 60 samples were collected. A 5 ml disposable syringe was used to draw 3 ml of blood from the wing vein. Following collection,

the samples were kept at room temperature in a slanting position for approximately 25 to 30 minutes, at an inclination of 20 to 30 degrees. During this time, the serum was extracted from every blood sample. The serum that had seeped out was meticulously removed from the remaining blood and gathered into sterile plastic bottles. Before analysis, bottles were labeled and kept at -20 ºC.

The haemagglutination inhibition (HI) test and ELISA were used to evaluate the antibody titers against NDV (Alexander and Senne, 2008). In the pathology lab of the University of Agriculture of Faisalabad, antibody titers against the ND virus were assessed in experimental birds using HI and ELISA as per the protocol (Allan and Gough, 1974).

Statistical analysis

According to Burgh's (1998) description, antibody titers against NDV were statistically translated into Geometric Mean Titers (GMT) for every group. To compare the mean titer values of the various groups with the controls, chi-square analysis was employed. Immunity (vaccinated against non-vaccinated) was examined as a binomial variable using logistic regression. Additionally, it was believed (converted to) that the ELISA data was binomially distributed, and it was analyzed as such (Sokal and Rohlf, 2012).

RESULTS

The HI test was used to detect the antibody titers against NDV in all groups, and ELISA was used to determine the titers in groups B, E, H, and L. The immunization status against ND was assessed by HI tests in all groups and ELISA in some groups, based on the titers of the various groups that received various treatments.

One day before given treatments, HI tests showed the values of GMT of different groups. i.e. A, B, C, D, E, F, G, H, I, J, K and L, as 9.12, 18.19, 95.49, 18.19, 47.86, 41.68, 23.98, 31.62, 165.95, 36.3, 18.19 and 36.3 respectively. On 7th day after given treatments, values of GMT of the above groups were found to be 18.19, 12.02, 165.95, 144.54, 72.44, 47.86, 41.68, 23.98, 144.54, 125.89, 380.18 and 218.77 respectively. On 30th day after given treatments, values of GMT of the above groups were 36.3, 20.89, 288.4, 218.77, 218.77, 23.98, 83.17, 47.86, 125.89, 436.51, 190.54 and 144.54 respectively. On 45th day post treatments, GMT values of above groups were found to be 5.24, 12.02, 7.94, 31.62, 41.68, 13.8, 20.89, 54.95, 95.49, 54.95, 125.89 and 72.44, respectively (Table 2).

Table 2. HI titers at 0, 7th, 30th and 45th day

Group	GMT at day			
	0	**7th**	**30th**	**45th**
A	9.12	18.19	36.3	5.24
B	18.19	12.02	20.89	12.02
C	95.49	165.95	288.4	7.94
D	18.19	144.54	218.77	31.62
E	47.86	72.44	218.77	41.68
F	41.68	47.86	23.98	13.8
G	23.98	41.68	83.17	20.89
H	31.62	23.98	47.86	54.95
I	165.95	144.54	125.89	95.49
J	36.3	125.89	436.51	54.95
K	18.19	380.18	190.54	125.89
L	36.3	218.77	144.54	72.44

ELISA was also performed for the groups B, E, H and L. Optic density (O.D) values of ELISA were converted to titer values. One day before the treatments, ELISA showed mean titer values 1378.4, 6083.4, 7127.6 and 6910.0, respectively for above groups. On 7th day of treatments titers found to be 1981.4, 6346.5, 1285.8 and 10051.6 respectively. On 30th day post treatment, titers were 1650.2, 4155.2, 266.4 and 7487.6 respectively. On 45th day after treatments titer values were found to be 654.0, 2838.2, 1990.8 and 4597.4, respectively (Table 3).

Table 3. Antibody titer determined by ELISA

Group	Antibody titer (ELISA) at day			
	0	**7th**	**30th**	**45th**
B	1378.4	1981.4	1650.2	654
E	6083.4	6346.6	4155.2	2838.2
H	7127.6	1285.8	266.4	1990.8
L	6910	10051.6	7487.6	4597.4

DISCUSSION

One of the main illnesses that affects the chicken industry is ND, which causes substantial mortality and financial loss for producers. Vaccination and immunomodulator supplements are the most effective ways to manage this illness. The goal of the current study was to assess the immune state of UniGold birds against ND in the form of antibody titers following vaccination via various methods in the presence and absence of the immunomodulator "Lisovit."

The immune system of the body is the body's line of defense against foreign particles and harmful organisms. A related system in the body is the immunological system. It is made up of multiple useful parts. It changes based on the particular immunogenic challenge. Numerous mechanisms that contribute to the development of the immune system have been identified. The immune system also involves several organs

and cells. Pathogenic bacteria are eliminated through phagocytosis by macrophages, neutrophils, eosinophils, and polymorph nuclear neutrophils (PMNs).

Depending on the circumstances and available resources, many conventional serological tests with high sensitivity can be used to monitor vaccination programmes effectively and assure their efficacy. One of the most popular and simple tests for estimating the amount of antibodies (Abs) produced by birds to fight the NDV is the HI test (Makkawi *et al.,* 2004).

The HI test is the most widely used serological approach in traditional medicine and is regarded as the reliable and standard laboratory test for ND. To maintain the protective levels of antibody titers in birds, anti-NDV antibody titers in birds and flocks are regularly checked and revaccinated. Anti-ND titers guarantee safety and safeguard against ND. Researches have discussed their impact of antibodies on breeders and transmission of maternal antibody titers to their progeny and children (Madadi *et al.,* 2014).

Birds' immune systems are crucial for protecting their bodies from various illnesses. Commercially available immunomodulators can be used to quickly achieve and sustain antibody titers up to a protective threshold. It is said that Lisovit (Biomin, Austria) strengthens the body's immunological response. Therefore, the goal of the current investigation was to determine how supplementing with Lisovit affected the immune response against NDV. The study's second goal was to compare the effectiveness of the two NDV vaccination routes; drinking water and eye-drop in terms of immune status. The third goal was to assess the birds' immune systems, both male and female.

The quickest and most effective way to generate immunological titers is to induce immune-stimulating medications, which are widely and reasonably priced. Immunologists, dietitians, and scientists have long placed a high value on the interaction between immune systems and immunological modulators. According to Wagner and Jurcic (1995), the concept of immune-stimulation provides a preventative and therapeutic approach intended to strengthen both specialized and generic immune systems. Immunomodulators that are sold commercially aid in immune system stimulation. Prophylactic and therapeutic measures are included in immune-stimulation to stimulate both non-specific and specific immunity. Maintaining appropriate antibody titers is necessary to protect against ND because of its high mortality rate. Vaccination and the use of immunomodulators can provide the required antibody titers. Immunostimulatory effects have been reported for levamisol, selenium, vitamin E, and lisovit (Bashir, 1994). The assessment of antibody titers against ND is done by ELISA and the HI test (Alexander and Senne, 2008).

The lysozyme clearly proves the great interest for researchers due to its potential helf to overcome the infections (Ferraboschi *et al.,* 2021). In our study the male bird groups A, C, and E who received a 200 mg/kg body weight supplement of Lisovit in their drinking water shown a higher level of immunity than the male bird groups B, D, and F who did not get Lisovit, according to the study's findings. Lisovit was added to Group A, which was not vaccinated, and compared to Group B, which was not vaccinated and did not get Lisovit. While group B, which was maintained as a control, had a stronger immunity status than group A, which was not vaccinated and received supplements of Lisovit, group A's titer was not at a level that was protective.

It is concluded that Lisovit alone is not enough to enhance the immune status up to protective level. Both vaccinations along with supplementation of Lisovit as immunomodulator are recommended to boost up the immunity level of birds up to a protective level against ND. Group A showed better immunity compared to group B at 7^{th} and 30^{th} day after treatment but showed decreased response at 45^{th} day after treatment. It was concluded that the effect of Lisovit remained up to thirty days after supplementation.

The results of present study indicated that the groups which were vaccinated and were given

Lisovit @ 200 mg/kg body weight showed higher antibody titers against ND as compared to those groups which were only vaccinated but not given Lisovit (immunomodulator). The findings of present study were in line with the findings of Qayyum *et al.* (2012) who also concluded that Lisovit supplementation had role in enhancement of immune status. The results of study of Qayyum *et al.* (2012) indicated that Lisovit at the dose rate of 200mg/kg b.wt. can be used to enhance antibody titres against ND and IBD vaccines, to reduce mortality rate and to improve FCR in broiler chickens. In a study conducted by Sindaye *et al*. (2023) it was observed that the Lysozyme administration in

the diet could improve intestinal morphology, immune eciency, and nutritional digestibility in laying hens. Moreover, it was observations that lysozyme was safe to use as a feed supplement for the production of laying hens. Sindaye *et al*. (2023) suggested that that the dietary supplementation with 200 to 300 mg/kg lysozyme should be suggested to farmers as a proper level of feed additive in laying hens breeding. The results were also in line with results of Mubarak *et al.* (2009) who concluded that combine use of vitamin E and Selenium have role to enhance immunity status.

Abdel-Latif *et al*. (2024) found that the exogenous dietary lysozyme supplementation by a dose of 90 mg/kg broilers' diet induced better effects on intestinal integrity, fecal bacterial counts, immune response and growth performance. Vitamin E has enhance immune responses in animal and human and confer protection against several infectious diseases (Lee and Han, 2018). Bastamy, *et al*. (2024) recorded several benefits of lysocome in poultry production. The cellular immune modulation showed higher opsonic activity in Lysosome Treatment in broiler. Also, higher local, IgA, and humoral, HI titers, for both Newcastle, and avian influenza H5 viruses were found in Lysosome treatment. In conclusion, microbial lysozyme could improve feed efficiency, intestinal integrity, lactobacillus counts, anti-inflammatory, and immune responses in broiler chickens.

CONCLUSION

The results of present study indicated that the groups which were vaccinated and were given Lisovit @ 200 mg/kg body weight showed higher antibody titers against ND as compared to those groups which were only vaccinated but not given Lisovit (immunomodulator).

AUTHOR'S CONTRIBUTION

H. Saifullah: Performed the experiments.
M. I. Saleem: Conceived and designed the experiments.
T. Ahmad: Analysed the data/ `Technical input.
M. Nadeem: Contributed materials/ tools.
A. Sharif: Wrote the paper.

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