

Comparative Efficacy of Newcastle Disease's Live Vaccines in Broilers Using Hemagglutination Inhibition (Hi) Test at Jaba Mansehra

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ABSTRACT

Two commercial Lasota strain Newcastle Disease (ND) vaccines namely Lasota vaccine-1 and vaccine-2 and two Mukteswar Newcastle disease vaccines namely Mukteswar vaccine-1 and vaccine-2 were evaluated for their efficacy and influence on productive performance of broilers. For this purpose, total of 75 broilers day-old chicks were distributed into five equal groups tagged as A, B, C, D and E respectively, each group was further divided into 3 subgroups containing 5 birds per subgroup respectively. The birds in groups B, C, D and E were actively immunized against ND with Lasota-1, Lasota-2, Mukteswar-1 and Mukteswar-2 respectively, on days 10 and 21 through ocular route, leaving A as control group left unvaccinated. The serum HI antibody response to these four vaccines was determined on day 21st and 35th day of the experiment. According to the recorded results, group C vaccinated with Lasota (vaccine-2) showed significantly high ($p < 0.05$) antibody titers throughout the experiment as an evidence of its high efficacy. Other performance parameters such as feed intake, water intake, total body weight and Feed Conversion Ratio (FCR) were recorded on day 7th, 14th, 21st and 35th day of the experiment. These performance parameters differed significantly ($p < 0.05$) throughout the study period. The unvaccinated broilers (control group) showed better results in terms of weight gain and FCR than the vaccinated groups and there were not found a noticeable difference in the productive performance of the broilers between the vaccinated groups. From the recorded results, it was concluded that group C of broilers administered with Lasota vaccine-2 proved to be more efficient in terms of antibody production in broilers, although all the tested vaccines produced protection titers and unvaccinated broilers had better productive performance as compared to vaccinated flock.

Keywords: Newcastle disease; Broilers; Lasota; Mukteswar; Feed Intake; Weight gain

INTRODUCTION

In this modern era, availability of food is challenge in correspondence to growing rate of population. All over the world, increasing demand of food is an issue worth noting. All over the world for decades, sector of poultry is recognized as source of good quality food and even today, to cope up the gap between the need and availability of food, poultry industry has been transformed from small rural flocks to more developed commercial farming of broiler even in developing countries like Pakistan. But there are certain factors that have come up against

the poultry industry including infectious viral as well as bacterial diseases which are hampering its progress. Newcastle disease, caused by Newcastle Disease Virus (NDV), member of Avian Para Myxo Virus type 1 (APMV-1) of genus Avula viruses and family Paramyxoviridae. This is one such challenge to poultry industry in the developing countries like Pakistan. On the basis of pathogenicity, strains of NDV are generally grouped as highly (velogenic), moderately (mesogenic) and weakly pathogenic (lentogenic). 5% from the total of filed strains in Pakistan are reported as velogenic, 40% reported as lentogenic and 55% reported as mesogenic. Newcastle disease has led to huge

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economic losses associate with high morbidity, high mortality and many other production related losses. It is epidemiologically evident that NDV is being evolved continuously and its isolates have been classified so far into more than 18 distinct genotypes. NDV may be fatal sometimes and birds don't show any symptoms till death or the symptoms may range from mild air succulitis to visceral and severe nervous damages, cause paralysis even death of bird. In Pakistan, annual incidence is reported to be upto 38% among the broilers and mortality has been upto 50% that may varies according to the pathogenicity of the strain. Different methods have been implemented to avoid, reduce and prevent the disease on national level in birds flocks. Vaccination is currently one of the most prevalent methods to overcome Newcastle disease. Practice of the vaccination in the Pakistan comprises primary vaccination with Lasota strain (a lentogenic vaccine) afterwards the application of Mukteswar and Komorav strains. Mukteswar vaccine is usually prepared by combining appropriate quantities of mineral oil, emulsifier and desired antigen within surfactant covered particles [1]. Mukteswar vaccines are suggested to initiate when chicks are eight weeks old, to increase the immunity that was produced by lentogenic or Lasota vaccines. It has been reported that Mukteswar vaccine, produced in Pakistan is neither highly pathogenic nor highly immunogenic. It has been reported that primary vaccination at day 7 followed by secondary dose at day 28 is best regarding immune response and protection potential.

The immune response of the broiler chicken to vaccines antigen is influenced by age of chicken at vaccination, type of vaccine and level of maternally derived antibodies. Day old chickens are unable to respond well to vaccination due to high level of maternally derived antibodies [2]. So a secondary vaccination dose is required to achieve the level of immunity as the level of antibodies fall gradually with age. It is suggested to vaccinate the young chicks before 12 days of age; otherwise the decline in maternal antibodies can make bird more susceptible to infection. HI assay is most frequently used immunoassay for the detection of NDV antibodies in the poultry. This assay relates to detection of such antibodies that block binding of chicken red blood cells to Hemagglutinin Neuraminidase (HN) protein of NDV. A greater increase in HI antibody titre is regarded as evidence of infection. Despite being so useful in reducing and combating Newcastle disease, according to a report, vaccination leads to reduced body growth, less body weight resulting in lower Feed Conversion Ratio (FCR) compared to non-vaccinated birds. The growth promoting effect of propolis extract and vaccinated with NDV vaccines showed the highest increase in body weight followed by those received NDV vaccines and control negative groups respectively [3].

Despite being so widely studied about, Newcastle Disease is still prevalent in Pakistan, posing huge threats to poultry farming. The purpose of present experiment was to compare the efficacy and potency levels of ND vaccines; Lasota (international) and Mukteswar (national) of different companies in broiler chickens using the Hemagglutination (HI) test and also their impact on productive performance of the broilers.

MATERIALS AND METHODS

Housing management

The study was carried out at the Poultry Research Institute Jaba, Mansehra to evaluate comparative efficacy of Newcastle Disease's live vaccines in broilers using Hemagglutination Inhibition (HI) test and their influence on growth performance that is Feed Intake (FI), Water Intake (WI), Weight Gain (WG) and Feed Conversion Ratio (FCR) of the broilers [4]. Before the arrival of the chicks, the house was initially cleaned, white washed and properly fumigated for disinfection. Rice husk was used as litter of about 3 inches depth for comfortable bedding of the broilers. House temperature was maintained around 95°F 85°F in brooding and 70°F after brooding and humidity was maintained around 55-60 % during the period of experiment. Proper biosecurity measures were taken into account.

Experimental birds

A total of 75 broilers day old chicks of Cobb-500 were purchased from local hatchery for this research study. Chicks were weighed on day of arrival at Poultry Research Institute Jaba and randomly distributed into 5 groups group A, group B, group C, group D and group E containing 15 birds in each group. All the five groups were kept in 5 different pens. Each group was further replicated into three subgroups containing 5 chicks in each replica Table-1.

	Group A (Control)	Group B Lasota 1	Group C Lasota 2	Group D Mukteswar 1	Group E Mukteswar 2
Subgroup 1	5	5	5	5	5
Subgroup 2	5	5	5	5	5
Subgroup 3	5	5	5	5	5

Table 1: Study design of the experiment with subgroups (replicates).

Vaccines

All the groups received same dietary and managerial conditions including feeding, temperature, ventilation and humidity. All the groups have received ad-libitum feeding throughout the experiment period. The difference among the groups was the vaccination of Newcastle Disease Virus (NDV) that was the strain and the manufacturing company of the vaccines. Following vaccination of NDV was given to all experimental groups [5].

Group A: Chicks of group A were considered as control group chicks and were not administered any vaccine throughout the experiment.

Group B: Chicks of group B were administered Lasota strain vaccine-1 of NDV through ocular route on day 10th and day 21st of the experiment.

Group C: Chicks of group C were administered Lasota strain vaccine-2 of NDV through ocular route on day 10th and day 21st of the experiment.

Group D: Chicks of group D were administered Mukteswar strain vaccine-1 of NDV through ocular route on day 10th and day 21st of the experiment.

Group E: Chicks of group E were administered Mukteswar strain vaccine-2 of NDV through ocular route on day 10th and day 21st of the experiment.

(Vaccine-1 and vaccine-2 used in the experiment came from different commercial companies)

Data collection

When the birds arrived, they were weighed and then distributed randomly into 5 groups. Then the birds were regularly weighed on day 7, 14, 21, 28 and 35 through weight balance and mean weight of birds in each group was recorded. The feed intake of birds of all groups was recorded on day 7th, 21st, 28th and 35th. The given feed was calculated and the left over feed in the feeder was also weighed at the end of the day. Feed intake was the difference between amount of feed given and amount of feed left in the feeder at end of the day. The feed consumed per bird was found as total feed consumed in a group divided by total number of chicks in each group. The feed conversion ratio was recorded on day 7, 21, 28 and 35. The FCR was recorded using the formula; FCR=Feed given/weight gain. 3 chicks per group were randomly selected in which 1 chick from each replica was taken for the blood collection through the wing vein of the chick with sterile syringes on 21st and 35th day of the experiment. The blood samples after collection poured into the clot activator glass tubes (BD, Germany) and were shifted to the Laboratory of Poultry Research Institute (PRI) Jaba for serum separation. The blood samples were centrifuged at 2500 rpm for 15 minutes at room temperature after which the supernatant fluid was collected using sterile pipette into the serum collection plastic tubes provided by the PRI Jaba. The HI tests for NDV were done in V bottom microplates with automated equipment, using 4 HA units of antigen. Serial two-fold dilutions of serum in antigen started at 1:5 were prepared using 50 µl per well. 50 µl of 0.5 % chicken erythrocytes were added to each well and the plates were incubated for 1 hour at 25°C before reading. The samples showing specific central button shaped settling of RBCs was recorded as positive and the maximum dilution of each sample causing Haemagglutination inhibition was considered as the end point. The HI titer of each serum sample was expressed as reciprocal of serum dilution. A geometric mean HI titer was determined on selected birds from each experimental group on day 21st and day 35th.

Statistical analysis

Collected data was analyzed with the use of Analysis of Variance (ANOVA) procedure through Completely Randomized Design

(CRD), using two factors. Comparison of means was worked out using Duncan’s Multiple Range (DMR) test.

RESULTS

Feed intake

Feed intake of all the five groups was measured weekly. From the obtained results it was found that feed intake of all the groups had no noticeable difference. The results revealed significant difference (p<0.05) among the groups throughout the experiment as shown in the Table 2.

Week	Group A	Group B	Group C	Group D	Group E	p value
1 st	175 ± 3	175 ± 2	174 ± 4	175 ± 3	171 ± 4	<0.05
2 nd	369 ± 5	363 ± 6	364 ± 5	365 ± 7	364 ± 5	<0.05
3 rd	607 ± 9	598 ± 8	598 ± 8	599 ± 9	598 ± 8	<0.05
4 th	814 ± 13	806 ± 14	808 ± 15	807 ± 13	806 ± 15	<0.05
5 th	1092 ± 22	2090 ± 23	1088 ± 21	1089 ± 22	1088 ± 21	<0.05

Table 2: The results revealed significant difference (p<0.05) among the groups throughout the experiment.

Water intake

Intake of water by broilers of all the groups was measured and recorded on weekly basis. According to the results, the groups showed not a noticeable difference in the water intake of the broilers of all the selected groups. There was significant difference (p<0.05) among the groups regarding water intake was observed among the groups throughout the study duration as shown in the Table 3.

Week	Group A	Group B	Group C	Group D	Group E	p value
1 st	366 ± 3	359 ± 5	358 ± 5	356 ± 5	361 ± 3	<0.05
2 nd	760 ± 6	751 ± 7	748 ± 6	749 ± 7	757 ± 5	<0.05
3 rd	1134 ± 12	1125 ± 13	1124 ± 12	1126 ± 14	1131 ± 13	<0.05
4 th	1574 ± 15	1564 ± 16	1563 ± 14	1562 ± 16	1571 ± 15	<0.05
5 th	2206 ± 21	2202 ± 22	2201 ± 23	2202 ± 21	2203 ± 22	<0.05

Table 3: Water intake (ml/bird) of broilers throughout the study period.

Total body weight (g/bird)

The results of weekly measurements of total body weights of all the groups showed significant difference ($p < 0.05$) throughout the study period. According to the recorded results, the control group of broilers had maximum body weight as compared to the other groups of broilers that were vaccinated throughout the study duration as shown in the Table 4.

Week	Group A	Group B	Group C	Group D	Group E	p value
0	44 ± 2	44 ± 2	44 ± 2	44 ± 2	44 ± 2	<0.05
1 st	183 ± 7	180 ± 5	179 ± 6	181 ± 5	178 ± 6	<0.05
2 nd	495 ± 9	485 ± 8	483 ± 8	482 ± 9	481 ± 8	<0.05
3 rd	894 ± 12	880 ± 11	879 ± 12	878 ± 13	881 ± 12	<0.05
4 th	1433 ± 15	1413 ± 16	1412 ± 14	1414 ± 15	1411 ± 16	<0.05
5 th	2039 ± 21	2007 ± 22	2010 ± 21	2011 ± 23	2008 ± 22	<0.05

Table 4: Total body weight (g/bird) of broilers throughout the study period.

Feed conversion ratio

The Feed Conversion Ratio (FCR) of broilers of all groups was noted on weekly basis. According to the results, control group of the broilers had better FCR as compared to the other groups of broilers that were vaccinated. There was a significant ($p < 0.05$) difference recorded in FCR between the groups throughout the study period as shown in the (Table 5).

Week	Group A	Group B	Group C	Group D	Group E	p value
1 st	1.25 ± 0.068	1.28 ± 0.032	1.28 ± 0.054	1.28 ± 0.074	1.27 ± 0.044	<0.05
2 nd	1.17 ± 0.032	1.18 ± 0.041	1.19 ± 0.047	1.21 ± 0.045	1.21 ± 0.050	<0.05
3 rd	1.51 ± 0.032	1.50 ± 0.039	1.50 ± 0.039	1.51 ± 0.035	1.49 ± 0.061	<0.05
4 th	1.50 ± 0.028	1.51 ± 0.045	1.51 ± 0.021	1.50 ± 0.039	1.52 ± 0.053	<0.05
5 th	1.79 ± 0.028	1.83 ± 0.029	1.81 ± 0.024	1.82 ± 0.029	1.81 ± 0.012	<0.05

Table 5: Feed conversion ratio of broilers throughout the study period.

Geometric mean titer

The Geometric Mean Titer (GMT) of all the groups was recorded on 21st and 35th day of the experiment. According to the obtained results, Group C showed a better GMT as compared to other groups on day 21st and day 35th. There was a significant ($p < 0.05$) difference in GMT of all the groups throughout the study period as shown in Table 6.

Day	Group A	Group B	Group C	Group D	Group E	p value
21 st	0	6.15	6.88	6.71	6.67	<0.05
35 th	0	12.22	12.27	11.89	11.51	<0.505

Table 6: Geometric Mean Titer of broilers of broilers throughout the study period.

DISCUSSION

The objective of the study was to compare the efficacy of Newcastle disease live vaccines i.e Lasota (International) and Mukteswar (National) in broiler chickens using Haemagglutination Inhibition (HI) test. In the present study, four groups of broilers B, C, D and E were administered NDV vaccines Lasota (vaccine-1 and vaccine-2) and Mukteswar (vaccine-1 and vaccine-2) respectively and group A was considered as control group. Vaccine-1 and vaccine-2 refer to different manufacturing companies. The recorded data revealed that unvaccinated (control) group showed high weight gain (606 ± 8.60) than the vaccinated groups. Among the vaccinated groups Lasota (vaccine-2) gave better results in terms of weight gain (598 ± 5.53) than Lasota Vaccine-1 (594 ± 8.52), Mukteswar vaccine-1 (597 ± 12.02) and Mukteswar vaccine-2 (597 ± 7.11). The present study correlates with the study of which reported weight gain to be comparatively less in vaccinated birds than the non-vaccinated ones. Our study is also consistent (in case of vaccinated birds) to the results of which concluded that body weights were significantly higher ($p < 0.05$) in birds vaccinated with Lasota. also reported similar results that Lasota is superior to Mukteswar vaccine in terms of weight gain.

In the present study, there was no significant difference ($p > 0.05$) between vaccinated and un-vaccinated birds throughout the experimental period. This indicates that vaccines did not have any effect on cumulative feed consumption. The recent study showed that birds which were left unvaccinated (control group) showed a better feed conversion ratio (1.79 ± 0.028) than the vaccinated birds. Among the vaccinated groups, the one inoculated with Lasota vaccine-2 gave a better feed conversion ratio (1.51 ± 0.021) than the ones administered with Lasota vaccine-1 (1.51 ± 0.045), Mukteswar vaccine-1 and vaccine-2 (1.50 ± 0.039 and 1.52 ± 0.053) respectively supported the present study by reporting a less feed conversion ratio in case of vaccinated birds compared to uninoculated birds. This is because of the heat and body stress in birds because of vaccination which resulted in the failure of birds to efficiently convert the feed into weight. In consent to our study, also found a significantly higher FCR ($p < 0.05$) in the non-vaccinated birds.

It may also be because the vaccine was administered after the complete decay of maternal antibodies. The birds received vaccine for the first time on day 21st throughout the experiment that's why they were unable to convert the feed into weight in a better way. Thus, FCR was higher on 35th day of the study.

Our study gives the evidence of efficacy of Lasota vaccine-2 in terms of Geometric Mean Titer (GMT) obtained through Haemagglutination Inhibition (HI) test. The recorded results gave higher geometric mean titer (12.27) in the birds inoculated with Lasota vaccine-2. The birds being administered with other vaccines in group C, D and E showed comparatively less titers (12.22, 11.89 and 11.51) respectively, followed by control group with zero titers throughout the experiment. This low titer may be because of heat stress and water deprivation that led to steroid production ultimately causing immunosuppression. These results are consistent with the study of according to which Lasota produced more immune response and high titer of antibodies than other vaccine strains also described the efficacy of NDV Lasota in terms of high antibody titers than other vaccines. Also compared the efficacy of Lasota strain depending upon the time of administration (as primary vaccine or after the decay of maternal antibodies). The results indicated that use of Lasota strain after the decay of maternal antibodies resulted in high antibody titer and robust antigen-specific immune response.

CONCLUSION

From the recorded results, it was concluded that group C of broilers administered with lasota vaccine-2 proved to be more efficient in terms of antibody production in broilers, although all the tested vaccines produced protection titers and unvaccinated broilers had better productive performance as compared to vaccinated flock. Among the vaccinated flock, there was not a noticeable difference in productive performance of broilers among the groups. However, it was recommended that the choice of which vaccine to use depends not only on the foregoing factors, but also on the conditions of a specific region, such as the availability of veterinary services, population distribution, previous experience, the communication structure and climate of the region.

AUTHOR'S CONTRIBUTION

Aisha Bakhtiar conducted the research, collection of data and write-up to fulfill the requirement of degree of Master of Philosophy. Naqash Khalid supervised the research work as co-supervisor, contributed in the study designing, helped in research article write up, in each step of data collection, proper presentation and interpretation of the results. Sardar Azhar Mehmood supervised the study designing and helped in data collection and analysis. Syeda Faryal Sakhawat, Zainab Arshad and Zanib Miskeen help in data collection and laboratory analysis. Muhammad Ayaz provides a platform to conduct the study, supervise overall research methodology, facilitate at every step of experiment and provided financial support.

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CONFLICT OF INTEREST

Authors didn't declare any conflict of interest.

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