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Immunomodulatory Effects of Flumequine and Enrofloxacin on Newcastle Disease Virus Vaccinated Broiler Chicks

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Abstract

Immunomodulatory effects of Flumequine and enrofloxacin were evaluated on humoral immune response of Newcastle disease virus (NDV) vaccinated broiler birds. It was observed that birds receiving flumequine as growth promoter had higher mean body weights, better feed conversion ratio, higher NDV haemagglutination inhibition (HI) antibody, lesser overall mortality, 100% protection against challenge with virulent NDV and no detrimental effects on their lymphoid organs compared to the cyclophosphamide treated and untreated chicks. It was concluded that the use of flumequine has good effect on growth and performance of the treated chicks. However enrofloxacin treatment of chicks as growth promoter does not have effects comparable to flumequine. The cyclophosphamide treatment adversely affected the performance of chicks.

Key Words: Flumequine, Enroflxacin, Chicks, Immunomodulation

Introcuction

Broiler production is an ideal enterprise for small scale investment owing to the fast growth of bird and quick returns from the market. Important growth promoters extensively used in poultry; are enzymes, buffers, probiotics, stabilizers of intestinal flora growth hormones, antibiotics (Flachowsky *et al*., 1992), organic acids, micro-flora enhancers, coccidiostats, carotenoids herbal products, metabolic peptides etc. (Swick, 1996). However, their use may result in immuno-modulation in the birds; either immuno-stimulatory or immunosuppressive (Muneer *et al*., 1988). Immunostimulation of chicks may lead to increased antibody production, enhanced graft versus host reactions, increased phagocytosis; inhibit macrophages and their potential to inhibit tumor growth (Spallyholz *et al*., 1973). Suppression of immune response of the hosts may be due to temporary or permanent damage to primary lymphoid organs (Thigpen *et al*., 1973; Muneer *et al*., 1988).

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The present study was planned to evaluate the use of Flumequine and Enroflxacin when administered at recommended dosage level, whether to have any detrimental effects on body weight, lymphoid organs and immunity of the broiler chickens as measured by antibodies produced by Newcastle disease virus vaccine.

Materials and Methods

A total of 192 day-old Broiler chicks were procured from M/S Hi-Tech Breeding Company, Lahore and Commercial non-medicated (Broiler Starter and broiler finisher) feeds were purchased from Big bird feed Mills (PVT) limted, Lahore. Atiquin containing 50% flumiquin, Enflox containing 25% Enrofloxacin and Cyclophosphamide an immunosuppressive agent were procured from market. A live freeze-dried Newcastle disease virus (Lasota Strain) vaccine manufactured by Lohmann animal health was used. Virulent field strain of NDV was obtained from microbiology department, UVAS, Lahore

Experimental Design

The chicks were randomly divided into two main group's i.e; medicated and non-medicated. The medicated group was further divided into two flumequine and enrofloxacin medicated chicks. The non-medicated chicks were divided into cyclophosphamide treated and untreated control groups. Each of the groups was further sub-divided into two and offered treatments according to the protocol as mentioned in table (1).

Fifty percent of the birds from each subgroup were challenged with virulent field virus having EID50 $10^{5.5}$. The challenged birds were kept under observation for 16 days to record the clinical signs of the disease or any mortality occurred.

Weight Gain and Feed Conversion Ratio (F.C.R)

Birds were weighed weekly and differences in body weight of chicks in various treatment groups and subgroups were recorded. Weights of bursa of Fabricius, thymus spleen and liver of birds were also recorded. FCR of chicks recorded after calculation by the formula.

$$\text{F.C.R.} = \frac{\text{Feed consumed (Gms)}}{\text{Weight again (Gms).}}$$

Table 1: Experimental design

Treatment	Age of Birds	Treatment Subgroups							
		1a	1b	2a	2b	3a	3b	4a	4b
NDV vaccine oculonasal route	7 and 21	+	_	+	_	+	_	+	_
Cyclophosphamide sub-cutaneously	1, 2 and 3	_	_	_	_	+	+	_	_
Flumequine 50% medication in drinking water	1 to 56	+	+	_	_	_	_	_	_
Enrofloxacine 25% medication in drinking water	1 to 56	_	_	+	+	_	_	_	_
Non-medicated control ration	1 to 56 termination	+	+	+	+	+	+	+	+
Challenge with virulent NDV strain oculonasal route	40[a]	+	+	+	+	+	+	+	+

\+ = Treatment given
\- = Treatment not given
a = Challenged 50% birds from each subgroup

Analysis of Serum samples

Blood samples were collected from all groups at weekly interval till 8th weeks to determine the pre- and –post- Vaccination and post-virus challenge antibody titres using haemagglutination inhibition (HI) test.

Chicken embryo propagated Lasota strain of NDV was used for both HA and HI test. Haemagglutination test & Haemagglutination Inhibition (HI) tests (Alexander and Chattel 1977).

Data Analysis

The data in all the experimental groups was compared by analysis of variance, and statistically significant differences among various treatment means were determined using least significant difference (LSD) test at 5% level of probability (Steel *et al*. 1997).

Results & Discussion

The health problems of the growing flocks have been traditionally managed by the use of antibiotics and bio security at the farms. Many types of growth promoters such as vitamins, antibiotics, probiotics, buffers, enzymes, growth hormones and stabilizers of intestinal flora are in common use for increasing the growth and production potentials of chickens (Flachowsky *et al*., 1992.) The effect of antibiotics and vaccines on immune response of chickens may sometimes be detrimental (Muneer *et al*., 1988) requiring their evaluation before use in commercial poultry. The present study evaluated the effect of Flumequine and enrofloxacin on the performances of birds and to compare immune suppressed chicks with untreated chicks.

The comparison of mean body weights of birds in various treatment groups, and subgroups are presented in table2 at different days. The results indicate that the birds vaccinated against ND had higher body weight gain when compared at different days. To those which were not vaccinated Newcastle Disease. Among the NDV Vaccinated birds, those receiving Flumequine medication had higher mean body weights as compared to chickens that had received enrofloxacin medication or those which were reared as non-medicated feed. Among the NDV vaccinated chickens, the mean body weight of birds that received cyclophosphamide treatment was significantly lower than the birds that received Flumequine 50% and enrofloxacin 25% medication and those receiving non medicated ration (control group). The differences in weight gains in chickens in various groups were significant at 5% level of probability using heart Significant Difference Test (LSD). It was observed that the Flumequine treated chicks continued to have higher mean body weights than the other groups throughout the experimental period. This suggests that the use of Flumenquine in broiler feed help in increasing the body weight gain. These findings are in accordance to the works of Bunyan *et al.,* (1977). It was observed that enrofloxacin treated. Chicks have lower mean body weight than the flumequine treated and untreated control groups throughout the experimental period. This indicates that the use of enrofloxacin in broiler chicks decreases the body weight gain. Lindmann (1984) reported similar findings for chloramphenical. It was observed that the cyclophosphamide treated chicks gained lower mean body weight than the antibiotics treated and untreated control groups throughout the experiment. Hirage *et al*. 1976. have also reported higher body weight gains in the untreated chicks than the cyclophosphamide treated chicks.

Table: 2: The comparison of mean body weights of birds in various treatment groups

Sub group	Treatment	Body wt. (gm) mean ± SE	Bursal wt. (gm) Mean ± SE	BBR	Spleen wt. (gm) mean ± SE	Thymic wt.(gm) mean± SE	Liver wt. (gm) mean ± SE
1a	Flumequine+NDV VACCINATED	2342.92±7.9	2.78±0.058	0.12	2.08±0.058	7.53±0.057	41.78±0.057
2a	Enrofloxcin+NDV VACCINATED	2285.46±24.04	2.19±0.057	0.02	1.78±0.033	7.36±0.058	3987±0.058
3a	Cyclophosphamide+NDV VACCINATED	1969.58d±3.63	0.49±0.058	0.11	1.59±0.058	7.43±0.057	37.06±0.058
4a	Non-medicated+NDV VACCINATED	2317.83ab±7.92	2.58±0.058	0.11	1.19±0.089	7.49±0.057	41.25±0.058
1b	Flumequine + Non-VACCINATED	2264.17bc±10.86	2.41±0.058	0.09	1.86±0.058	7.51±0.058	38.63±0.057
2b	Enrofloxacin+Non-VACCINATED	2218.75c±10.82	1.93±0.058	0.02	1.53±0.058	6.94±0.058	34.98±0.057
3b	Cyclophosphamide+Non- VACCINATED	1911.67d±0.833	0.43±0.058	0.11	1.49±0.057	7.21±0.057	35.61±0.058
4b	Non-medicated+Non- VACCINATED	2209.17c±45.62	2.38±0.057	-	1.75±0.058	7.29±0.058	37.47±0.058

X = 24 (Sample size) Y = (10Sample Size)
Wt. = Weight Gms = Grams
M±SE = Mean+Standard Error NDV = Newcastle Disease Virus
Abcdefgh= Any 2 means carrying the same superscript are not significantly different from each other at 5% probability level Using LSD test.

At day 56, a total of 10 birds from each treatment subgroups were randomly selected, weighed, sacrificed and their bursa of Fabricius, spleens, thymus and liver removed and weighed. A comparison of mean bursa, splenic, thymic, and liver weights along with the mean live body weight of birds is presented in table 3.

Table 3: Comparison of Mean Body, Bursal, Splenic, thymic, liver weights and Bursal body weight ratio of Chickens in Various Treatment Subgroups at Day 56.

Sub group	Treatment	Mean body wt. (Gms)XM±SE	Mean bursal wt. (Gms)YM±SE	Mean Splenic wt. (Gms)YM±SE	Mean Thymic wt. (Gms)Y M±SE	Mean Liver wt. (Gms)YM±SE	Bursal Body weight Ratio
1a	Flumequine 50%+NDV vaccinated						
2a	Enrofloxacin 25%+NDV vaccinated	371.25^{ab} ± 5.12	607.50^{bc} ± 4.73	914.17^{b} ± 3.00	1282.50^{b}± 21.32	1794.17^{b}± 3.63	2048.08^{ab}± 6.28
3a	Cyclophosphamide+NDV vaccinated	315.83^{d}± 5.42	552.92^{e}± 2.53	859.79^{d}± 6.42	1220.00^{c}± 8.32	1730.42^{e}± 4.35	1830.83^{d}± 1.10
4a	Non-medicated+NDV vaccinated	377.50^{abc}± 1.91	615.42^{b}± 1.82	917.50^{b}± 4.39	1287.08^{b}± 10.01	1800.42^{b}± 1.10	2055.00^{ab}± 10.03
1b	Flumequine 50% + Non-vaccinated	381.67^{ab}± 5.12	598.33^{c}± 1.10	912.92^{b}± 3.25	1279.17^{b}± 2.73	1780.42^{cd}± 3.97	2028.54^{cd}± 11.09
2b	Enrofloxacin 25%+Non vaccinated	369.79^{b}± 2.01	584.58^{d}± 1.82	899.17^{c}± 3.97	1270.00^{b}± 4.73	1767.08^{d}± 9.85	2008.33^{c}± 16.27
3b	Cyclophosamide+Non vaccinated	311.25^{d}± 2.60	539.37^{f}± 2.86	844.58^{c}± 2.92	1205.42^{c}± 5.79	1710.00^{f}± 7.11	1801.67^{d}± 4.23
4b	Non-medicated+ Non vaccinated	373.12^{bc}± 5.34	588.33^{d}± 1.50	904.58^{bc}± 3.97	1274.17^{b}± 2.92	1776.66^{cd}± 7.23	2022.92^{bc}± 14.58

M±SE = Mean ± Standard Error
NDV = Newcastle Disease Virus
a b c d e f = Any 2 means carrying the same superscript are not significantly different from each other at 5% probability level

The bursal weights of the birds that received cyclophosphamide treatment were general lower than those which did not received cyclophosphamide. Generally the mean bursal weight of chickens vaccinated against NDV (Flumequine 50% medicated) were higher as compared to NDV non-vaccinated chickens in various groups. Highest mean bursal weight was observed in chicks of subgroup 10x Flumequine treated and the lowest in chicks of subgroup 3b. These findings are in agreement with the observations of Hijden 1995 who reported the mutagenic response of lymphoid cells of European eel treated with flumequine. Dafwang *et al.* 1996 reported that the bursal weight in chicken administered with growth promoting antibiotics was increased. The enrofloxacin treated chicks had lower mean bursal weight than the flumequine treated and untreated control groups These findings are similar to the observations of Al-Ankari and Homeida 1996 for oxy tetracycline and sulphadimidine. Cyclophosphamide treated chicks had lower mean bursal weight (Munir 1994 and Fulton *et al.* 1996 also reported that cyclophosphamide induced bursal atrophy in the treated chicks.

Highest mean splenic weights were recorded in chickens in subgroup 1a (Flumequine 50% medicated and NDV vaccinated and the lowest mean splenic weights were of subgroup 3b. (Cyclophosphamide treated, non-vaccinated). The highest mean splenic weight was observed in the flumequine treated chicks followed by enrofloxacin treated cyclophosphamide treated and untreated control groups. These findings are different from the observation of Al-Ankari and Homeida 1996.

The highest mean thymic weight was recorded in the chicken from subgroup la, and lowest in 2b. It was observed that the mean thyumic weight of the NDV vaccinated chickens were higher than those of NDV non-Vaccinated birds. Significant differences between the thymic weight of chicks treated with Flumequine and enrofloxacine were observed. Whereas, there were non-significant difference among the mean thymic weight of chicks treated with cyclophosphamide and those which were not Fulton *et al.*, 1996. Reported that cytoxan had no effects on the treachea, lung, liver, kidney and Thymus. These findings are also congruent with the observations of Munir *et al.* 1994.

Highest mean liver weight was recorded in chickens of subgroup a (Flumequine 50% medicated and NDV vaccinated and the lowest was of subgroup ab (Enrofloxacin 25% medicated, non-vaccinated). In general, Liver weights were higher in groups vaccinated against NDV than non-Vaccinated. The liver weights of the vaccinated chicks were heavier than the non-Vaccinated chicks these observations are different from the findings of Asker *et al*., 2004. Who reported that relative weight of weight, of liver were not affected by vaccination or treatment of chicks. The findings of present study indicate that in vaccinated birds, flumequine treatment gave good results a6 compared with enrofloxacin treated. The cyclophosphamide treatment also significantly lowers the liver weight gains of chicks. Yoshida *et al*., 1999. Reported increased number of mitosis in the livers of mice treated with flumequine

Feed conversion ration (FCR) of different subgroups at day 56is presented in Table (4) Flumenquine treated subgroups, showed higher FCR than enrofloxacin treated birds. Cyclphaphamide treated birds had poor FCR as compared to chicks in the cyclophosphamide untreated subgroups. In case of subgroup 1a findings of present study are similar as reported by Rakowska *et al*. 1993 and different from Lin *et al.*, 1991 who reported that there was no effect on feed conversion rates of groups on growth promoting antibiotics Lincomycin and spectinomycin.

Table4: Feed Conversion Ratio of different treatment subgroups

Treatment subgroups	Ration Consumed (grams)	Mean weight Gain (Grams)	Feed Conversion Ratio
1a	4512	2302.22	1.96
1b	4468	2223.27	2.01
2a	4802	2276.15	2.11
2b	4799	201.7	2.18
3a	4127	1928.58	2.14
3b	4171	1870.54	2.23
4a	4447	2261.56	1.68
4b	4486	2177.93	2.06

Treatments Subgroups

1a	=	Flumequine 50% Medication +NDV Vaccinated
1b	=	Flumequine 50% Medication +Non- Vaccinated
2a	=	Enrofloxacin 25% Medication +NDV Vaccinated
2b	=	Enrofloxacin 25% Medication +Non- Vaccinated
3a	=	Cyclophosphamide treatment + NDV Vaccinated
3b	=	Cyclophosphamide treatment + Non- Vaccinated
4a	=	Enrofloxacin 25% Medication +NDV Vaccinated
4b	=	Non- Medicated +Non- Vaccinated

The GM HI titers of chickens 1a, 2a, 3a, 4a, 1b, 2b, 3b, and 4b, on day 16 post challenge were recorded as 8.9, 8.4, 4.7, 8.6, 1.5, 0.0, 0.00 and 1.25 respectively. The groups which were treated with cyclophosphamide either vaccinated against NDV or not showed either quite lower or insuperably negligible titres The highest HI antibody titers were recorded in the sera of chick from subgroup 1a (Flumequine treated and NDV vaccinated). The differences in the mean HI titers of birds on day zero and 7 were not significant. Significant differences among the GM HI titers of birds in various treatment subgroups were observed from day 14to on ward till the completion of study. The highest GM HI titers were recorded. In the birds in subgroup 1a (8.9) and the lowest values were in subgroup. 2b (0) and 3b (0). The NDV vaccinated birds had significantly higher HI> titers than NDV non-vaccinated birds. Moreover, the birds which had received cyclophosphamide treatment exhibited either extremely low or negligible HI titres as compared to the birds. This did not receive cyclophosphamide treatment. There were non significant differences among the mean HI titers of birds from subgroup 1b (1.5), 4b(1.25) and 2b(0), 3b(0). The mean HI titres of the vaccinated birds offered flumequine 50% medication was significantly higher than the vaccinated birds kept on enrofloxacin 25% medication and non treated ration. The highest GM HI titres were recorded in the sera of chicks treated with flumequine and lowest in the sera of chicks treated with cyclophosphamide there was significant different between the HI titre of chicks treated with flumequine and enrofloxacin These findings are congruent with Rzedzicki *et al*., 1991 who reported variable antibody response after The administration of drugs like Flumequine, enrfloxacin, Dimerazine Bacitracin chlortetracycline and chloramphenicol. However, findings observations of Shojadoost *et al.*, 1999 Who reported that there was non significant difference in the mean antibody titre between birds given antibiotics and untreated control.

The post- challenge mortality percentages in birds from subgroups 1a, 2a, 3a, 4a, 1b, 2b, 3b, and 4b, in 16days period were recorded as 8,25,75,8,83,100,100 and 83 respectively. The birds in subgroups 1a (flumequine medication and NDV Vaccinated) 2a (enrofloxacin medication and NDV vaccinated) and 4a (Non –medicated and NDV vaccinated) resisted the virulent NDV challenge. The overall mortality was lower in flumequine treated chicks after challenge and maximum in cyclophosphamide treated chicks. The results are in accordance to the observations of Rouse and Szenberg 1974 who reported extremely high post challenge mortality in cyclophosphamide treated birds.

The analysis of post-challenge sera from various groups indicated that the flumequine has good effect on the immune system of chicks Cyclophosphamide treatment interfered with HI antibody production against NDV. These findings are in agreement with the observation, of Rouse and Szenberg 1974. Who reported that the cyclophosphamide treated chicks failed to produce antibodies.

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