



Research Article

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Cow Urine Distillate as an Immunomodulatory Agent in Broiler Chick

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Abstract

The present study was conducted to assess the immunomodulatory effect of feeding cow urine distillate (CUD) in comparison to a known positive immunomodulator, Levamisole HCl as feed additive on humoral and cell mediated immune response in broiler birds. For monitoring humoral immune response against NDV vaccine HI test was done. For monitoring cell mediated immune response status in all the four groups, contact sensitivity test using DNFB (2, 4- dinitrofluorobenzene) as contact sensitizer was used. On all weekly intervals post NDV vaccination, higher MHI antibody titer was observed in levamisole treated group than any other groups. There was significantly ($p < 0.01$) higher MHI titer observed in the levamisole treated group T2 than T1 (CUD) treated group and two control groups at most ages post vaccination. The study indicated immunopotential effect of CUD but to a much lesser extent than levamisole, a proven immunostimulant and CUD can be recommended in broiler ration at optimum dose level against NDV.

Keywords: Antibody titer, Broiler, CUD, Immunomodulator, Levamisole

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1. Introduction

Experimentally, it has also been proved that among urine from various species the urine of the Indian cows is most effective¹. Immunomodulation is gaining importance for immuno-potential in hosts against various infections². The cow urine distillate is found to have immunomodulatory effect in mice as it enhances both T- and B-cell proliferation and also increases the level of IgG³. Recently, the cow urine has also been granted U.S. patents (No. 6896907 & 6410059) for its synergistic properties with antibiotics, antifungal and anti-cancer drugs as bio-enhancer⁴. It has provided the base for further research on immunomodulatory properties of indigenous cow urine. It has also been reported that CUD enhances B and T lymphocyte blastogenesis, increases IgG antibody titer in avian species^{5,6}. Keeping in view all the above facts, the present investigation was planned to study the

immunomodulatory effect of cow urine distillate (CUD) on humoral and cell mediated immune response against NDV vaccination in broiler chicks when administered orally.

2. Materials and methods

Eighty (80 No.) day-old Vencobb chicks were procured and maintained under standard farm condition in Avian Research and Development Centre (ARDC), College of Veterinary Science & Animal Husbandry, Ranchi. Initially, all chicks were kept under hover for first 6 days and on 7th day those were vaccinated with Newcastle disease (NDV) vaccine (F1 strain) and also recorded weight and grouped randomly into 4 groups of 20 chicks each. Group T1 was treated with CUD, Group T2 with levamisole, Group T3 was kept as vaccinated control and Group T4 was made the unvaccinated control respectively. CUD was prepared by distillation of indigenous cow urine after collection from the farm at College of Veterinary Science & Animal Husbandry, Ranchi farm laboratory.

CUD was given at a dose of 10 ml/ litre of drinking water. Broiler grower feed fed up to 4 weeks and broiler finisher feed fed ad lib till 6th weeks in all groups offered same quantities of feed and residue of feed weighed to know the actual amount of feed consumed every week. For monitoring of humoral immune response (HI test) Chicks of all groups immunized as well as controls were bled on 0, 7th, 14th, 21st, 28th, 35th and 42nd day of age respectively by adopting all aseptic precaution. Blood were collected without anticoagulant and allowed to clot and oozed out serum samples were collected in serum vials and inactivated at 56°C for 30 min.

Unvaccinated fowls were screened for obtaining suitable fowl RBC for Haemagglutination test (HA). Blood sample collected in Alsever's solution was centrifuged at 1500 rpm for 10 min. The packed red blood cells were re-suspended in PBS to make 1 % v/v suspension as per method by Allan and Gough⁷. HA and HI test were conducted as per Buxton and Frazer⁸ with slight modifications. Cell mediated immune response based on delayed hypersensitivity reaction showed manifestation of the T cell effector arm of the immune system. The test was done as per Tiwary and Goel⁹ with slight modifications. Statistical analyses for different observations of different parameters were done as per the method by Snedecor and Cochran¹⁰.

3. Results and Discussion

Results

Maternal antibody titers against NDV vaccination in the sera of day old and 7 day old chicks were found to be 1.505 ± 0.067 and 0.903 ± 0.077 . Maternal antibody was found to be waned up regularly at weekly interval. Mean haemagglutination inhibition (MHI) antibody titer (\log_{10} values) of sera against NDV vaccination of chicks in different treatment groups have been presented in Table 1. There were significant effects of treatments on MHI titer after vaccination up to 35 days post vaccination i.e. 42nd day of age of chicks. At day 7 after vaccination in treatment groups, higher MHI titer was observed in group T2 (1.756 ± 0.123) followed by T1 (1.657 ± 0.103) as compared to T3 (1.304 ± 0.100) and T4 (0.552 ± 0.100). The mean differences among the treated groups were found to be statistically significant among all the four groups as found on critical difference test.

The skin thickness of sensitized chicks was recorded two weeks later (i.e. on 42nd day) and challenged with 1/10 of the above dose (sensitizing dose). This resulted in reaction with initial erythema and induration followed by vesication, thickening and covering with soft scales. The overall mean skin thickness of sensitized normal and treated group of chicks was measured with vernier calipers as re presented in Table 2. There was non-significant effect of treatments on skin thickness in contact sensitivity test with DNFB at initial stage i.e. 6 h post challenge but their effect had significant influence on 24, 48 and 72 h post challenge. The response as increase in skin thickness was more among chicks of Levamisole (T2) and cow urine distillate (T1) treated groups than vaccinated (T3) and non-vaccinated control (T4) groups.

Discussion

Immunomodulatory effect of cow urine or its distillate has been reported by many workers¹¹⁻¹⁵ and therefore this has made the base for present research. The dose of CUD selected in the present study is according to the recommendation by Kumar et al.¹².

Effect of treatment on antibody titer

In the present study, it was found that there was a progressive increasing trend of MHI antibody titer up to 21 days of age in all groups except in unvaccinated control group which simulated with the finding of Chakraborty and Chatterjee¹³, Mehta¹⁴, Kumari¹⁵ and Singh¹⁶ while working with other immunomodulators. Increase in antibody titer can be attributed to enhanced proliferation and activation, transformation of B-lymphocytes into plasmocytes, responsible for immunoglobulin level. The result obtained in the present study was in accordance to finding of Chauhan et al.¹⁷ and Garg et al.¹³ who reported significant increase in serum antibody titer (IgG level) in NDV vaccinated chicks using ELISA. Immunopotentiating effect of levamisole on humoral and cell mediated immune response upon vaccination in chicks has been reported by Chakraborty and Chatterjee¹³, Kalita et al.¹⁸, Cao et al.¹⁹

and Prasad²⁰. In the present study, the result obtained in levamisole treated group of chicks also showed significant effect on MHI antibody titer in comparison to CUD suggesting its superior immunopotentiating effect over CUD on humoral immune response upon vaccination. In the present study, the increased cell mediated immune (CMI) response correlated with the findings of Awadhiya et al.¹³ and Srikumar et al.²¹.

Table 1. MHI antibody titer (log₁₀ value) of sera of different treatment groups at different intervals post-vaccination.

Days after NDV vaccination				
7	14	21	28	35
1.656±0.103 ^c	2.057±0.148 ^c	1.756±0.092 ^c	2.207±0.100 ^c	2.358±0.092 ^c
1.756±0.123 ^c	2.308±0.100 ^c	2.157±0.109 ^d	2.659±0.092 ^d	2.809±0.063 ^d
1.304±0.100 ^b	1.655±0.128 ^b	1.355±0.103 ^b	1.756±0.100 ^b	1.806±0.109 ^b
0.552±0.100 ^a	0.351±0.092 ^a	0.000±0.000 ^a	0.000±0.000 ^a	0.000±0.000 ^a

Values bearing different superscripts differ significantly, each observation is an average of six observations, (p<0.01)

Table 2. Mean skin thickness (mm) of broiler chicks of different treatment groups at various hours post DNFB challenge.

Groups	0	6	24	48	72
T1	0.563±0.043 ^a	0.750±0.032 ^a	1.210±0.077 ^b	96±0.038 ^b	1.250±0.046 ^b
T2	0.583±0.053 ^a	0.792±0.042 ^a	1.271±0.075 ^b	79±0.068 ^b	1.333±0.042 ^b
T3	0.550±0.025 ^a	0.688±0.028 ^a	0.938±0.043 ^a	88±0.028 ^a	1.021±0.050 ^a
T4	0.546±0.050 ^a	0.667±0.026 ^a	0.896±0.038 ^a	46±0.038 ^a	0.958±0.053 ^a

Values bearing different superscripts differ significantly; Each observation is an average of six observations, (p<0.01).

4. Conclusion

Cow urine distillate (CUD) possesses immunomodulatory effect as judged by increase in HI antibody titer against NDV. After analyzing the immunopotentiating effect of CUD on humoral and cell mediated immune response with NDV virus vaccination, its use as an immunomodulating agent at proper dose level may be advocated.

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