SUMMARY AND CONCLUSIONS

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RESEARCH PROJECT

ON

"RELATIONSHIP OF MHC GENES POLYMORPHISM WITH COCCIDIAL RESISTANCE IN CHICKEN"

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SUMMARY AND CONCLUSIONS OF THE PROJECT

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Name of the Organization: College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur
Principal Investigator: Dr. Mohan Singh Thakur

1. SUMMARY

The present investigation was conducted on Kadaknath, Caribro-91(Cari Vishal), Jabalpur Dual Coloured, Hubbard, Naked Neck birds and Cobb broiler chicken under the research project funded by Madhya Pradesh Biotechnology Council, Bhopal, Department of Animal Genetics and Breeding, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur. Sixty day old broiler chicks each of Kadaknath, Jabalpur Dual Coloured, Hubbard and Cobb broiler chicks procured from the hatchery were used in the study while fertile eggs of Caribro-91(Cari Vishal) and Naked Neck broiler chicken were brought from Central Avian Research Institute, Izzatnagar (U.P.) and were hatched in the Department of Poultry Science, College of Veterinary Science and Animal Husbandry, NDVSU, Jabalpur.

The battery brooder house was fumigated by using a combination of 35 ml of formalin (40 per cent formaldehyde) and 10 g potassium permanganate solution for one cubic meter of space. On arrival (day 1) they were wing-banded, weighed and randomly allocated to the treatments. The birds were maintained in the brooder batteries kept inside a well ventilated room in the college experimental poultry shed unit under proper coccidian free conditions. The usual precautions for raising chicks under coccidian free conditions were observed. The standard chick diet free of any anti-coccidial additives and sanitized tap water were given to the birds *ad lib* throughout the experiment.

The *Eimeria tenella* inoculums were prepared separately in three different stages i.e. harvesting of oocysts, sporulation of oocysts and storage of inoculums. The number of sporulated oocysts in the suspension was estimated and the volume was adjusted to contain the 10,000 sporulated oocysts/ml of suspension using McMaster counting chamber.

The chicks from all the experiment groups were weighed at day old and at weekly interval upto 8 weeks of age. The difference in the body weight of Kadaknath, Jabalpur colored birds, Cobb, Caribro-91, Hubbard and Naked neck under control and treatment group upto 3rd week of age was non-significant. However, after infection of *Eimeria tenella* with dose of 10,000 sporulated oocycts, the reduction in body weight was observed in treatment groups from 4 to 8 weeks of age in all the six genetic groups.

The average body weight from 1 to 8 weeks of age in control and treatment groups of Kadaknath, Jabalpur dual colored, Cobb, Hubbard, Naked Neck and Caribro-91 (Cari Vishal) birds were ranged from 54.68 to 658.71 g and 53.71 to 518.21 g, 67.61 to 777.58 g and 70.58 to 705.82 g, 134.29 to 2161.43 g and 141.39 to 1914.73 g, 65.41 to 2045.42 g and 63.11 to 1696.57 g, 113.80 to 1624.85 g and 115.24 to 1426.11 g, and 101.04 to 1413.08 g and 102.21 to 1201.66 g, respectively. The coccidial infection significantly affected the body mass gain and feed conversion ratio of the birds.

Lesion score was determined as per the method suggested by Johnson and Reid (1970). The lesion score ranged from +1 to +4 among the birds/groups under study. In Jabalpur dual coloured and Kadaknath it ranged from +1 to +2, while Naked neck and Hubbard showed +1 to +3. Caribro-91 and Cobb had the lesion score upto +4. The finding of the lesion score revealed that Kadaknath and Jabalpur dual coloured found to be more resistant to coccidial infection amongst the different genetic groups, while Caribro-91 and Cobb were most susceptible genetic groups. The genetic group involving Naked Neck and Hubbard was in intermediate range of susceptibility.

Oocyst index were determined by microscopic examination of mucosal scrapings from the caeca on 4^{th} to 14^{th} day post infection as per the method of Hilbrich (1978). The oocysts index varied from 1 to 3. The number of oocysts per field ranged from 15 to 100. Oocyst index in Jabalpur dual coloured, Kadaknath and Hubbard were in the range of 1 to 2, while those of Naked Neck, Caribro-91 and Cobb were 1 to 3.

Oocyst per gram (OPG) of birds from all six genetic groups was estimated using McMaster counting chamber by following the method of Davies *et al*. (1963), from day 5 to 9 post infection. The shedding of oocysts was recorded as early as on the 5^{th} day post challenge. The oocysts number shed varied at different stages of challenge. The oocysts production was highest on day 7^{th} and then the OPG started to decline upto day 10 post challenges.

The Mortality amongst the different genetic groups ranged from 0 to 15 %. No mortality was observed in Kadaknath and Jabalpur dual coloured birds, while maximum mortality was recorded in Caribro-91 (15%).

Genotyping was carried out using PCR-SSP (Polymerase Chain Reaction with Sequence Specific Primers). A 235bp, exon-2 region of chicken MHC BL-β II family gene was amplified using a set of five primers *i.e.*, B2 (222bp), B13 (141bp), B15 (222bp), B19 (213bp) and B21 (213bp). Genotyping by PCR-SSP revealed a total of 15 genotypes in the studied sample size of experimental birds. The number of genotypes found in Cobb, Kadaknath and Caribro-91, Naked Neck, Hubbard and Jabalpur Dual Coloured were 9, 8, 10, 10, 8 and 10, respectively. Allelic frequencies of B_2 and B_{13} were found to be highest in Kadaknath while frequencies of B_{15} and B_{19}

alleles were highest in Caribro-91. B_{21} allele was absent in Kadaknath and Jabalpur dual coloured birds.

The mean lesion score showed increasing trend till day 7 post challenge and then declined at day 14 post challenge. The highest mean lesion score on day 7 post challenge was recorded in Cobb (3.25±0.25), followed by Hubbard (2.75±0.29), Caribro-91 (2.75±0.25), Naked Neck (2.50±0.29), Jabalpur dual Coloured (1.75±0.25) and Kadaknath (1.50±0.29). These results indicated that Cobb genetic group was more susceptible for coccidial infection as compared to other five genetic groups, whereas Kadaknath group was most resistant to coccidial infection.

Oocyst index was maximum on days 7 post challenge and minimum on day 14 post challenge in all the six genetic groups. The highest mean oocysts index was found in Cobb as compared to other five genetic groups and the lowest mean oocysts index was observed in Kadaknath. On comparison, the Cobb genetic group was more susceptible for coccidial infection than other five genetic groups, whereas Kadaknath was found to be most resistant to coccidial infection.

The association between cytokine genes expression level in relation to coccidial resistance in different breeds/lines at different intervals (4, 7 14 days post infection) was studied using real-time PCR. The expression level of mRNA encoding cytokines genes quantified in intestinal lymphocytes following *E. tenella* infections were upregulated at different intervals of infection showed significant variation in level of cytokine genes. All the ten cytokine genes showed relative up-regulation in the six genetic groups in responses to coccidiosis

The phylogenetic analysis showed that the six breeds/lines were divided into two clusters. The first cluster consisted of Cobb, Naked Neck, Caribro-91 and Hubbard. Kadaknath and Jabalpur dual coloured chickens were grouped under second cluster. Kadaknath is more closely related to Jabalpur dual coloured chickens as compared to other genetic groups.

The effect of antibody response to SRBC in five identified commercial lines and Kadaknath with age of infection, challenge period (first vs. second) and their interactions were significant. After the first infection, the number of oocysts shed varied among the broiler lines; however, the line variation was dependent on the age of infection. The Cobb broiler and Caribro-91 (Cari Vishal) birds showed the poorest performance in antibody response to SRBC and challenge with coccidiosis. These two breed had low antibody titre to SRBC as well as an increased susceptibility to coccidiosis as compared to other chicken breed/lines. The Naked Neck broiler and Hubbard broilers were comparable in their responses to SRBC and coccidial challenges. The Kadaknath and Jabalpur dual coloured birds showed maximum response to SRBC as well as minimum lesion scores and oocyst Index as compared to other breeds/lines. These two breed were comparatively more resistance to coccidial challenges.

2. CONCLUSIONS:

From the results obtained in the present study it can be concluded that:

i. Allelic frequencies of B2 and B13 were found to be highest in Kadaknath while frequencies of B15 and B19 alleles were highest in Caribro-91. B21 allele was absent in Kadaknath and Jabalpur dual coloured birds.

ii. A total of 15 genotypes were observed in the entire population under study. The number of genotypes found in Cobb, Kadaknath and Caribro-91, Naked Neck, Hubbard and Jabalpur Dual Coloured were 9, 8, 10, 10, 8 and 10, respectively. The genotype B13B21 was absent in all the genetic groups.

iii. Kadaknath and Jabalpur dual coloured populations were in HW equilibrium while Cobb, Hubbard, Naked Neck and Caribro-91 were not in HW equilibrium at this MHC locus.

iv. The birds with genotype B2B21 and B13B19 gained higher body weight and also least affected by coccidial infection in comparison to other genotypes among the entire six genetic group.

v. The relative fold expression of cytokine genes increased with dose and was lowest on day 14 post challenge. The relative expression of these cytokine genes could play a crucial role by driving higher immune responses in susceptible birds to coccidial infection.

vi. Among the six genetic groups, Kadaknath breed was found to have more genetic divergence indicating that this breed is genetically distinct from the other chicken breed included under study.

vii. The lowest mean for lesion score, oocyst index and OPG were found in Kadaknath as compared to Jabalpur dual coloured, Cobb, Hubbard, Naked Neck and Caribro-91 genetic groups. The Cobb genetic group was most susceptible whereas **Kadaknath genetic group was most resistant to coccidial infection**.

viii. The present work provides a clue to the resistance capacity of the local Kadaknath birds as compared to the multicross varieties, suggesting for the incorporation of local germplasm into the new varieties.

ix. These results will definitely contribute to the further understanding of the genetic and immunological basis of resistance to coccidial infection in poultry. **These findings suggest that there may be a room for some latitude in utilization of Kadaknath breed of chicken for the development of coccidial resistance commercial lines/strains of birds in future, thereby increasing the production efficiency of farmers flock.**