MICROSATELLITE, IMMUNOCOMPETENCE AND CANDIDATE GENE EXPRESSION PROFILING OF RHODE ISLAND RED CHICKEN AND ASSOCIATION OF MICROSATELLITE ALLELES AND IMMUNOCOMPETENCE TRAITS WITH LAYER ECONOMIC TRAITS

Thesis

Submitted to the
DEEMED UNIVERSITY
Indian Veterinary Research Institute
Izatnagar - 243 122 (U.P.), India

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy
(Animal Genetics and Breeding)

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"Your Lord hath decreed that ye worship none but Him and that ye be kind to parents. Whether one or both of them attain old age in thy life, say not to them a word of contempt, nor repel them, but address them in terms of honor. And out of kindness lower to them the wing of humility and say: 'My Lord! Bestow on them Thy Mercy even as they cherished me in child-hood.'" (Quran 17:23-24)

Dedicated to....

Great Almighty

&

Abuji & Ammiji

Abu Hurairah (R) narrates that a man came to the Prophet (pbuh) and asked him, "Who amongst his near one had the greatest right over him?" The Prophet replied, "Your mother". The man then asked, "Who after that?" to which the Prophet replied again, "Your mother". Asked who is next, the Prophet again replied, "Your mother". The man asked who after that, the Prophet said, "Your father".

केन्द्रीय पक्षी अनुसंधान संस्थान
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Dated: 31.07.2015

Certificate

Certified that the research work embodied in this thesis entitled "Microsatellite, immunocompetence and candidate gene expression profiling of Rhode Island Red chicken and association of microsatellite alleles and immunocompetence traits with layer economic traits" *submitted by* Dr. Abdul Rahim, Roll No. 1524, *for the award of* Doctor of Philosophy *Degree in* Animal Genetics and Breeding *at Indian Veterinary Research Institute, Izatnagar, is the original work carried out by the candidate himself under my supervision and guidance.*

It is further certified that Dr. Abdul Rahim, Roll No. 1524, *has worked for more than 30 months in the Institute and has put in more than 300 days attendance under me from the date of registration for the Doctor of Philosophy Degree in this Deemed University, as required under the relevant ordinance.*

(SANJEEV KUMAR)
Chairman
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Certificate

We the undersigned members of Advisory Committee of **Dr. Abdul Rahim,** *Roll No.* **1524** *a candidate for the degree of* **Doctor of Philosophy** *with the major discipline* **Animal Genetics and Breeding,** *agree that the thesis entitled* **"Microsatellite, immunocompetence and candidate gene expression profiling of Rhode Island Red chicken and association of microsatellite alleles and immunocompetence traits with layer economic traits"** *may be submitted in partial fulfillment of the requirement for the degree.*

We have gone through the contents of the thesis and are fully satisfied with the work carried out by the candidate, which is being presented for the award of **Doctor of Philosophy** *Degree of this Institute.*

It is further certified that the candidate has completed all the prescribed requirements governing the award of **Doctor of Philosophy** *Degree of the Deemed University, Indian Veterinary Research Institute, Izatnagar.*

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Name Dr S-P-TAILOR
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Date : 31·10·15

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ABBREVIATIONS

%	=	percentage
@	=	at the rate of
~	=	approximately
<	=	less than
>	=	greater than
≤	=	less than equal to
≥	=	greater than equal to
°C	=	degree centigrade
χ^2 test	=	Chi-square test (Goodness of fit test)
β	=	Beta
A	=	Adenine
Ab	=	Antibody
ADL	=	Avian Diseases and Oncology Laboratory
AFE	=	Age at first egg
ANOVA	=	Analysis of variance
ASM	=	Age at Sexual maturity
Avg.	=	Average
bp	=	base pairs
BW	=	Body weights
BW16	=	Body weight at 16 weeks of age
BW20	=	Body weight at 20 weeks of age
BW40	=	Body weight at 40 weeks of age
BW64	=	Body weight at 64 weeks of age
C	=	Cytosine
Ca	=	Calcium
CARI	=	Central Avian Research Institute
cDNA	=	Complementary deoxy ribonucleic acid
CD test	=	Critical Difference test
CF	=	Crude fibre
cm	=	Centimeter
cM	=	Centi Morgan
CMI	=	Cell mediated immunity
conc.	=	concentration
CP	=	Crude protein
Ct	=	Threshold cycle
d	=	Days
df	=	degree of freedom
DEPC	=	Diethyl pyrocarbonate
DNA	=	Deoxy Ribonucleic Acid
dNTP	=	Deoxy nucleotide triphosphate

dpi	=	days post inoculation
Dr.	=	Doctor
EDS	=	Egg dropping syndrome
EDTA	=	Ethylene Di Ammine Tetra acetic acid
eg.	=	for example
EP40	=	Egg production upto 40 weeks of age
EP64	=	Egg production upto 64 weeks of age
et al.	=	et alii (and others)
etc.	=	et cetera (and so forth)
EW28	=	Egg weight at 28 weeks of age
EW40	=	Egg weight at 40 weeks of age
EW64	=	Egg weight at 64 weeks of age
F	=	Forward primer
FAO	=	Food and Agricultural Organisation
FES	=	Fertile egg set
Fig.	=	Figure
g	=	Grams
G	=	Guanine
GDP	=	Gross domestic product
h^2	=	Heritability
HA	=	Haemagglutination
H_e	=	Expected heterozygosity
H_o	=	Observed heterozygosity
h	=	hours
H-W	=	Hardy-Weinberg
I	=	Shannon's Information Index
IBD	=	Infectious bursa disease
IC	=	Immunocompetance
IDT	=	Integrated DNA Technologies
i.e.	=	*id est* (that is)
IgG	=	Immunoglobulin-G
IgM	=	Immunoglobulin-M
IL1- β	=	Interleukin-1 beta
iNOS	=	Inducible nitric oxide synthase
IU	=	International unit
K Cal/Kg	=	Kilo calorie per kilogram
log	=	Logarithm
LS means	=	Least squares means
MCW	=	Microsatellite Chicken Wageningen
MD	=	Marek's Disease
ME	=	Metabolizable energy
mg	=	milligrams
MAGE	=	MetaPhor agarose gel electrophoresis
µg	=	microgram

$MgCl_2$	=	Magnesium Chloride
MHC	=	Major Histocompatibility Complex
min(s)	=	minutes
µl	=	microlitre
ml	=	millilitre
mm	=	millimeter
µM	=	micro molar
Mm	=	milli molar
MAS	=	Marker Assisted Selection
mRNA	=	Messenger ribonucleic acid
MS	=	Microsatellite
MSE	=	Mean error sum of squares
MSS	=	Mean sum of squares
N	=	Number of observation
N_a	=	Observed number of alleles
NaCl	=	Sodium chloride
NaOH	=	Sodium hydroxide
N_e	=	Effective number of Alleles
NE	=	Not estimated
Nei's H	=	Nei's average hetrozygosity (unbiased estimate)
NFW	=	Nucleus free water
No.	=	Number
NTC	=	Non template control
º	=	Degree (unit to measure radial angle)
O.D.	=	Optical Density
PAGE	=	Poly acrylamide gel electrophoresis
PBS	=	Phosphate Buffer Saline
PCR	=	Polymerase Chain Reaction
pH	=	Concentration of hydrogen ion
PIC	=	Polymorphic information content
PL	=	Punjab Layer
qRT-PCR	=	Quantitative Reverse Transcriptase Real time polymerase chain reaction
QTL	=	Quantitative Trait Loci
R	=	Reverse Primer
RAPD	=	Random Amplified Polymorphic DNA
RBC	=	Red blood corpuscles
RD	=	Ranikhet Disease
RFLP	=	Restriction fragment length polymorphism
r_G	=	Genetic correlation
RIR	=	Rhode Island Red
RIR^C	=	Control strain of Rhode Island Red
RIR^S	=	Selected strain of Rhode Island Red

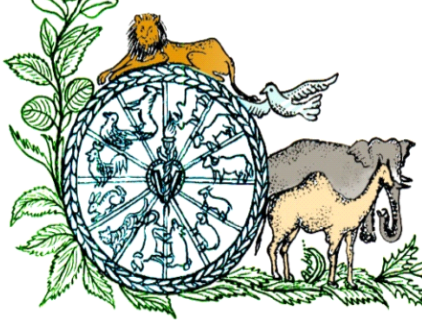
RNA	=	Ribonucleic Acid
rpm	=	Rotation per minute
r_P	=	Phenotypic correlation
SAS	=	Statistical Analysis Software
SDL	=	Synthetic Dam Line
SDS	=	Sodium Do-decyl Sulphate
SE	=	Standard error
sec(s)	=	second(s)
SOV	=	Source of variance
SRBC	=	Sheep Red Blood Cells
SRID	=	Single Radial Immunodiffusion Assay
T_a	=	Annealing temperature
T	=	Thymine
Taq	=	Taq DNA polymerase
TBE	=	Tris borate EDTA
TES	=	Total egg set
TLR15	=	Tool like receptor 15
Tris	=	Trihydroxymethyl aminomethane
U	=	Unit
USA	=	United States of America
UV	=	Ultra Violet
V	=	Volts
Vit.	=	Vitamin
viz.	=	Videlicet (namely)
VNTR	=	Variable Number Tandem Repeats
v/v	=	Volume by volume
W	=	Watt
wks	=	Weeks
WLH	=	White Leghorn Breed
wt.	=	Weight
w/v	=	Weight/volume

LIST OF TABLES

LIST OF FIGURES

CONTENTS

Introduction



1 INTRODUCTION

The wild Red jungle fowl *(Gallus gallus*) is widely considered as the ancestor of today's modern domestic chicken *(Gallus gallus domesticus)* and it was first introduced in India in about 3000 BC. The divergence between the Red Jungle Fowl and the domestic fowl suggested that the later has originated around 8000 year back (Romanov and Weigend 2001). Chicken genome has undergone tremendous changes due to intense selection over this period. Chicken received maximum attention as a result vast varieties of chicken ranging from fancy breeds to high yielding commercial germplasm were developed.

India ranks third in egg production and fifth in meat production with total population of approximately 729 million (19th Livestock Census, 2012). Poultry industry alone share approximately 0.6 % of total GDP of India (FAO 2009) and 10% to the total livestock sector. Per capita availability of egg and meat in India is only 55 eggs and is 2.22 kg meat per head per year against ICMR recommendation of 180 eggs and 11 kg poultry meat per head per year (Borah and Halim, 2014). Globally the per capita availability of meat is 12.22 kg per year and eggs is about 9.1 kg per year. This huge gap may be filled by genetic improvement in productivity of birds through selection. Selective breeding is a well-known tool for increasing genetic potential as well as productivity. Selection based on part period egg production is an important criterion for improving annual egg production in various layer and broiler breeding enterprises and has brought about 85 - 90% change over last 50 years (Sharma and Chatterjee, 2006). Selection changes the genetic structure of population by changing the gene and genotypic frequencies and hence, the genetic parameters are also liable to change in every generation (Falconer and Mackey, 1996). The main emphasis of commercial breeder largely depends on layer economic traits like body weight, age and weight at sexual maturity, egg production and egg weight etc.

Knowledge of genetic parameters like heritability, genetic and phenotypic correlation is therefore essential to evaluate the population under selection in every generation for these traits and is pre-requisite for deciding and formulating future effective breeding strategies.

Good health and survivability of birds along with optimum productive performance is one of the most important factors for deciding the success and feasibility of poultry production. Development of modern strains of layers and broiler through long-term genetic selection programs were focused only on high production potentials. Increasing selection pressure on economically important traits in high yielding populations led to severe decline in immune response capabilities and increased susceptibility to numerous diseases (Tomar *et al*., 2012), which in turn impairs genetic improvement in production traits. Genetic improvement of immunocompetence status of birds with high production potential will be a target for poultry improvement in the future.

Currently the intensive production system is highly applicable in chickens than any other agriculturally important species. More importance to the production traits and existence of negative genetic correlation between production and protection traits predispose poultry to a variety of potentially pathogenic organisms. As a result, vaccination and antibiotic use become mandatory and it incurs more input cost. Expectedly, because of continuous, huge antigenic exposure, vaccination and antibiotic treatment, the pathogenic strains develop resistance and show no response to chemotherapeutic agents, posing serious threat to birds.

Genetic selection seems to be promising approach for improving disease resistance of birds (Gavora and Spencer, 1983; Lamont, 1998). Genetic improvement of immunocompetence or disease resistance by direct selection is difficult due to quantitative nature of traits, low heritability and difficulties in reliable measurements. Many genetic and environmental factors control immune response in chickens. Disease control by improvement in the genetic resistance is one of the reliable, environmentally sound and economic strategies (Cheng, 2003). Disease resistance is a quantitative trait and controlled by Quantitative trait loci (QTL). Selection could be for /against a particular pathogen and/ or for general immunocompetence (Yonash *et al.,* 1996). Genetic resistance in poultry is controlled by highly-evolved avian immune system (Zhou *et al*., 2001). The non-specific components of immune system including lysozyme plays important role in the body's defense against pathogens. Similarly,

the specific components of immune system involving humoral response and cell-mediated response are considered to be the important facets of immunocompetence. Immune response and disease resistance in chickens has been found to be affected by multiple genetic and environmental factors (Gavora, 1993). Genetic control of antibody response and cell mediated immunity has been reported to be polygenic (Lamont and Dietert, 1990, Das *et al*., 2014a, b, c) having low to moderate heritability estimates.

Various selection strategies have been practiced to enhance disease resistance of bird (Sarker *et al.,* 2000, Pinard-van der Laan *et al.,* 2004). Better immunocompetence in indigenous chicken is supported by various studies suggesting higher complement activity, higher serum lysozyme level and antibody response (Kundu *et al.,* 1999, Baelmans *et al.,* 2005, Singh *et al.,* 2010). One of the important non-pathogenic multi-determinant antigens to monitor immune responsiveness in poultry is sheep red blood cell (Siegel and Gross, 1980). Birds eliciting higher antibody response against SRBCs also produce more antibodies to a variety of antigens (Parmentier *et al.,* 1998). Lysozyme, an abundant and widespread non-specific bactericidal substance, plays an important role in the body's defense against infection through its direct bacteriolytic action (Biggar and Sturgess, 1977). The IgG, most abundant immunoglobulin in serum, is regarded as an indicator of general immune response (Pinard van der Laan *et al.,* 1998). Positive correlations have been found between anti-SRBC immune response and serum IgG level (Chao and Lee, 2001, Sivaraman *et al.,* 2003). Therefore, understanding of the genetic control of antibody production may provide an opportunity for genetic enhancement of vaccine mediated immunity and thereby resistance to diseases. Because of increased cost of vaccination or medication and consumer awareness regarding drug residues in meat, developing lines or strains with superior immunocompetence status and high production potential is important. It also conserves natural resources and decreases repeated expenditure on health management.

Immune system is mediated by cellular interactions and molecules secreted by them play major role in deciding the type and magnitude of immune response. Cytokines are central regulator of immune responses as it mediates cell signaling to produce different responses. Cells produce wide range of cytokines and it varies with respect to cells and pathogens. Based on their inherent property, cytokines represent promising candidates for treating infections that

are difficult to control by antibiotics (Dinarello, 2000; Min *et al*., 2001; Li and He, 2004). Avian innate immunity provides first line of host defense to microbial infections. Toll-like receptor (TLR-15), cytokines (IL-1β), inducible nitric oxide synthase (iNOS) are essential effectors molecules of innate and acquired immunity and are crucial signaling molecules in cellular communication. Chicken IL-1β, belonging to the IL-1 super family of cytokines, was one of the first chicken cytokines described. Chicken IL-1β increases antibody production and mediates an inflammatory response similar to its mammalian counterpart (Sterneck *et al.,* 1992). After parasitic infestation the expression of IL-1β gene differed between chicken inbred lines disparate for the MHC (Kim *et al.,* 2008a). Nitric oxide is an important mediator of intra and intercellular signaling and it is diverse in bodily functions. It is produced by macrophages stimulated by cytokines or microbial components. It plays an important role in immune responses because of its antimicrobial and anti-tumor functions (Eisenstein, 2001). Nitric oxide is produced by a family of enzymes called as nitric oxide synthases (NOS), which is of three types based on tissue type viz. eNOS (endothelial NOS), iNOS (inducible NOS), nNOS (neuronal NOS). Of these, inducible nitric oxide synthase (iNOS), an immune defense enzyme mainly activated by PRRs (pattern recognition receptors) signaling pathways and it catalyzes the reaction of nitric oxide conversion from L-arginine (Aktan, 2004). Expression of the iNOS gene in chickens varies with genetic background (Dil and Qureshi, 2003, Kumar *et al.,* 2011) and also after Eimeria infection (Kim *et al.,* 2008a). TLR15 is a novel, avian –specific TLR, play an important role as effectors molecules of innate and acquired immunity. Expression of TLR15 mRNA was highest in bone marrow, bursa, and spleen and have role in immunity (Higgs *et al.,* 2006). Understanding the differences in basal expression levels of genes related to innate immunity helps to unearth their specific pattern of expression.

Recent advances in molecular biology have opened new horizons in assessment of genetic variability at the DNA level. More recently the chicken genome became the first livestock genome to be sequenced with six fold coverage and is the main driving force for chicken genome analysis. The chicken genome comprises of 39 pairs of chromosomes, which are divided into 8 pairs of cytologically distinct macro-chromosomes along with sex chromosome (Z and W chromosome) and 30 pairs of micro-chromosomes. The chicken genome size is

estimated to be 1.2×10^9 base pairs and approximately 4,000 cM in length (Bloom *et al*., 1993; Groenen *et al*., 2000). Therefore, 1 cM is approximately equivalent to 300 kb of DNA in the chicken. In contrast, 1 cM in humans is about 1,000 kb of DNA, and thus, the chicken genome is about one-third the size of the human genome. Three international reference populations for chicken genetic mapping include the East Lansing, the Compton, and the Wageningen populations (Bumstead and Palyga, 1992; Crittenden *et al.,* 1993; Groenen *et al.,* 1998). A consensus chicken genetic map based on the three populations has been established with about 1,965 genetic markers mapped to 50 linkage groups and covering almost the entire 4,000 cM (Groenen *et al*., 2000).

Molecular genetics characterization of any livestock/ poultry species is a pre-requisite for chalking out its genetic improvement programme using effective approach like marker-assisted selection (MAS). The discovery of the polymerase chain reaction (PCR) had a major impact on the research of eukaryotic genomes and contributed to the development and application of various DNA markers (Marle-Koster and Nel, 2003). DNA/Molecular marker is simply identifiable DNA sequences, found at specific locations on genome, and are transmitted from one generation to next generation by the standard laws of inheritance. Genetic markers are also helpful for tracking the inheritance of linked segments of the genome in pedigrees. A significant association between the inheritance of a particular marker allele and quantitative traits loci is pre-requisite for genetic improvement programme like marker-assisted selection (MAS). Various molecular markers such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Randomly Amplified Polymorphic DNA), minisatellite (VNTR), microsatellites and SNP (single nucleotide polymorphism) etc are used for this purpose.

Among these, microsatellite markers are more popular, numerous, ubiquitous, effective and recommended because they are polymorphic, have repetitive DNA sequences, distributed randomly throughout the genome, and display moderate to high levels of variation and co-dominant inheritance (Tautz, 1989). Litt and Luty (1989) coined the term 'Microsatellite' to characterize the simple sequence repeats by PCR. Microsatellite sequences, also known as Short Tandem Repeats (STR), simple sequence repeats (SSR), sequence tagged microsatellite repeats (STMR), are tandemly repeated motifs of 1-6 nucleotides in length (Hillel *et al*.,

2003), are widely used as valuable genetic markers due to their dense distribution in the genome, wide variability, co-dominant mode of inheritance and easy genotyping. They have been extensively used in assessing genetic structure, diversity and relationship analyses and are ideal for deciphering genetic variability (Zhou *et al*., 2008). They provide a powerful tool for MAS, QTL research, genome scanning and genetic clustering analysis (Sewalem *et al*., 2002). A few microsatellite loci have been found to be associated with growth and egg production traits in chicken (Chatterjee *et al,* 2010a*,* Das*,* 2013).

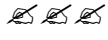
Rhode Island Red (RIR) is a brown-egger, dual-purpose chicken breed, preferred by small poultry farmer and well adapted to local environment and is considered useful for developing multicolored strains for backyard/ rural system. Central Avian Research Institute (CARI), Izatnagar had imported about 1400 fertile eggs from USA in 1980, which were hatched at institute's hatchery and were subjected to genetic improvement through selection and breeding. The strain was well adopted, acclimatized and genetically improved over last 35 years covering 29 generations of selection and being maintained as selected strain (RIR^S) as well as Random bred control strain (RIR^C) population. RIR^S population is showing positive genetic response in egg production. Periodic evaluation of its performance is necessary to exploit its production performance and for development of multicolored strains for rural poultry production. In view of the above background knowledge, the present study was undertaken with following **objectives**:-

1. To analyze the effects of various genetic and non-genetic factors on layer economic traits in Rhode Island Red (RIR) chicken.

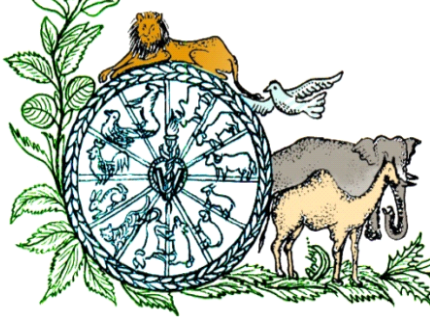
2. To determine allelic polymorphism at egg production-associated microsatellite loci in Rhode Island Red chicken and their association with layer economic traits.

3. To estimate immunocompetence traits and their association with layer economic traits in Rhode Island Red chicken.

4. To analyze relative expression of important immunity related genes in various tissues of Rhode Island Red chicken by quantitative reverse transcription PCR (qRT-PCR).



Review of Literature



2 REVIEW OF LITERATURE

Recent literatures on relevant aspects in each of four objectives were collected from the National Library on Veterinary Sciences at IVRI, Izatnagar and from on-line databases.

Literature in the areas of immunocompetence, layer performance, microsatellite studies and gene expression profiling, relevant to such studies in Rhode Island Red (RIR), has been reviewed and presented below in an objective-wise manner:

2.1 Objective 1: To analyze effects of various genetic and non-genetic factors on layer economic traits in Rhode Island Red (RIR) chicken

2.1.1 Fertility and hatchability

Poultry production at all scales of operation is wholly dependent on the supply of day-old chicks. Fertility and hatchability are two major parameters of reproductive performance towards regeneration of stocks and are influenced by several genetic and non-genetic factors such as storage temperature, care, age, quality of eggs, age of pullets, season, nutrition, pre-incubation warming, and humidity etc. Fertility refers to the percentage of incubated eggs that are fertile while hatchability is the percentage of fertile eggs that hatch. Fertility and hatchability are interrelated heritable traits that vary among breed, variety and individuals of breed or variety. It is therefore important to understand the factors that influenced the fertility and hatchability of eggs

Zelleke *et al.* (2005) compared the reproductive performance in chicken by artificial insemination and reported percent fertility as 82.97±0.67, 84.66±0.63, 85.48±0.60 and 96.11±1.35% in RIR, WLH and RIR x WLH and WLH x RIR crossbreds, respectively. The

percent hatchability were 76.67±1.66, 86.67±2.45, 97.72±2.44, 97.12±2.79 on fertile egg set basis and 59.49±2.30 73.42±2.09, 84.09±1.09, 93.33±1.64 on total egg set basis, respectively.

Bhardwaj *et al.* (2006) studied the reproductive performance of Kadaknath, Aseel, Rhode Island Red, Brown Cornish and their crosses Kadaknath x Brown Cornish, Brown Cornish x Kadaknath, Aseel x Rhode Island Red and Rhode Island Red x Aseel and found that the percent fertility, hatchability were higher in Rhode Island Red, Rhode Island Red x Aseel cross than other purebreds and crossbreds. The percent fertility was 70.44, 73.22, 75.21, 71.92, 62.61, 71.35, 72.43 and 76.39, respectively. Correspondingly the percent hatchability on total egg set basis were 44.75, 41.90, 38.01, 35.54, 51.43, 55.91, 57.25, 64.91 and on fertile egg set basis were 57.75, 58.00, 50.39, 49.49, 78.82, 79.13, 78.33 and 84.93, respectively.

Malago and Baitilwake (2009) reported that percent fertility (± SD) of local, Rhode Island Red and crossbred chickens was 92.0 ± 4.14, 91.1 ± 4.42, and 94.5 ± 2.21%, respectively.

The percent fertility in RIR-selected and control strains, CARI Sonali (HR) and CARI Devendra (CD) chicken were 87.13%, 87.04%, 85.64% and 73.78%, respectively (CARI Annual Report, 2010-11).

Ahmed *et al.* (2012) studied the comparative evaluation of fertility and hatchability of different crosses of chicken with White Leghorn including Rhode Island Red for backyard poultry and reported 87.00% fertility and 83.9% hatchability on fertile egg set (FES) basis in Rhode Island Red cross.

Das *et al.* (2014c) reported the overall percent fertility were 75.86%, 79.03%, 79.34%, 70.83%, 60.64% in RIR^S, RIR^C, RIR^W, CARI-Sonali and CARI-Debendra chickens, respectively. Percent hatchability based on total egg set (TES) were 57.46%, 68.51%, 67.80%, 55.58%, 53.09% and on fertile egg set (FES) were 75.65%, 86.82%, 85.27%, 78.44% and 87.54%, respectively.

Khan *et al.* (2014) studied the significant ($P \leq 0.05$) effect of storage time (0, 2, 3, 5, 7 and 9 days) on egg quality and hatchability characteristics of Rhode Island Red (RIR) hens. The overall percent fertility, percent hatchability based on total egg set (TES) and fertile egg set (FES) basis upto 9 days storage were 78.26%, 51.41% and 65.69% respectively.

Debnath *et al.* (2015) studied the reproductive performance i.e. percent fertility and hatchability in four different hatches of selected pure strain of RIR chicken under standard incubation and hatching conditions and reported that the percent fertility ranged from 71.28 to 84.55; overall percent fertility being 76.98%. The percent hatchability estimated on total egg set (TES) basis ranged from 55.96 to 65.85% and on fertile eggs transferred (FET) basis ranged from 76.25 to 83.28, overall being 61.44% and 79.85%, respectively.

2.1.2 Age at first egg (AFE) or age of sexual maturity

Kumar *et al.* (2002) reported AFE as 149.18 ± 0.14 days, Jilani *et al.* (2005) and Jilani *et al.* (2007) reported age of sexual maturity as 146.16±0.33 days in commercial strain of Rhode Island Red chicken.

Saini *et al.* (2011) reported age of sexual maturity in white leghorn PL-2, RIR, Dahlem Red and Naked neck laying chicken as 157±3.9, 152.00±3.8, 151.00±6.3 and 153.00±1.6 days, respectively.

Khawja *et al.* (2013) reported that the comparative performance of age of sexual maturity in Fayoumi, Rhode Island Red (RIR) and their reciprocal crossbred RIFI (RIR male X Fayoumi female) and FIRI (Fayoumi male X RIR female) were 135.00±3.51, 147.00 ±1.15, 146.00 ± 2.51 and 149.00±1.51 days.

Das *et al.* (2014c) estimated AFE as 148.86±0.78, 177.23±1.92, 169.33±2.55, 135.06±1.27 and 177.73±2.25 days in RIR^S, RIR^C and RIR^W purebreds and CARI-Sonali and CARI-Debendra chicken, respectively.

2.1.3 Body weight at different weeks of age

Kumar *et al.* (2002) studied RIR strain and estimated body weight as 1517.69±0.40g and 1783.43±0.73g, respectively at 20 and 40 weeks of age.

Jilani *et al.* (2005) studied 740 pullets of RIR commercial strain obtained in three hatches and the body weight were recorded as 1395.68±5.05g and 1673.24±5.64g at 20 and 40 weeks of age, respectively.

Swain *et al.* (2005) recorded body weight at 20 and 40 weeks of age as 1261.7g and 1434.5g in twenty-four number of RIR pullets under controlled group.

Jilani *et al.* (2007) recorded body weight at 8, 20 and 40 weeks of age as 550.21±1.65g, 1395.68±5.05g, 1673.24±5.64g, respectively in RIR strain.

Veeramani *et al.* (2008) reported the body weight in IWP strain of White Leghorn chicken at 16, 40 and 64 week of age as 1.131±0.0008kg, 1.651±0.0010kg and 1.712±0.0009kg, respectively.

Chatterjee *et al*. (2007a) studied two Indian native chicken Kadaknath and Aseel for their growth, body confirmation and immunocompetence traits. Body weight at 2, 4, 8, 12 and 16 weeks of age averaged 51.8±0.48g, 125±2.27g, 275±9.15g, 583±18.18g and 861±19.50g for Kadaknath, and 65.1±1.04g, 154±2.39g, 393±8.52g, 796±13.12g and 1218 ± 19.0g for Aseel, respectively under deep litter management system.

Chatterjee *et al*. (2010b) reported body weight at 40 weeks of age as 1232g, 2580g, 2595g, 1853g and 1596g in Kadaknath, Vanraja male line, Vanraja female line, Aseel and Gramapriya female line, respectively.

Saini *et al.* (2011) studied the randomly selected 50 birds each of four different strains of egg laying chicken viz., single comb white leghorn PL2, RIR strain, Dahlem Red strain and naked neck strain and estimated body weights at 20 and 40 weeks of age in these four strains to analyze difference among strains for body weights. The body weight at 20 and 40 week of age in RIR, white leghorn PL2, Dahlem Red and naked neck strain were 1369±20.7g and 1836±34.2g; 1035±20.6g and 1204±36.9g; 1300±22.9g and 1752±33.6g and 1075±20.5g and 1322 ±34.6g, respectively. The Rhode Island Red being a dual purpose breed has higher body weight while white Leghorn PL2 has significantly lower body weight. White leghorn PL2 and naked neck strains had no significant difference of body weight at 20 and 40 week of age because of their common genetic origin.

CARI, Annual Report (2010-11) reported that the body weights of RIR selected, RIR control of 27th generation, CARI-Sonali and CARI-Debendra crosses at CARI, Izatnagar were, 1570.25±4.78, 1198.41±7.49, 1447.77±70.29 and 2214.36±17.36g at 20 weeks of age, 1825.84±6.56, 1516.67±10.39, 1681.44±12.59 and 2928.39±31.20g at 40 weeks of age, respectively

Rajkumar *et al.* (2012) recorded the body weight in Nack neck hens at 20, 40, 52, 64 and 72 weeks of age as 2455.60±1.66g, 3046.89±3.29g, 3297.44±3.28g, 3542.89±3.33g and 3805.13±5.48g, respectively.

Das (2013) reported various growth traits in Rhode Island Red chicken and their crosses at different weeks of age including body weight at 16th, 20th weeks in both sex and 40th weeks of age in female only as 1446.63±12.72g; 1589.74±10.25g; 1744.78±8.86g in RIR^S, 1088.38±8.23g, 1204.75±20.02g, 1775.74±21.83g in RIR^C, 1319.17±35.91g, 1442.18±32.80g, 1766.87±29.01g in RIR^W, 1452.99±18.46g, 1482.72±15.58g, 1747.65±14.44g in CARI-Sonali and 2321.54±18.97g, 2398.88±16.07g, 2122.34±25.54g in CARI-Debendra chicken, respectively.

Das *et al.* (2014c) recorded overall body weight at 16^{th} weeks as 1446.63±12.72g (1639.10±18. 08 in males 1254.16±17.61 in female) in Rhode Island Red chicken and at 20th weeks of age only in female as 1589.74± 10.25g.

Das *et al.* (2014d) estimated body weights at 0 day, 2, 4, 6, 8, 12 and 16-weeks of age as 35.91 ±0.37, 190.26±5.57, 322.85±6.82, 482.43±11.54, 828.75±19.75 and 1206.07 in RIR-White strain chicken and reported male were heavier than females.

CARI , Annual Report (2014-15) reported that the body weights of selected and control RIR chicken at 20, 40 and 64 weeks of age were 1417.4±3.76g and 1172.9±6.96g; 1721.4±5.93g and 1552.9±10.31g and 1933.1±23.4g and 1683.5±43.1g, respectively.

2.1.4 Egg weight at different weeks of age

Kumar *et al.* (2002) recorded that egg weight at 35 weeks of age was 50.82g in commercial strain of RIR chicken.

Wardecka *et al.* (2003) reported that Rhode Island Red and Green-legged Partridge (GlP) differed in egg laying performance considerably during the first 100 days of laying i.e. mean egg weight at 33 weeks of age was 59.4 g for RIR and 48.9g for GlP strains, respectively.

Jilani *et al.* (2005) and Jilani *et al.* (2007) recorded 35 week's egg weight in commercial strain of RIR chicken was 54.32±0.0089g.

Malago and Baitilwake (2009) reported EW40 as 60.58 ± 4.55g and 58.42 ± 6.88g in RIR and its crossbred, respectively.

Saini *et al.* (2011) estimated egg weight at 40 weeks of age as 55.2±0.67, 51.6±0.62, 54.7±0.61 and 57.0±0.62g in White Leghorn PL-2, RIR, Dahlem Red and Naked neck chicken under individual cage management system.

Faruque *et al*. (2010) estimated egg weight at 40 weeks of age as 42.94g, 40.32g and 44.15g in Non-descript Desi (ND), Hilly (H) and Naked Neck (NN) chicken, respectively under intensive management system.

Khawaja *et al.* (2013) estimated egg weight at 40 weeks of age as 56.82±0.54, 43.34±0.69, 47±0.60 and 47.5±0.56g, respectively in Rhode Island Red, Fayoumi and their reciprocal (RIFI and FIRI) crossbred.

Das *et al.* (2014c) recorded egg weight at 28 weeks of age as 44.98±0.20, 43.46±0.50, 44.45±0.67, 46.66±0.33, 46.78±0.59g and egg weight at 40 weeks of age as 51.75±0.19, 50.45±0.47, 51.23±0.62, 53.46±0.31, 53.52± 0.55 in RIR^S, RIR^C, RIR^S, CARI-Sonali and CARI-Dabendra chicken, respectively.

CARI, Annual Report (2014-15) estimated egg weights at 28, 40, 64 and 72 weeks of age in selected and control strain of RIR chicken as 43.42±0.08 and 42.53±0.13g; 50.93±0.09 and 49.44±0.15g; 53.2±0.38g and 50.2±0.82g and 53.4±0.4g and 50.2±0.82g, respectively.

2.1.5 Egg production up to 40 (EP40) and 64 weeks of age

Kumar *et al.* (2002) recorded 40 weeks egg production as 112.49±0.54 eggs in commercial strain of RIR chicken.

Jilani *et al.* (2005) and Jilani *et al.* (2007) reported average egg production up to 40 weeks of age as 107.19±0.10 eggs in commercial strain of RIR chicken.

Saini *et al.* (2011) recorded EP40 under individual cage management system as 52±3.2eggs in White Leghorn PL-2, 74± 3.2eggs in Rhode Island Red, 59±3.4eggs in Dahlem Red and 61± 2.3eggs in Naked neck chicken.

Khawaja *et al.* (2013) recorded average number of eggs per hen during production phase from 18 to 72 weeks of age as 141±2.00 in Rhode Island Red and 123±2.05 in Fayoumi chicken breeds.

Das *et al.* (2014c) recorded average egg production upto 40 weeks of age as 96.45± 0.99 eggs in selected strain of RIR, 60.79± 2.43eggs in control strain of RIR, 71.61± 3.24eggs in white strain of RIR, 111.13± 1.61eggs in CARI-Sonali and 66.23± 2.85eggs in CARI-Dabendra chicken, respectively.

Annual Report, CARI (2014-15) estimated egg production upto 40, 64 and 72 weeks of age in selected and control strain of RIR chicken as 110±0.60 eggs and 61.57±0.97eggs; 211.0±5.1eggs and 141.3±4.6eggs and 243.6±2.4 and 196.8±6.8eggs, respectively.

2.1.6 Factors affecting body weights and layer economic traits

2.1.6.1 Sex effect

Padhi *et al*. (2012) studied 1022 birds of Vanaraja, Control broiler, Vanaraja commercial and found significant ($P \leq 0.05$) difference between male and female chicks at day old, 2, 4 and 6 weeks of age in Vanaraja. However, male line of Vanaraja (PD1) and Control Broiler showed significant ($P \leq 0.05$) differences between male and female chicks for 2, 4 and 6 weeks of age.

Das *et al*. (2014c) reported that significant effect of sex on chick weight and body weights and males demonstrated higher body weight than the females.

Das *et al*. (2014d) studied the influence of sex on body weights at different ages and reported that sex had significant effect on body weights from 8-week onwards and males demonstrated higher body weight than the females.

2.1.6.2 Sire effects

Sivaraman *et al.* (2005) reported significant effect of sire on body weights at 4, 5 and 6 weeks of age in synthetic dam line (SDL) of broiler chicken.

2.1.7 Genetics Parameters

Effective selection and breeding program depends upon knowledge of genetic parameters such as heritability and correlations. The literature scanned revealed wide variation in the parameters for Rhode Island Red chicken and various other chicken breeds. The estimated genetics parameters are specific to a particular population which is dependent upon gene frequency and the environmental circumstances to which it belongs.

2.1.7.1 Heritability

The heritability (h^2) is the portion of total variance attributed to the average gene effects (Lush, 1949) and expressed as proportion of additive genetic variance to the total phenotypic variance in the narrow sense. Several methods of estimation of heritability have been described in literature (Lush, 1949, Lerner, 1950, Kempthorne, 1957, Falconer and Mackey, 1996). The genetic interpretation of variance components have been described by Lerner, 1950. The heritability described by half sib and full sib may be biased due to sex-linked, maternal and non-additive genetics effects.

2.1.7.2 h^2 estimates of body weights and their correlations with growth and layer economic traits

Kumar *et al.* (2002) reported heritability estimates based on sire component of variance in RIR strain as 0.10.07 for body weight at 20 weeks (20BW), 0.18±0.08 for age at sexual maturity (ASM), 0.56±0.14 for egg weight at 35 weeks of age (35EW), 0.58±0.14 for body weight at 40 weeks of age (40BW) and 0.14±0.07 for edd production upto 40 weeks of age (EP40). The genetic and phenotypic correlations of ASM with 40BW and 35EW were positive but negative and significant with EP40, 40BW with EP were negative but positive and significant with 35EW. The EP40 was negatively correlated with EW35 at genetic and phenotypic levels.

Shivakumar (2003) recorded body weights at 5 to 6 week of age on 225 chicks from two hatches, progeny of 19 sires from SDL broiler line and estimated the heritability, genetic

and phenotypic correlations among body weights, which were found to be medium to high in magnitude.

Jilani *et al.* (2005) estimated high h^2 based on sire component of variance for BW20, BW40, ASM, EP40 and EW35 as 0.41±0.12, 0.40±0.12, 0.50±0.13, 0.70±0.16 and 0.40±0.12, respectively in Rhode Island Red chicken. The r_G and r_P of among body weights were positive and significant. The 20BW were negatively and significant correlated with age at sexual maturity at genetic and phenotypic levels. The r_G and r_P of ASM with egg production and egg production with egg weight were negative and significant in 740 pullets of RIR strain.

Jilani *et al.* (2007) estimated h^2 based on sire component of variance in elite Rhode Island Red chicken population as 0.45±0.13, 0.41±0.12, 0.40±0.12, 0.50±0.13, 0.70±0.16 and 0.40±0.12 for BW8, BW20 BW40, ASM, EP40 and EW35, respectively and reported positive and significant genetic and phenotypic correlations of BW8 with BW40. The BW20 had negative and significant correlation with ASM, both on genetic and phenotypic levels.

Sivaraman *et al*. (2005) recorded 303 chicks, of both sexes, for body weight at 4, 5 and 6 weeks of age on synthetic dam line of broiler chicken and found that the heritability estimates for body weights were high ranged from 0.425±0.201 to 0.796±0.274. The genetic and phenotypic correlations among body weights were positive and high ($P \leq 0.01$).

Rajkumar *et al*. (2011) analyzed the growth and production up to 64 weeks of age on 894 chicks for juvenile traits and on 212 birds for production traits. The estimated heritabilities were medium to high for body weights, low to high for egg weights and low for egg production traits. The genetic correlation between 4 and 6 weeks of body weights was highly significant and positive (r_G=0.96) indicating the high degree of association between these traits.

Ahmad and Singh (2007) conducted studies on 559 pullet, progenies of 149 dams mated with 58 sires obtained in a single hatch, of White Leghorn chicken and estimated heritability from sire, dam and sire + dam components of variance as 0.16±0.14, 0.32±0.19 and 0.24±0.08 for ASM, 0.34±0.17, 0.35±0.19 and 0.34±0.09 for BW12, 0.34±0.18, 0.58±0.22 and 0.46±0.11 for BW36, 0.29±0.15, 0.18±0.17 and 0.24±0.09 for EP40 and 0.08±0.15, 0.58±0.23 and 0.34±0.09 for EW32, respectively.

Chaudhary *et al.* (2009) studied the growth and performance traits on 1733 pullets of two selected strains and one control line of White Leghorn chickens and estimated moderate to high h^2 for body and egg weights and low to moderate for age at sexual maturity and egg production. Genetic correlation of early body weights with egg number; egg mass and egg weight were positive and negative with ASM and rate of lay.

Barot *et al.* (2008) recorded body weights at 20, 40, 56 and 72 weeks of age, AFE, egg production up to 40 and 72weeks of age and egg weights at 32, 40, 56 and 72 weeks of age on pullets of synthetic White Leghorn belonging to 30 to 40 sires families under long term selection for egg production over five generations and reported high heritability for growth and egg weight and low to moderate for egg production traits. The genetic correlations among growth traits were strong and positive. The genetic and phenotypic correlations of age at first egg with body weight and egg production were low in magnitude and either positive or negative, while the correlations between body weights and egg weights showed positive trend. The genetic correlation of egg production with body weight and egg weight revealed negative association between the traits.

Anees *et al.* (2010) estimated genetic and phenotypic parameters of economic traits in White Leghorn and reported low to moderate heritabilities of ASM, egg weight and egg production and high heritability for body weights. Positive correlation between egg weight and body weights and negative between ASM and EN40 at both genetic and phenotypic scales.

Qadri *et al.* (2013) estimated moderate to high heritabilities for AFE, BW16, BW28 and BW40 and low to high for EP40 in IWN strain and IWP strain of White leghorn chicken, respectively. Genetic and phenotypic correlations of AFE with EP40 were negative and high in magnitude. Genetic correlations between AFE and egg weights at different ages were in general positive and showed increasing trend from low to high with advancement in age in IWN strain, whereas in general positive and high in IWP strain.

2.1.8 Mortality

Azizul and Reza (1980) observed 45.45% mortality up to 52 week of age in RIR, 13.63% in upgraded birds and 9.03% in local birds. On the other hand, Hutt (1938) reported a lower mortality in upgraded birds in comparison to indigenous stock of improved birds.

Sivaraman *et al*. (2005) recorded mortality on 303 chicks of both sexes over the period from 0 to 4, 4 to 5 and 5 to 6 weeks of age, which was 2.77 %, 0.63 % and 0.0 %, respectively.

Khawaja *et al*. (2012) estimated the percent mortality as 12.00±0.29, 6.00±0.29 during 0-8 weeks and 8-20 weeks period in RIR chicken.

Das *et al.* (2014c) calculated the percent mortality in selected strain of Rhode Island Red chicken for different periods and reported it to be approx. 5.15% during first week, 4.80% during brooding, 5.60% during growing and 7.73% during laying stages.

Debnath *et al.* (2015) reported the overall percent mortality during 0 to 6 weeks of age as 5.24% in selected strain of RIR chicken.

2.2 Objective 2: To determine allelic polymorphism at egg production-associated microsatellite loci in Rhode Island Red chicken and their association with layer economic traits.

Genetic characterization of a breed by molecular markers is a prerequisite for developing strategies for its optimum utilization. Molecular markers, revealing polymorphism at the DNA level are now key players to the geneticists and breeders to evaluate the existing germplasm. Various molecular markers are used for this purpose. But microsatellite markers are the marker of choice in livestock genetic characterization studies showing a higher degree of polymorphisms and co-dominant inheritance (Tautz, 1989) and ease of identification than other markers. It provides a powerful tool for mapping QTL, marker assisted selection (MAS), genome scanning, and genetic clustering analysis (Van Kaam *et al.,* 1999; Sewalem *et al.,* 2002).

2.2.1 Microsatellites markers

2.2.1.1 ADL0020 Microsatellites marker

Panday *et al.* (2002) genotyped ADL0020 along with 14 other microsatellite loci to assess the genetic variability of three indigenous poultry breeds and reported four allele in Aseel and Nicobari and five alleles in Miri chicken. The effective no. of alleles, polymorphic information content , expected heterozygosity and direct count heterozygosity were 2.34,

0.57, 0.58 and 0.41 in Aseel, 3.11 0.67, 0.69 and 0.65 in Miri and 3.14, 0.68, 0.69 and 0.61 in Nicobari fowl, respectively.

Vijh and Tantia (2004) genotyped ADL0020 loci along with 25 other microsatellite in 32 random selected birds of Nicobari, 20 of Miri, 36 of Aseel and 46 of Kashmir Favorolla indigenous breeds of poultry and observed five alleles and average heterozygosity was 0.58, respectively.

Panday *et al.* (2005) genotyped ADL0020 along with 29 microsatellite loci to measure genetic variation in Ankleshwar chicken breed and found four observed and 2.714 effective numbers of alleles. Alleles ranged from 95 to 105 base pairs in size having Shannon's information index and PIC values as 1.111 and 0.558. The observed and expected heterozygosity and Nei's values were 0.850, 0.640 and 0.632, respectively.

Chatterjee *et al*. (2008a) genotyped ADL0020 along with four other MS loci in six crossbred population of White Leghorn and reported three alleles having sizes as 97, 107, 112 bp. The estimated PIC value, observed and expected heterozygosities were 0.54, 0.61 and 0.60, respectively.

Kim *et al*. (2008b) genotyped ADL0020 along with 16 other microsatellite markers located on chromosome no. 1 in Korean native chicken and reported two alleles having sizes as 101 and 105bp. The PIC and expected heterozygosity were 0.7582 and 0.8020, respectively. Frequency of two alleles was 0.262 and 0.257.

Chatterjee *et al*. (2008b) genotyped ADL0020 along with four other MS loci in six crossbred population of White Leghorn having 15 birds in each population and reported three alleles having sizes as 97, 107, 112 bp. The genotypic frequencies varied from 0.09 to 0.33 and allelic frequencies varied from and 0.23 to 0.53, respectively.

Chatterjee *et al*. (2010b) genotyped ADL0020 microsatellite loci along with 14 MS loci in two indigenous native breeds Kadaknath and Aseel and three different chicken lines (Vanaraja male line, Vanaraja female line and Gramapriya female line) to assess genetic diversity and reported six alleles having sizes as 98, 100, 102, 108,112 and 116 bp and mean PIC

value as 0.656. The observed and effective numbers of alleles were 5 and 4.16; observed heterozygosity (Ho) and expected heterozygosity (He) were 0.74 and 0.76, respectively.

2.2.1.2 ADL0023 Microsatellites marker

Panday *et al.,* (2002) genotyped ADL0023 loci in Aseel, Miri and Nicobari three native poultry breeds reported five alleles in Aseel and Miri and three in Nicobari chicken. The effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity were 3.13, 0.68, 0.69 and 0.48 in Aseel, 2.51, 0.60, 0.61 and 0.57 in Miri and 2.49, 0.59, 0.60 and 0.55 in Nicobari fowl.

Vijh and Tantia (2004) genotyped ADL0023 loci along with 25 other microsatellite in 32 random selected bird of Nicobari, 20 of Miri, 36 of Aseel and 46 of Kashmir Favorolla indigenous breed of poultry and reported eight number of observed allele and average heterozygosity was 0.76respectively

Panday *et al.* (2005) genotyped ADL0023 along with 29 microsatellite loci to measures of genetic variation in Ankleshwar chicken breed and found eight alleles ranging from 164-182bp and effective number of alleles as 2.714. The estimated Shannon's information index, PIC value, observed heterozygosity, expected heterozygosity and Nei's value were 1.111, 0.558, 0.850, 0.640 and 0.632, respectively.

Chatterjee *et al*. (2008a) genotyped ADL0023 along with four other MS loci in six crossbred populations of White Leghorn and reported five alleles having sizes as 166, 170, 178, 182, and 194bp. The estimated PIC value, observed and expected heterozygosities were 0.58, 0.64 and 0.95, respectively.

Khan *et al*. (2010) genotyped ADL0023 in three different chicken breeds along with three other microsatellite loci using MetaPhor agarose and found different alleles ranging from 175-200bp in Kadaknath, 178bp in White Leghorn and 178-189bp in Ankleshwar chicken breed. The average PIC was 0.59.

Chatterjee *et al*. (2008a) genotyped ADL0023 along with four other loci in six crossbred population of White Leghorn with 15 birds in each population and reported 3

alleles having sizes of 176, 184, 204 bp. The genotypic and allelic frequencies were varied from 0.01 to 0.52 and 0.21 to 0.49, respectively.

Chatterjee *et al*. (2010b) genotyped ADL0023 microsatellite loci along with14 loci in two indigenous native breeds i.e. Kadaknath and Aseel and three different chicken lines (Vanaraja male line, Vanaraja female line and Gramapriya female line) to assess genetic diversity and reported 5 alleles having sizes as 166, 170, 178, 182 and 194bp having PIC value of 0.712. The observed and effective numbers of alleles were 5 and 4.76; observed heterozygosity (Ho) and expected heterozygosity (He) were 0.91 and 0.79, respectively.

2.2.1.3 ADL0102 Microsatellites marker

Panday *et al.,* (2002) genotyped ADL0102 along with 14 other microsatellite loci to assess the genetic variability of three indigenous poultry breeds and reported three alleles in Aseel, five alleles in Nicobari and seven alleles in Miri chicken having effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity as 2.61, 0.61, 0.62 and 0.97 in Aseel, 4.22 0.76, 0.77 and 0.64 in Nicobari and 4.88, 0.79, 0.81 and 0.75 in Miri fowl, respectively.

Vijh and Tantia (2004) genotyped ADL0102 loci along with 25 other microsatellite in 32 randomaly selected birds of Nicobari, 20 of Miri, 36 of Aseel and 46 of Kashmir Favorolla indigenous breed of poultry and observed 8 alleles with average heterozygosity of 0.71.

Panday *et al.,* (2005) genotyped ADL0102 along with 29 microsatellite loci to measures of genetic variation in Ankleshwar chicken breed and found eight alleles ranging from 164-182bp and effective numbers of alleles as 2.714, the Shannon's information index, PIC value, observed heterozygosity, expected heterozygosity and Nei's value were 1.111, 0.558, 0.850, 0.640 and 0.632, respectively.

Chatterjee *et al*. (2008a) genotyped ADL0102 along with four other loci in six crossbred population of White Leghorn and reported three alleles having sizes as 92, 108 and 118bp and corresponding PIC value, observed and expected heterozygosity were 0.58, 0.65 and 0.69.

Chatterjee *et al*. (2008a) genotyped ADL0023 along with four other loci in six crossbred population of White Leghorn with 15 birds in each population and reported three alleles having sizes as 92, 108 and 118 bp. The genotypic and allelic frequencies varied from 0.09 to 0.33 and 0.23 to 0.39, respectively.

Chatterjee *et al*. (2010b) genotyped ADL0102 microsatellite loci along with14 loci in two indigenous native breeds, Kadaknath and Aseel and three different chicken lines (Vanaraja male line, Vanaraja female line and Gramapriya female) line to assess genetic diversity and reported five alleles having sizes as 96, 104, 108, 114 and 120bp and PIC value as 0.573. The observed and effective numbers of alleles were 5 and 4.41; observed heterozygosity (Ho) and expected heterozygosity (He) were 0.59 and 0.70, respectively.

Das (2013) screened ADL0102 along with 23 microsatellites using 8% denaturing UREA-PAGE in selected line of Rhode Island Red chicken and reported four alleles having sizes and frequencies as 136bp and 0.200, 146bp and 0.400, 166bp and 0.200, and 174bp and 0.200. The estimated PIC value, Nei's heterozygosity, number of observed alleles, number of expected alleles, Shannon's information index, observed heterozygosity, expected heterozygosity were 0.6720, 0.7200, 4, 3.5714, 1.3322, 0.8000 and 0.7579, respectively.

2.2.1.4 ADL0176 Microsatellites marker

Panday *et al.,* (2002) genotyped ADL0176 along with 14 other microsatellite loci to assess the genetic variability of three indigenous poultry breeds and reported eight alleles in Aseel, seven alleles in Nicobari and four alleles in Miri chicken having corresponding effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity as 4.72, 0.78, 0.79 and 0.85 in Aseel, 5.64, 0.82, 0.83 and 1.00 in Nicobari and 2.83, 0.64, 0.66 and 0.60 in Miri fowl, respectively.

Vijh and Tantia (2004) genotyped ADL0176 loci in 32 randomly selected bird of Nicobari, 20 of Miri, 36 of Aseel and 46 of Kashmir Favorolla indigenous breed of poultry and observed 12 alleles and the average heterozygosity was 0.78.

Panday *et al.* (2005) genotyped ADL0176 along with 29 microsatellite loci to measure genetic variation in Ankleshwar chicken breed and found five alleles ranging from 188-210bp

and effective numbers of alleles as 3.703. The corresponding Shannon's information index, PIC value, observed heterozygosity, expected heterozygosity and Nei's value were 1.43, 0.686, 0.816, 0.740 and 0.730, respectively.

Haunshi and Sharma (2006) genotyped ADL0176 loci along with 9 other microsatellites in 76 backcross progenies of Nacked neck population and found three different alleles having sizes of 198, 190, 181bp, respectively. The most frequent allele was of 190bp.

Chatterjee *et al*. (2008b) genotyped ADL0176 along with 4 other loci in six crossbred population of White Leghorn with15 birds in each population and reported 6 alleles with sizes of 138, 150,160, 168, 176 and 188 bp. The genotypic and allelic frequencies varied from 0.04 to 0.32 and 0.23 to 0.33, respectively.

Chatterjee *et al*. (2008a) genotyped ADL0176 MS locus along with four other loci in six crossbred population of White Leghorn and reported six alleles having sizes as 138, 150,160, 168, 176 and 188bp. The mean PIC value, observed and expected heterozygosity were 0.72, 0.75 and 0.66.

Chatterjee *et al*. (2010b) genotyped ADL0176 microsatellite loci along with14 other MS loci in two indigenous native breeds, Kadaknath and Aseel and three different chicken lines (Vanaraja male line, Vanaraja female line and Gramapriya female line) to assess genetic diversity and reported 6 alleles having sizes as 190, 196, 200, 202, 206 and 218bp with mean PIC value as 0.694. The observed and effective numbers of alleles were 6 and 4.98; observed heterozygosity (H_o) and expected heterozygosity (H_e) were 0.90 and 0.80, respectively.

Das (2013) screened ADL0176 along with 23 microsatellites using 8% denaturing UREA-PAGE and silver staining in control and selected line of Rhode Island Red chicken and reported three alleles in selected strain of RIR chicken having sizes and frequencies as 200bp and 0.500, 202bp and 0.400, and 236bp and 0.100. The estimated PIC value, Nei's heterozygosity, number of observed alleles, number of expected alleles, Shannon's information index, observed heterozygosity, expected heterozygosity were 0.4918, 0.5800, 3, 2.3810, 0.9433, 0.2000 and 0.6105, respectively.

Arya (2012) genotyped ADL0176 using 3.4% MetaPhor Agarose along with 46 other microsatellites to study the genetic variability of microsatellite in high and low egg producing sub-populations of white leghorn chicken and reported only 1 allele of 188 bp in both sub-populations.

Suh *et al.* (2014) screened ADL0176 locus along with 29 microsatellite loci in 450 chickens (50 birds per breed) belonging to 9 different breeds using capillary electrophoresis and reported eight numbers of alleles across nine chicken breeds, which included six Korean native chicken breeds (Korean Reddish Brown, Korean Yellowish Brown, Korean Grayish Brown, Korean Black, Korean White, Korean Ogye) and three imported breeds (White Leghorn, Rhode Island Red, Cornish,) ranged from 186 to 208 and mean PIC value was 0.703. The average expected and observed heterozygosity frequencies across breeds were 0.739 and 0.295, respectively.

Deshmukh *et al.* (2015) genotyped ADL0176 locus using 3.4% MetaPhor along with 24 microsatellite loci in 76 randomly selected birds i.e. 20 of Hill fowl, 14 of Rhode Island Red, 14 of Kadaknath,14 of White Leghorn and 14 of White Cornish. Three alleles were reported which ranged in size from 194 to 216 base pairs in all selected birds except White Cornish, where 2 alleles of 194 to 204bp were seen.

2.2.1.5 ADL0210 Microsatellites marker

Panday *et al.,* (2002) genotyped ADL0210 along with 14 other microsatellite loci to assess the genetic variability of three indigenous poultry breeds and reported five alleles in Aseel, six alleles in Nicobari and eight alleles in Miri chicken having effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity as 2.44, 0.58, 0.59 and 0.60 in Aseel, 3.36 0.70, 0.71 and 0.43 in Nicobari and 5.33, 0.81, 0.83 and 0.55 in Miri fowl, respectively.

Vijh and Tantia (2004) genotyped ADL0210 locus along with 25 other microsatellite in 32 randomly selected Nicobari, Miri, Aseel and Kashmir Favorolla native breeds of chicken and observed 9 alleles and average heterozygosity was 0.56, respectively.

Panday *et al.,* (2005) genotyped ADL0210 along with 29 microsatellite loci to measure genetic variation in Ankleshwar chicken breed and found five alleles which ranged from 115-131bp and effective numbers of alleles was 2.085. The corresponding Shannon's information index, PIC values observed heterozygosity, expected heterozygosity and Nei's value were 0.999, 0.479, 0.342, 0.527 and 0.520, respectively.

Haunshi and Sharma (2006) genotyped ADL0210 loci along with 9 other microsatellite in 76 backcross progenies of Nacked neck population and found 2 different alleles having sizes as 131, and 124bp, respectively. The most frequent allele was of 131bp.

Chatterjee *et al*. (2010a) genotyped ADL0210 along with 8 other loci in six crossbred population of White Leghorn and reported four alleles having sizes as 124, 128, 130, and 134bp with frequencies as 0.13, 0.31, 0.34 and 0.22, respectively.

Chatterjee *et al*. (2010b) genotyped ADL0210 microsatellite loci along with 14 loci in Aseel and Kadaknath native breed and three different chicken lines to assess genetic diversity and reported three alleles having sizes as 128, 134, and 142bp and mean PIC value as 0.456. The observed and effective numbers of alleles were 3 and 2.65; observed heterozygosity and expected heterozygosity were 0.99 and 0.62, respectively.

Das (2013) screened ADL0210 MS locus along with 23 microsatellites using 8% denaturing UREA-PAGE in selected line of Rhode Island Red chicken and reported five alleles having sizes and frequencies as 128bp and 0.083,130bp and 0.333, 134bp and 0.333, 140bp and 0.167 and 150bp and 0.083. The estimated PIC value, Nei's heterozygosity, number of observed alleles, number of expected alleles, Shannon's information index, observed heterozygosity, expected heterozygosity were 0.6921, 0.7361, 5, 3.7835, 1.4452, 0.1667 and 0.7681, respectively.

2.2.1.6 MCW0007 Microsatellites marker

Panday *et al.* (2002) resolved MCW0007 MS locus along with 14 other microsatellite loci in indigenous chicken breeds and reported three alleles in Aseel and Miri and two alleles in Nicobari chicken breeds having effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity as 2.80, 0.64, 0.65 and 0.74 in

Aseel, 1.99, 0.49, 0.50 and 0.23 in Nicobari and 2.57, 0.61, 0.62 and 0.95 in Miri fowl, respectively.

Vijh and Tantia (2004) genotyped MCW0007 locus along with 25 other microsatellites in Nicobari, Miri, Aseel and Kashmir Favorolla native breeds of chicken and reported seven alleles with average heterozygosity as 0.63.

Panday *et al.,* (2005) genotyped MCW0007 along with 29 microsatellite loci in Ankleshwar chicken breed and found four alleles, which ranged from 295-317 bp in size and effective numbers of alleles was 2.980. The Shannon's information index, PIC value observed heterozygosity, expected heterozygosity and Nei·s value were 1.205, 0.604, 0.692 and 0.675, respectively.

Chatterjee *et al*. (2008b) genotyped MCW0007 and reported 3 alleles having sizes as 292, 298, and 320bp. The genotypic and allelic frequencies varied from 0.01 to 0.48 and 0.21 to 0.54, respectively.

Chatterjee *et al*. (2008a) genotyped MCW0007 along with four other loci in six crossbred population of White Leghorn and reported three alleles having sizes as 292, 298 and 320bp. the PIC value, observed and expected heterozygosity were 0.55, 0.61 and 0.71,respectively

Chatterjee *et al*. (2010b) genotyped MCW0007 and reported two alleles having sizes as 290 and 318bp and mean PIC values as 0.364. The observed and effective numbers of alleles were 2 and 1.95. The observed heterozygosity and expected heterozygosity were 0.83 and 0.49, respectively.

Arya (2012) resolved MCW0007 microsatellite loci in 3.4% MetaPhor Agarose and reported two alleles of 227 and 303bp in both high and low egg production subpopulations of white leghorn chicken.

Deshmukh *et al.* (2015) genotyped MCW0007 locus using 3.4% MetaPhor along with 24 microsatellites loci in Rhode Island Red chicken and reported two alleles having sizes and frequencies as 316 bp and 0.27 and 340bp and 0.73. The estimated PIC value, observed heterozygosity and expected heterozygosity 0.453, 0.546 and 0.416, respectively.

2.2.1.7 MCW0014 Microsatellites marker

Panday *et al.,* (2002) genotyped MCW0014 locus in three indigenous poultry breeds and reported five alleles in Aseel, two alleles in Nicobari and five alleles in Miri chicken with effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity as 2.01, 0.50, 0.51 and 0.37 in Aseel, 1.66, 0.39, 0.40 and 0.41 in Nicobari and 2.71, 0.63, 0.65 and 0.43 in Miri breed, respectively.

Vijh and Tantia (2004) genotyped MCW0014 locus and reported eight alleles. The average heterozygosity was 0.47, in four native chicken breeds.

Panday *et al.* (2005) genotyped MCW0014 and found four alleles, which ranged in size from 174-188bp. The effective numbers of alleles was 2.602 .the Shannon's information index, PIC value, observed heterozygosity, expected heterozygosity and Nei's values were 1.105, 0.541, 0.539, 0.624 and 0.616, respectively.

Bao *et al.* (2007) genotyped MCW014 locus along with other 28 loci in 14 Chinese indigenous chicken breed and one red jungle fowl and reported 11 alleles with allele size ranging from 160 -186bp. The expected heterozygosity and Polymorphic Information Content were 0.6707 and 0.50, respectively.

Bao *et al.* (2008) genotyped MCW014 locus along with other 28 loci to access Genetic diversity and phylogenetic relationships among 568 individuals of two red jungle fowl subspecies (*Gallus gallus spadiceus* in China and *Gallus gallus gallus* in Thailand) and 14 Chinese domestic chicken breeds, and found 11 alleles ranging from 160 to 186 base pairs. The expected heterozygosity and Polymorphic Information Content were 0.6774 and 0.63, respectively

Chatterjee *et al*. (2010a) genotyped MCW0014 MS locus along with 8 other loci in six crossbred population of White Leghorn and reported 2 alleles having sizes as 180 and 192bp.The PIC value as 0.38 and frequency of alleles were 0.92and 0.08, respectively.

Chatterjee *et al*. (2010b) reported four alleles at MCW0014 MS locus with sizes of 180, 182, 190 and 206 bp among three different chicken lines and two native Aseel and Kadaknath breed.

Das (2013) screened MCW0007 using 8% denaturing UREA-PAGE in selected line of Rhode Island Red chicken and reported two alleles having sizes and frequencies as 173bp and 0.200, 175bp and 0.200 and 177bp 0.600. The estimated PIC value, Nei's heterozygosity, number of observed alleles, number of expected alleles, Shannons information index, observed heterozygosity, expected heterozygosity were 0.4992, 0.5600, 3, 2.2727, 0.9503, 0.0000 and 0.5895, respectively.

Keambou *et al.* (2014) genotyped MCW0014 along with other 24 loci in Ankleshwar chicken breed and found 9 different alleles ranging from 124 to 142 base pairs. The effective number of alleles and PIC values were 5.040 and 0.780, respectively.

Ceccobelli *et al.* (2013) analyzed MCW0014 locus along with 27 microsatellite loci in five Italian local chicken breeds (Ancona, Livorno, Modenese, Romagnola and Valdarnese bianca) to assess genetic diversity, population structure and the genetic relationships among them and reported 7 allele ranging from 164-182bp.

Arya (2012) analyzed MCW0014 microsatellite locus on 3.4% MetaPhor Agarose and reported only 1 allele of 246 bp in both high and low egg production subpopulations of WLH chicken.

Deshmukh *et al.* (2015) genotyped MCW0014 loci using 3.4% MetaPhor and reported two alleles having sizes and frequencies of 200bp and 0.29 and 220 and 0.71bp in RIR chicken. The estimated PIC values, observed and expected heterozygosity were 0.250, 0.431 and 0.328, respectively.

2.2.1.8 MCW0041 Microsatellites marker

Panday *et al.* (2002) genotyped MCW0041 using 6% denaturing PAGE to asses genetic variability in three indigenous poultry breeds and reported four alleles in Aseel and Miri and two alleles in Nicobari chicken. The effective no. of alleles, polymorphic information content, expected heterozygosity and direct count heterozygosity were 3.03, 0.66, 0.67 and 0.60 in Aseel, 1.99, 0.49, 0.50 and 0.90 in Nicobari and 2.54, 0.60, 0.62 and 1.00 in Miri breed, respectively.

Vijh and Tantia (2004) genotyped MCW0041 MS locus along with 25 other microsatellites in Nicobari, Miri, Aseel and Kashmir Favorolla native chicken breeds and reported 8 alleles. The average heterozygosity was 0.69, respectively.

Panday *et al.* (2005) genotyped MCW0041 locus and found four alleles which ranged from152-172bp. The effective numbers of alleles was 1.570. The Shannon's information index, PIC value, observed heterozygosity, expected heterozygosity and Nei's values were 0.773, 0.346, 0.300, 0.369 and 0.365, respectively.

Chatterjee *et al*. (2010a) genotyped ADL0041 along with 8 other loci in six crossbred population of White Leghorn with15 birds in each population and reported 3 alleles having sizes as 150, 160, and 170bp. The PIC value was 0.52. The frequency of three alleles were 0.62, 0.15 and 0.23, respectively

2.2.1.9 ADL0069 Microsatellites marker

Bao *et al.* (2007) genotyped MCW0069 MS locus along with other 28 loci in 14 Chinese indigenous chicken breeds and one red jungle fowl. They reported 9 alleles with allele size ranging from 158 -176bp. The expected heterozygosity and Polymorphic Information Content were 0.7646 and 0.60, respectively.

Bao *et al.* (2008) genotyped MCW0069 MS locus along with other 28 loci to estimate genetic diversity and phylogenetic relationships among 568 individuals of two red jungle fowl subspecies (*Gallus gallus spadiceus* in China and *Gallus gallus gallus* in Thailand) and 14 Chinese domestic chicken breeds, and found 9 alleles ranging in size from 158 to 186 base pairs. The expected heterozygosity and Polymorphic Information Content were 0.7584 and 0.63, respectively

Arya (2012) genotyped MCW0069 MS locus using 3.4% Metaphor Agarose along with 46 other microsatellites to study the genetic variability of microsatellite in high and low egg producing subpopulations of white leghorn chicken and reported two alleles of 126bp and 160bp in high egg production and only one allele of 110bp in low production sub-populations of WLH chicken. The estimated PIC value, expected and observed heterozygosity were 0.471, 0.000, 0.352 in high egg production sub-population.

Ceccobelli *et al.* (2013) analysed 27 microsatellite loci in five Italian local chicken breeds (Ancona, Livorno, Modenese, Romagnola and Valdarnese bianca) to assess genetic diversity, population structure and the genetic relationships among them. They genotyped MCW0069 locus and reported 7 alleles ranging in size from 158-170bp.

Deshmukh *et al.* (2015) genotyped MCW0069 loci using 3.4% MetaPhor and reported two alleles having sizes and frequencies of 174bp and 0.62 and 194bp and 0.38 bp in RIR chicken. The estimated PIC value, observed and expected heterozygosity were 0.361, 0.154 and 0.492, respectively.

Abebe *et al.* (2015) genotyped MCW0069 MS locus along with 29 other microsatellites loci to assess the genetic diversity of five local Swedish chicken breeds detected by microsatellite markers and reported 6 alleles across population. The observed and expected heterozygosity frequencies were 0.739 and 0.359, respectively. The PIC value was 0.767.

2.2.1.10 MCW0103 Microsatellites marker

Bao *et al.* (2007) genotyped MCW0103 MS locus along with other 28 loci in 14 Chinese indigenous chicken breed and one red jungle fowl. They reported 2 alleles with allele size ranging from 266 -270bp. The expected heterozygosity and polymorphic information content were 0.3945 and 0.25, respectively.

Bao *et al.* (2008) genotyped MCW0103 along with other 28 loci to assess genetic diversity and phylogenetic relationships among 568 individuals of two red jungle fowl subspecies (*Gallus gallus spadiceus* in China and *Gallus gallus gallus* in Thailand) and 14 Chinese domestic chicken breeds, and found 2 alleles ranging from 266 to 270 base pairs. The expected heterozygosity and Polymorphic Information Content were 0.4506 and 0.29, respectively

Arya (2012) analyzed MCW0103 microsatellite loci on 3.4% MetaPhor Agarose and reported four alleles of 359, 401, 439 and 479 bp in high egg sub-populations with mean PIC value as 0.352 and only one allele of 359bp in low egg production sub-population of WLH.

Ceccobelli *et al.* (2013) analyzed 27 microsatellite loci in five Italian local chicken breeds (Ancona, Livorno, Modenese, Romagnola and Valdarnese bianca) to assess genetic

diversity, population structure and the genetic relationships among them. They genotyped MCW0103 locus and reported 2 alleles ranging from 266-270bp.

Suh *et al.* (2014) genotyped 50 individuals per breed at MCW0103 locus along with 29 microsatellite loci in 450 chickens belonging to 9 different breeds using capillary electrophoresis, which included six Korean native chicken breeds (Korean Reddish Brown, Korean Yellowish Brown, Korean Grayish Brown, Korean Black, Korean White, Korean Ogye,) and three imported breeds (White Leghorn, Rhode Island Red, Cornish), respectively. Two alleles were reported across nine chicken breeds ranging from 269 to 273 and corresponding average PIC value was 0.258. The average expected and observed heterozygosity frequency across breed were 0.305 and 0.278, respectively.

Deshmukh *et al.* (2015) genotyped MCW0103 MS locus using 3.4% MetaPhor Agarose along with 24 other microsatellites to study the genetic variability of 76 randomly selected birds *i.e*. 20 of Hill fowl, 14 of Rhode Island Red 14 of Kadaknath, 14 of White Leghorn and 14 of White Cornish and reported only one allele of 324 bp in all breeds.

2.2.2 Association of microsatellites genotypes with growth traits

Sewalem *et al.* (2002) genotyped 101 microsatellite markers on chromosome 1 in F_2 chicken population which was established by crossing of a broiler sire-line and an egg laying (White Leghorn) line and found that the microsatellites LEI0068, LEI0146, and MCW0018 were associated with body weights at 3, 6 and 9 weeks of age and microsatellite ROS0025 affected the body weight at 6 weeks of age only.

Pandya *et al. (*2005) studied LEI-146 and MCW-43 microsatellites association with growth traits in Bantam, White Leghorn and Bantamised White Leghorn chicken. The birds having 1,1 and 1,3 genotypes for LEI0146 microsatellite marker, had lowest body weight at all the ages, while birds with genotypes 2,2 and 3,3 were having highest body weight at all the ages. Birds with1,3 and 4,4 genotypes for MCW043 microsatellite marker were having highest body weight while birds with 1,2 and 2,3 genotypes at this locus were having lowest body weight at all the ages.

Atzmon *et al*. (2006) genotyped 76 microsatellite markers and established association between microsatellite markers and growth related traits and reported that MCW0102 was significantly associated with BW at seven weeks of age in a commercial broiler line.

Nones *et al*. (2006) found that LEI0068 and LEI0079 MS markers were associated with BW at 35 and 42 days and MCW0058 with BW at 42 days in F_2 experimental population, developed by two generations of crossbreeding between a broiler sire line and a layer line.

Chatterjee *et al.* (2008b) analyzed five microsatellites to assess the association with growth traits in six crossbred layers chicken. The genotypes at many microsatellites were found to have significant ($P \leq 0.5$.) effect on body weights at BW20, BW28 and BW40. MCW007, ADL020, ADL023 and ADL176 microsatellites were found to be significantly correlated with BW. Genotype 16 of ADL176 was found significantly associated with low BW40, while the genotype 11, 12, 13, 14, 25, 36 and 44 revealed moderate body weights at this week. Genotype 11 of MCW007 was having relatively higher BW at all the age groups. In case of ADL020 microsatellite, genotype 11 showed relatively higher BW40.

Kim *et al*. (2008b) Studied 17 polymorphic microsatellite markers on chromosome one used for allelic association tests with phenotypic traits in Korean native chicken and reported that UMA1.117, ADL0020, UMA1.019, LMA1 and ADL0238 loci showed significant differences in allelic distribution for the trait of body weights. MCW0160 showed a significant difference between high and low groups for egg weight.

Boschiero *et al.* (2009) studied the associations between nine microsatellites viz., LEI0143, ADL0123, ADL0210, MCW0230, ADL0147, LEI0251, MCW0213, MCW0110 and MCW0104 and performance, carcass and organs' traits in chicken. Significant association between markers studied and different traits were found.

Chatterjee *et al*. (2010a) studied the genetic variability of nine microsatellites and its relationship with growth traits in six crossbred population of White Leghorn chicken and reported that only MCW0041 genotypes were significantly ($P \leq 0.05$) associated with body weights at 28 and 40 weeks of age. Genotypes 11 and 33 had the highest body weight at 28 and 40 weeks of age, whereas other genotypes (12, 13, and 23) produced lower body weight at these ages.

Nassar *et al*. (2012) analysed seven microsatellite markers in the 6th selected generations of Cairo B-2 line and compared with the control line (C line). The allelic frequencies of the simple sequence repeats (SSR) loci, ADL0328, were higher (six alleles) in the Cairo B-2 line, males and females, while the C line showed only five alleles and reported that heavier birds had more alleles for the ROS0025, MCW0010, MCW0018, c3-46151949, c5-4999025, and MCW0097, than the lighter birds.

Das (2013) reported that MCW0014, MCW0051 and ADL0176 genotypes were significantly ($P \leq 0.05$) associated with body weight at 40 weeks of age in selected strain of RIR chicken. Heterozygotes BD≃ DD of MCW0051 locus had significantly ($P \leq 0.05$) higher BW40 than CD heterozygote of the locus. Homozygote AA of MCW0014 locus had significantly ($P \leq 0.05$) higher BW40 than CC and BB homozygotes at this locus and Heterozygote CE of ADL0176 locus had significantly ($P \leq 0.05$) higher BW40 than DD H" CC homozygotes at this locus.

2.2.3 Association of microsatellites genotypes with layer economic traits

Chatterjee *et al.* (2008a) studied the variability at five microsatellites markers in six crossbred (IWH x IWI, IWI x IWH, IWK x IWH, IWH x IWK, IWI x IWK, and IWK x IWI) chicken of White Leghorn and reported that out of five microsatellites studied, only ADL023 microsatellite was found significantly ($P \leq 0.05$) associated with EP64, EP72 and EW28. Genotype 11, 12, 13 and 23 produced more number of eggs at EP64, EP72 than the genotype 22. EW28 were higher in genotype 12, 13 and 23 and lower in genotypes 11 and 22.

Kim *et al.* (2008b) studied 17 microsatellites to compare the allelic frequencies in high and low production population in Korean native chicken and reported that ADL0234, UMA1.125 and ADL0101 showed significant differences in allelic distribution for AFE. ADL0101 and ADL0238 showed significant differences in allelic distribution for egg production traits

Chatterjee *et al.* (2008b) assessed the association of MCW007, ADL020, ADL023, ADL102 and ADL176 in six crossbred layers chicken. All the microsatellites except ADL102 showed significant association with AFE. Genotype 16 at ADL176 and genotype 13 at

MCW007 loci were found to be significantly associated with low AFE. In case of ADL020 microsatellite, 11 and 23 genotype showed higher AFE. The genotypes 12, 13 at ADL023 microsatellite revealed low AFE than other genotypes.

Chatterjee *et al.* (2010a) reported significant association ($P \leq 0.05$) between three microsatellites loci, MCW0041, ADL0210, and MCW0110 with egg production traits. Genotype 11 of MCW0110 showed high EP28. Genotypes 11 and 33 at MCW0041 showed the highest BW28 and BW40. Genotype 33 at MCW0041 and genotypes 23 and 34 at ADL0210 had higher EP64. Similarly, genotypes 12, 23, and 33 of MCW0041 and 23 and 34 at ADL0210 produced significantly higher EP72. Genotype 34 at ADL0210 and 11 at MCW0110 were two potential candidate loci for egg production traits.

Arya (2012) studied 47 microsatellites in white Leghorn chicken. Out of these 47 markers, 9 (19%) were found to be polymorphic between the sub-populations and 12 alleles at six microsatellite loci were sub-population-specific. The number of sub-population-specific allele(s) at one locus ranged from one (at MCW0037 and MCW0183) to three alleles (at MCW0069 and MCW0103). Of these, 7 alleles were specific to high egg production (HEP) sub-population and 5 alleles were specific to low egg production (LEP) sub-population. Maximum number of sub-population-specific alleles were three alleles at MCW0103 locus for HEP sub-population. They concluded that, for HEP sub-population, MCW0069 and MCW0067 were found to be the most promising sub-population-specific alleles with high allelic frequencies. Overall, the MCW0069 locus was the most promising marker for egg production traits.

Das (2013) studied 24 microsatellite loci to assess the genetic diversity between RIR^S and RIR^C and reported significant ($P \leq 0.05$) microsatellite genotypic effect for MCW0044 on EP40, MCW0075 on AFE, MCW0005 on EW28, MCW0014 on EW28, ADL0102 on EP40 and ADL0158 on EP40 in selected strain of RIR chicken and no significant effect found for any locus on any trait in control strain of RIR chicken. In RIR^S, heterozygote DG at MCW0044 locus had significantly ($P \leq 0.05$) higher EP40 than CF heterozygote at the locus. Heterozygote CF at MCW0075 locus had significantly ($P \leq 0.05$) lower AFE than BE

heterozygote of the locus. Heterozygotes AE≃BD at MCW0005 locus had significantly (P≤0.05) higher EW28 than CE heterozygote ≃ BB homozygote at this locus. Homozygote AA of MCW0014 locus had significantly (P≤0.05) higher EW28 than BB ≃ CC homozygotes at this locus. Homozygote DD of ADL0102 locus had significantly (P≤0.05) higher EP40 than BE ≃ AD heterozygotes at this locus. Homozygote DD of ADL0158 locus had significantly (P≤0.05) higher EP40 than CG ≃ DH or DH ≃ DG heterozygotes at this locus.

2.3 Objective 3: To estimate immunocompetence traits and their association with layer economic traits in Rhode Island Red chicken

Immune system is a set of mechanism that protects an organism from invasion or infection by identifying and killing foreign or foreign like substances, including pathogens such as bacteria, viruses, and fungi as well as offer protection from parasites. Immune response is broadly defined as the defense of the host as a result of its immune system to pathogens and parasites. Immunocompetence is the ability of a body to produce an immune response against any antigen. The immunocompetence status of any breed speaks its general response to invading pathogens. The scientific literature on immunocompetence traits and their inheritance pattern inter relationship and influence of IC levels on growth and layer economic traits have been reviewed below:-

2.3.1 Immunocompetence traits

Estimation and evaluation of various immunocompetence traits to view the overall status of immune response in various populations of chickens has been depicted in the following section. It is necessary for improvement of both production as well as immunocompetence traits of birds for incorporating in breeding and selection programs to improve diseases resistance. Immunocompetence profile can be evaluated by assessing a few important parameters related to various facets of immunity such as antibody response to SRBC, serum IgG level and serum lysozyme activity etc. Various genetic groups /varieties /breeds/ species have shown significant differences in these traits (Shivakumar, 2003; Sivaraman *et al.,* 2003; Singh *et al.,* 2003; Singh *et al*., 2009; Gupta *et al*., 2010; Kokate, 2013; Jaiswal *et al.,* 2014; Das *et al.,* 2014a,b,c).

2.3.2 Immunocompetence traits in chicken

2.3.2.1 Antibody response to SRBC

Sheep red blood cells (SRBCs) is one of the most frequently used antigens for the following reasons. First, SRBC is non-pathogenic antigen as the environmental microbes do not interfere with measurement of antibody response to SRBCs. Second SRBC is a nautral, multi-determinant and strongly immunogenic antigen. The main reason for choosing it as the selected antigen is that background genes, which are not linked to immune response gene at MHC and *Igh* loci, were shown to contribute to antibody production (Puel *et al.,* 1995). Third, the methods used to measure antibody level and to detect the specific antibody producing cells in the SRBC-tiggered immune response have been established and well documented. Lastly, selection for antibody production to SRBC has been considered as an attractive approach to improve general diseases resistance to multiple pathogens (Gross *et al*., 1980). Among these the divergently selected lines for humoral immune response against SRBC, the classic ones are the Biozzi lines of mouse and Siegel lines of chicken (Biozzi *et al.,* 1972; Siegel and Gross,1980). Birds eliciting higher antibody response against SRBCs also produce more antibodies to a variety of antigens. The use of antibody response to SRBC in a multitrait selection programme is well known concept in avian immunology that reveals various aspects of immune response and their genetic basis (Kean *et al*., 1994).

The administration of antigen (SRBC) has been done by various routes viz., intramuscular (i/m) in ISA Warren chicks (Van der Zijpp, 1983; Pinard *et al*., 1992) and intra venous (i/v) in White Leghorn chicken (Siegel and Gross, 1980; Gupta *et al.,* 2010; Das *et al*., 2014a, b, c) and Indian Aseel and Kadaknath native chicken (Singh *et al.,* 2009; Jaiswal *et al.,* 2014; Kokate, 2013). Three routes of SRBC administration *viz.,* i/v, i/m and i/p were compared in new Hampshire chicken breed by Van der Zijpp *et al.* (1986) and reported that the route of injection had significant effect on total antibody titre to SRBC at 3, 5, 7, and 12 dpi. The highest titre was obtained on 5 dpi by intra venous route. Sex and age had no significant effect on titre. There were no significant differences in antibody titre against SRBC in early and late feathering of chicks of broiler lines from 8 to 35 days of age.

Kundu *et al*. (1999) studied the primary antibody response to sheep erythrocytes on 5, 12 and 19 dpi by haemagglutination test in Indian native breeds, Aseel, Kadakanath, Naked Neck and Frizzle fowl along with the imported breeds Dahlem Red, White Leghorn, Synthetic dam line broiler (SDL) and Naked Neck broiler. The HA titre values were relatively higher for indigenous chicken breeds followed by Dahlem Red and broilers. In most of the breeds, peak response was on 5 dpi and after that a gradual decline was observed.

Sivaraman *et al.* (2005) assessed the humoral immune response in synthetic dam line (SDL) of broiler chicken by estimating the antibody response against sheep SRBCs. The Least squares means for HA titre was 6.289 ± 0.246. Males showed a lower response to SRBC than females but the difference was not significant.

Kumar (2006) studied sheep RBCs response in Aseel chicken, the antibody titres on 5^{th} dpi averaged 12.80 ±0.74 in females and 11.96 ± 0.64 in males respectively: overall average being 12.38 ± 0.60. Further, older birds had higher mean antibody titres (13.0 ± 2.13) than the younger birds (10.88 ± 0.54).

Sharma (2007) estimated the persistency of antibody response to SRBC for four different age groups in Guinea fowl, by using HA titre at 5, 9, 12 and 15 dpi. Peak HA titre were observed at 11 week on 3 dpi followed by 9 week on 5 dpi. The HA titre at different dpi showed quite consistent trend at all ages and reasonable persistency from 5 dpi to 12 dpi with a peak at 8 dpi. At 15 dpi, the decrease was sharp.

Chatterjee *et al.* (2007a) reported that humoral immune response to SRBC was significantly ($P \leq 0.05$) higher in Aseel (77.25 ± 0.18 log2) than Kadaknath (5.70 ± 0.25 log2).

Chatterjee *et al*. (2007b) reported that the humoral immune response to SRBC of inbred and non-inbred populations of Dahlem Red differed significantly ($p \leq 0.05$) from each other. The immune response to SRBC was highest in non-inbred group (NB) group (8.79±1.44) followed by full sib mated group (FS) (7.60±1.78) and half sib mated group (HS) (6.23±1.54) groups and the heritability estimate of this trait was moderate (0.27±0.19).

Saini *et al*. (2008) evaluated the humoral response to T-cell dependent antigen SRBC in three strains of single comb White Leghorn (PL1, PL2 and PL3) and two Rhode Island Red (RIR-B and RIR-C) strains of chicken by using haemagglutination titre (HA), at 0, 4, 9, 14

and 21 days post primary inoculation of SRBC. They concluded that, antibody production in response to SRBC started immediately after exposure to foreign antigen (SRBC) and reached peak level at 4-9 days after exposure and then started declining with the lapse of time. Among RIR strains they reported that RIR-C had higher base total HA titre than RIR-B strain. The highest HA titre at 4 days PPI was recorded in strain RIR-C (5.20) indicating highest production of total antibodies in response to SRBC. Between RIR strains, RIR-B and RIRC have same level of total antibodies titre (4.70).

Mahrous *et al*. (2008) reported that with respect to primary immune response to SRBC, the naked neck (Nanaff), frizzle (nanaFf) and naked neck-frizzle (NanaFf) chicks had significantly higher total anti-SRBCs antibody titer. Similar trend was noticed for secondary immune response.

Singh *et al.* (2009) evaluated the humoral immune response against SRBC and estimated least squares mean of HA titre as 7.49±0.25 in 179 chicks of Kadaknath native breed.

Gupta *et al.* (2010) studied immunocompetence profiled in HSRBC and LSRBC lines of white Leghorn chicken divergently selected for humoral response to sheep erythrocytes. Least squares mean of HA titre was 8.06±0.22 in HSRBC and 7.87±0.26 in LSRBC lines. All the traits in both the lines were significantly affected by hatch.

Rajkumar *et al.* (2010) evaluated the effect of naked neck (Na) gene on immune competence traits in three genotypes (NaNa, Nana and nana) of the naked neck chicken under a tropical climate of Southern India. The humoral response as measured by antibody titre to SRBC was significantly higher in NaNa (7.00±0.29) followed by Nana (6.88±0.65) and nana (4.62±0.38).

Tomar *et al.* (2012) studied the humoral immune response to sheep RBCs, in indigenously developed five broiler parent lines (coloured plumaged CSML and CSFL, white plumaged SML and SDL and naked neck lines) through long-term selection for 5 week body weight. The highest titre against sheep RBCs were exhibited by Naked neck (7.923±0.383) and least in SML (6.280±0.296). The effect of line were found significant ($P \leq 0.05$) on response to sheep RBCs.

Kokate *et al.* (2013) studied antibody titres against sheep RBCs in Aseel, Kadaknath and White leghorn chicken, on 5th dpi was 10.84±0.18, 11.62±0.21 and 11.94±0.15, respectively. Further, males of Kadaknath and Aseel birds had slightly higher titre than females.

Revagade *et al.* (2013) evaluated the humeral response through antibody response to SRBCs in tropical oriented Naked neck and Frizzle major genes. There was no significant difference between varieties for the humoral immune response. Mean titres (log_2) estimated on 5 dpi for Naked neck were 8.810±0279, and corresponding value for Frizzle as 8.429±0.279, respectively.

Das *et al*. (2014a) estimated antibody response against Sheep RBCs in CARI-Debendra multicoloured chicken. The least square mean of HA titre on 5th dpi was 5.739±0.436.

Das *et al*. (2014b) evaluated antibody response against Sheep RBCs and estimated least square means of HA titre as 6.001±0.441 on 5th dpi in CARI-Sonali chicken.

Das *et al*. (2014c) estimated antibody response against Sheep RBCs in three pure strains of Rhode Island Red (RIR) chicken, viz. RIR^S, RIR^C & RIR^W, and its two crosses, viz. CARI-Sonali and CARI-Debendra. The least square means of HA titre were 8.837±0.473, 10.393±0.473, 6.511±0.504, 6.012±0.455 and 5.789±0.452, respectively.

Jaiswal *et al.* (2014) studied humoral immune response against sheep RBCs in Kadaknath native chicken, the antibody titres on 5th dpi averaged in females as 7.65 ± 0.33 and 8.19 ± 0.31 in males respectively: overall average being 7.93 ± 0.24.

Nath *et al.* (2014) studied the crossbreeding parameter for humoral immune response against SRBC response using a complete 4×4 diallele design for four synthetic broilers lines namely coloured synthetic male line (CM), white synthetic male line (WM), coloured synthetic female line (CF) and naked neck line (NN). The genetic groups had strong effect on ($P \leq 0.01$) on mean SRBC which ranged from 4.32 to 10.59 on 7-day post-inoculation. Among 16 groups, with 4 purebred and 16 crossbred groups, the crossbred progenies of NN×CM and CM×WM produced the highest mean antibody response (10. 57 ± 0.53) against SRBC.

Das *et al*. (2015) estimated antibody response against Sheep RBCs in selected pure strains and a random bred control population of Rhode Island Red (RIR) chicken. The Overall means ± SD of HA titre were recorded as 9.55 ± 4.06.

2.3.2.2 Serum lysozyme level

Lysozyme is a low molecular weight protein, having cationic charges and possesses non-specific bacteriolytic activity which cleaves the link between N-acetyl-muramic acid and N-acetyl-glucosamine of a mucopolsacchride in the bacterial cell wall (Salton, 1957). It has important role in the body's defense against infection and acts as an antibacterial agent, which is mediated through stimulatory effect on macrophages phagocytic function (Thacore and Willet, 1966) as well as its direct bacteriolytic action (Biggar and Sturgess 1977). Recently, it has been asserted that serum lysozyme activity reflect the homeostatic expression of the reticulo-endothelial system, which is one of the most fundamental defense mechanism against infection. Lysozyme exists in high concentration in the egg white of many birds. The egg and developing embryo do not produce immunoglobulins until about 7 days before hatching. It is possible that the high lysozyme content in the egg maintains protective vigilance until the embryo develops the capability to produce immunoglobulins.

Sato and Watanabe (1976) analyzed lytic activity of serum and egg white lysozyme from White Leghorn chicken against *Micrococcus* l*ysodeickticus*. Lytic activity of egg white lysozyme was found to be thousand times more than the serum lysozyme activity. Bessarabov and Krykanov (1985) observed the positive correlation between dam's serum lysozyme level and natural resistance of progeny to diseases in chicken.

Nath *et al.* (1999) studied the serum lysozyme level in Naked neck (NN) birds and their crosses with normally feathered (nn) birds at the age group of 15-18 weeks. They observed that overall mean for serum lysozyme was 3.24 ± 0.09 µg/ml. Males showed significantly higher level of lysozyme (3.54 ± 0.12 µg/ml) than females (3.18 ± 0.12 µg/ml). The serum lysozyme level in progenies of NN×NN, NN×nn and nn×NN were 3.81 ± 0.19, 3.60 ± 0.12 and 2.67 ± 0.12 µg/ml, respectively and the differences were significant.

Shivakumar (2003) estimated the serum lysozyme level in IWG and IWJ genotypes of WLH chickens using *Micrococcus lysodeickticus* as substrate and observed considerable

difference in the values, which were 2.18 ± 0.04 and 1.26 ± 0.04 µg/ml in IWG and IWJ lines, respectively.

Kumar (2006) estimated the serum lysozyme level in Aseel which ranged from 1.23 to 10.34 µg/ml which averaged 3.40 ± 0.23 µg/ml and 3.44 ± 0.20 µg/ml in male and female, respectively and overall average was 3.42 ± 0.19 µg/ml.

Singh *et al.* (2009) estimated least squares mean of serum lysozyme level to be 2.13±0.03 µg/ml in Aseel. Significant ($P \leq 0.05$) sexual dimorphism was observed in serum lysozyme levels in HSRBC line and influence of sex on serum lysozyme concentration varied in divergent lines.

Gupta *et al.* (2010) estimated least squares mean of serum lysozyme concentration as 2.85±0.08 µg/ml in HSRBC and 2.77±0.09 µg/ml in LSRBC lines and reported that it was significantly affected by hatch in both the lines.

Chaudhary (2010) reported relatively higher serum lysozyme level in Aseel chicken, and it ranged from 1.90- 12.82 µg/ml, the average serum lysozyme level was 4.85 ± 0.20 and 4.48 ± 0.22 in male and females, respectively. The overall average was 4.66 ± 0.16.

Kumar and Kumar (2011) demonstrated higher immunocompetence status of Aseel native chicken than those reported for most of the other chicken breeds/ poultry species. The least squares mean of serum lysozyme was 3.42±0.19 µg/ml. Influence of sex was not significant on serum lysozyme.

Tomar *et al.* (2012) evaluated the serum lysozyme level in 5 parents broiler line viz., coloured plumaged CSML and CSFL, white plumaged SML and SDL and naked neck lines and reported that SDL broiler parent line had highest serum lysozyme (4.180±0.049 mg/ml) concentration among the five germplasm analyzed.

Kokate *et al.* (2013) analyzed the serum lysozyme level and reported that least squares mean of serum lysozyme concentration were higher in Aseel (21.28±0.78 µg/ml) followed by in Kadaknath (16.91±0.93 µg/ml) and in White leghorn chicken (9.42±0.68 µg/ml).

Das *et al.* (2014a) estimated least squares mean of serum lysozyme concentration as 6.031±0.213 µg/ml in CARI-Debendra chicken.

Das *et al.* (2014b) estimated least squares mean of serum lysozyme concentration as 5.692±0.324 µg/ml in CARI-Sonali chicken.

Das *et al.* (2014c) reported relatively higher estimates of serum lysozyme level in RIR^W (6.996±0.435) followed by RIR^S (6.336±0.437), RIR^C (5.174±0.428), CARI-Debendra (6.000±0.47) and CARI-Sonali (5.692±0.404) chicken.

Jaiswal *et al*. (2014) studied serum lysozyme concentration in 174 chicks of Kadaknath which ranged from 0.60 to 12.91µg/ml. Females demonstrated higher estimates (5.18 ± 0.34) than males (4.95 ± 0.33), respectively. The overall least square mean as 5.07 ± 0.29µg/ml.

Das *et al.* (2015) estimated the overall serum lysozyme concentration as 5.69± 2.93µg/ml, in RIR chicken.

2.3.2.3 Serum IgG Level

Among all the immunoglobulin classes in serum, IgG is the most abundant antibody and constitutes approximately 80% of the total immunoglobulin. The bird's ability to mount antibody responses to other antigen is primarily revealed by serum IgG concentration and is easily traceable in all body fluids. Ahrestani *et al.* (1987) estimated the serum IgG level in different breeds of chicken by Single Radial Immune Diffusion (SRID) method. Aseel demonstrated significantly higher (20.51 ± 0.22 mg/ml) level than WL (7.53 ± 0.22 mg/ml). Further, higher concentrations in first week of age had been ascribed to the presence of maternal antibodies, which reduced as the age advanced.

Chhabra and Goel (1980) estimated the serum IgG level of white leghorn birds by single radial immunodiffusion (SRID) method and reported a range between 1.0 and 13.5 mg/ml with a mean of 5.09 mg/ml.

Ahrestani *et al*. (1987) estimated the serum IgG level in Aseel and White Leghorn. Aseel demonstrated significantly higher level (20.51±0.22 mg/ml) than White Leghorn chicken (7.53 ±0.22 mg /ml).

Sivaraman *et al.* (2005) studied synthetic dam line (SDL) of broiler chickens for immunological traits and overall least squares means for serum IgG was 6.287±0.194 mg/ml.

Saini *et al*. (2007) studied SRBC response in the RIR strains, and found that Ig G level was higher in RIR-C (2.03 vs.1.93) among RIR strains and there were non-significant differences between strains. Between RIR strains, RIR-C had higher IgG titre than RIR-B.

Gupta *et al.* (2010) profiled immunocompetence in HSRBC and LSRBC lines of white Leghorn chicken divergently selected for humoral response to sheep erythrocytes. Least squares means of serum IgG were 33.91±1.68 mg/ml in HSRBC and 31.65±1.28 mg/ml in LSRBC lines. It was significantly affected by hatch traits in both the lines.

Singh *et al.* (2009) assessed least squares mean of serum IgG concentration as 10.07 ± 0.20 mg/ml in Kadaknath chicks.

Chaudhary (2010) found average serum IgG concentration as 11.73 ± 0.49 in Aseel and it was higher in females (12.64 ± 0.75) than males (10.82 ± 0.64).

Kumar and Kumar (2011) demonstrated higher immunocompetence status of Aseel native chicken than those reported for most of the other chicken breeds/ poultry species.

Das *et al*. (2014c) estimated higher serum IgG concentration in RIR^C (7.780±0.361) followed by RIR^W (7.749±0.390), RIR^S (6.597±0.361), CARI-Sonali (5.151±0.398) and CARI-Debendra (6.002±0.398 µg/µl) chicken, respectively.

Jaiswal *et al*. (2014) reported overall serum IgG concentration as 12.15± 0.48 mg/ml in Kadaknath native breed; it was higher in males (12.47 ± 0.61) then females (11.82 ± 0.64).

Das *et al*. (2015) estimated overall serum IgG concentrations as 6.98 ± 2.95 mg/ml in selected and control line of RIR chicken.

2.3.3 Influence of sex on IC traits

Kundu *et al*. (1999) reported that males tend to have higher antibody titers than females at 5th dpi in Aseel, Kadakanath, Naked Neck, White Leghorn and Naked Neck broilers whereas Frizzle, Dahlem Red and SDL broilers showed the reverse trend.

Sivaraman *et al*. (2005) reported that non-significant effect sex on any of the IC traits in SDL broiler chicken. Males demonstrated lower response to SRBC and serum lysozyme

concentration but a higher serum IgG than females.

Kumar (2006) reported higher average serum IgG concentration in males than females. Age and sex of the bird had no significant effect on serum IgG level, although males revealed higher values.

Kumar and Kumar (2011) reported non-significant influence of sex on HA titre, although males revealed higher mean antibody titre and serum lysozyme level than females.

Jaiswal *et al*. (2014) observed that sex had no influence on the HA titre, serum lysozyme and IgG concentration, although, males exhibited slightly higher HA titre and serum IgG concentration than female, However, females demonstrated higher serum lysozyme concentration (5.18 ± 0.34) than males (4.95 ± 0.33).

Kokate (2013) reported non-significant effect of sex on IC traits in Aseel, Kadaknath and White leghorn chicken. Although, males exhibited higher HA titre, serum lysozyme and IgG concentration than female in Aseel, Kadaknath and WLH chicken.

Das *et al.* (2014a) studied the immunocompetence status in CARI-Debandra chicken and reported non-significant effect of sex on HA titre, serum lysozyme level and serum IgG level although males demonstrated higher estimates of HA titre, serum lysozyme and serum IgG concentration than female birds.

Das *et al.* (2014b) reported non-significant effect of sex on all IC traits in CARI-Sonali chicken although males demonstrated higher estimates of HA titre, serum lysozyme than female birds and serum IgG concentration was somewhat equal in both sexes.

2.3.4 Inheritance pattern of various immunological traits

2.3.4.1 Heritability (h^2)

The h^2 estimate studied in various chicken populations reported by various authors for IC traits were variable and low to moderate in range

Shukla *et al.* (1996) reported that the h^2 estimate of anti-SRBC response antibody titre (log_2) at 7 dpi in White Leghorn chicks was 0.35 ± 0.16.

Sivaraman *et al.* (2005) reported that the immunological traits showed low heritability estimates except for HA titre where it was moderate (0.261±0.163) in a synthetic dam line of broiler chicken.

Singh *et al.* (2009) reported that the heritability estimates of cell mediated immune response and serum lysozyme concentration were medium (0.248±0.208) and high (0.622±0.315), associated with higher standard errors, respectively and could not estimated for HA titre and serum IgG concentration in Kadaknath native chickens.

Gupta *et al*. (2010) reported that the heritability estimates of various IC traits were very low (0.009±0.059 to 0.049±0.083) in the HSRBC line and could not be estimated in LSRBC line of White Leghorn chicken.

Singh *et al.* (2011) recorded low to moderate heritability estimates for cell mediated immune response, serum lysozyme concentration and serum IgG level were 0.292±0.194, 0.096±0.123 and 0.141±0.141, respectively, but associated with higher standard errors and could not be estimated for HA titre in Aseel native chicken.

Jaiswal *et al.* (2014) estimated low to moderate heritability for HA titre (0.146 ± 0.205) and serum IgG concentration (0.257 ± 0.228) and high for serum lysozyme concentration (0.734 ± 0.308) although associated with high standard errors in Kadaknath native chicken**.**

Das (2013) estimated the heritability for HA titre, serum lysozyme and serum IgG concentrations which were low to moderate in magnitude and the estimates were 0.105 ± 0.458, 0.214 ± 0.450 and 0.398 ± 0.589 in selected pure strain of RIR and 0.307 ± 0.508, 0.312 ± 0.430 and 0.223 ± 0.459, respectively, in random bred control strain of RIR strain.

2.3.4.2 Genetic (r_G) and phenotypic (r_P) correlations

Wide variability in genetic correlations among IC traits was reported by Kean *et al.,* (1994).

Genetic correlation (r_G) between HA titre and serum lysozyme were not estimable in high SRBC line and in low line the value was 0.05 ± 0.78 (Shivakumar, 2003). Earlier reports in broilers indicated positive and low (0.02 ± 0.58) r_G values (Sivaraman *et al.,* 2003). The standard errors of r_G between immunological traits were also very high and the r_G among

serum lysozyme and IgG concentrations did not follow any specific trend, whereas HA titre exhibited positive correlation with other immunological traits in synthetic dam line of broiler chicken (Sivaraman *et al*., 2005). The r_G between HA titre and serum lysozyme was positive but out of range in HSRBC line of IWG genotype, thus not precise (Gupta *et al.*, 2010). HA titre had highly negative r_G with lysozyme concentration, associated with high standard error in Kadaknath native chicken (Jaiswal *et al.,* 2014). Das (2013) estimated low and positive r_G with serum lysozyme in RIR^S chicken.

Genetic correlation between HA titre and serum IgG level in high and low-SRBC lines were positive and more than unity. In IWJ-high SRBC line, the r_G was not estimable but was -0.16 ± 1.65 in IWJ-low SRBC line (Gupta *et al.,* 2010). HA titre had highly positive r_G (0.911 ± 0.969) with with serum IgG level in Kadaknath native chicken (Jaiswal *et al.,* 2014) associated with high standard error. Das, (2013) reported negative and moderate r_G with serum IgG in selected pure strain of RIR chicken.

Genetic correlation (r_G) between serum lysozyme and serum IgG levels was high and positive in Aseel chicken, although higher standard error associated with these estimates made them less precise (Singh *et al.,* 2011), very low and negative r_G with serum IgG level in Kadaknath chicken (Jaiswal *et al.,* 2014). Das (2013) reported positive and moderate r_G with serum IgG, but the estimate were less precise due to high standard errors, which might be due to less number of observations.

The phenotypic correlations (r_p) among IC traits were reported generally small and negative (Kean *et al*., 1994, Sivaraman *et al.,* 2005, Singh *et al*., 2009, Kokate *et al*., 2013, Jaiswal *et al*., 2014)

Sivaraman *et al*. (2005) reported for a synthetic dam line of broiler chicken that the r_P values among immunological traits were not significantly different from zero. Similarly, Sivaraman *et al.* (2005); Jaiswal *et al*. (2014); Das *et al*. (2014a) and Das *et al*. (2014b) reported very low phenotypic correlation among IC traits. Phenotypic correlations estimated among the three IC traits (HA, serum lysozyme and serum IgG levels) were very low and not significantly different from zero in Kadaknath chickens (Singh *et al.,* 2009) and Aseel chicken (Singh *et*

al., 2011). Das *et al.* (2014b) reported phenotypic (r_p) correlations among various IC traits which showed no definite trend, which might be due to small sample size as have also been reported in RIR chicken. Singh (2005) reported phenotypic correlations among three immunological traits (HA titre, serum levels of lysozyme and IgG) as positive and low to medium in magnitude in IWG strain of white Leghorn chicken. Kumar (2006) also estimated phenotypic correlations among the three immunocompetence traits (HA titre, serum levels of lysozyme and IgG) in Aseel native chicken, which were positive and low to medium in magnitude. Das (2013) reported positive and low r_p among IC traits in selected strain of RIR and negative and low in random bred control line of RIR chicken.

The **r_P value between HA titre with serum lysozyme level** was positive but very low in magnitude (Shivakumar, 2003). Phenotypic correlation between HA titre and serum lysozyme activity was 0.166 in Kadaknath native chicken (Jaiswal *et al.*, 2014). Das *et al*. (2014a) reported negative r_p between HA titre and serum lysozyme concentration (-0.215) in CARI-Debendra chicken. The corresponding values were noted to be 0.056 in HSRBC line of WLH and 0.015 in LSRBC line of WLH (Gupta *et al.*, 2010).

The **r_P value between HA titre with serum IgG level** was positive but very low in magnitude (0.005) in CARI-Debendra chicken (Das *et al.,* 2014a). Negative and very low r_p between HA titre and IgG concentration (- 0.074) has been reported in Kadaknath chicken (Jaiswal *et al*., 2014). The value was 0.118 in HSRBC line of IWG-WLH genotype and - 0.16 in LSRBC line (Gupta *et al.*, 2010).

The **r_P value between serum lysozyme levels with serum IgG level** was positive but very high in magnitude (0.42 to 0.72) in all the S1 divergent SRBC lines of IWG genotypes (Shivakumar, 2003). Das *et al.* (2014a) reported negative r_p between serum lysozyme and serum IgG concentration (–0.117) in CARI-Debendra chicken. Sivaraman *et al.* (2005) reported that the phenotypic correlations among immunocompetence traits were not significantly different than zero. Phenotypic correlation estimated between serum IgG concentration and serum lysozyme activity was 0.099 in a random-bred Aseel chicken (Kumar, 2006). The corresponding value was - 0.061 in HSRBC line and - 0.056 in LSRBC line (Gupta *et al.*, 2010).

2.3.5 Association of immunocompetence traits with growth and layer economic traits

Egg number and egg weight in chicken were higher in high titre to SRBC line than low titre line (Van der Zijpp and Nieuwland, 1986).

Martin *et al.* (1990) observed very low phenotypic correlation of SRBC response with growth traits of chicken in lines selected for high or low antibody response to sheep RBCs. Kundu, (1997) observed no consistent trend either in magnitude or in direction of phenotypic correlations for different immunocompetence traits with growth traits.

Siegel and Gross (1980) reported positive correlations between the AFE and antibody response to SRBC in WLH chickens.

Parmentier *et al*. (1998) reported that the high immune response chicken line had significantly lower body weight at 38 week of age than control line and low line selected for antibody response to SRBCs.

Eid *et al.* (2010) reported negative phenotypic correlations between antibody titres at 7 (-0.049), 14 (-0.008) and 21 (-0.041) dpi and body weight at seven weeks of age in both broiler (ISA Hubbard and Ross 308) strains, respectively.

Yunis *et al.* (2002) assessed genetic control of antibody (Ab) response to E. coli, IBD virus, and ND virus and reported effects of high and low line of antibody (Ab) responses on body weights.

Sivaraman *et al*. (2005) studied the phenotypic correlation between body weight at 4, 5 and 6 weeks of age of both sexes and immunological traits in synthetic dam line of broiler chicken which were not significantly different from zero, except for lysozyme which exhibited positive correlation with body weight at 4 weeks of age. The body weight showed medium to high positive r_G with CMI and HA, and negative r_G with serum IgG.

Reddy *et al.* (2005) analyzed the SRBC antibody response in high, medium and low feed efficiency groups of selected (IWH) line and control population of White Leghorn chicken. All feed efficiency groups for 0 dpi, antibody response did not differ significantly in IWH line, but SRBC response was highest in high group on 7th dpi followed by medium and then low feed efficiency group. In control line, all three feed efficiency groups did not differ significantly

at 0 and 7th day.

Saini *et al.* 2007 studied the Immune response to sheep red blood cells and its relationship with other production traits in selected strain of White Leghorn. The HA titre at 5 pdi had negative phenotypic and positive genetic correlations with body weights at 12, 24, 28 and 40 weeks of age while it had positive genetic correlations with all egg production traits (Egg specific gravity at 28 and 36 weeks, egg weight at 28 and 36 weeks and egg production upto 40 weeks of age) except with age at sexual maturity. The phenotypic correlation of HA titre with all the egg production traits were negative except very low correlations with specific gravity at 28 weeks and age at sexual maturity. Genetic correlations of MER (IgG level) titre were positive only with specific gravity at 28 weeks, egg number upto 40 weeks and egg weight at 28 and 32 weeks of age.

Das *et al*. (2014a) studied the immunocompetence profile and their association with production traits in CARI-Debendra chicken. The level of HA titre had no significant effect on any of the production trait. Serum lysozyme level had significant effect on BW40 and serum IgG had significant effect on EW28. Birds with medium or low serum lysozyme level revealed higher BW40 than birds with high level. Birds with high serum IgG concentration demonstrated higher EW28 than birds with low and medium levels.

Das *et al.* (2014b) evaluated the Immunocompetence traits and their association with layer production traits in CARI-Sonali commercial layer chicken. Significant ($P \leq 0.05$) effect of HA titre and serum lysozyme levels on EW40 and AFE respectively. Birds with high/ medium HA titre levels revealed significantly ($P \leq 0.05$) more EW40 than those with low level of HA titre. Birds with medium serum lysozyme level had significantly ($P \leq 0.05$) lower AFE than high level of serum lysozyme.

Das *et al*. (2015), HA titre and serum IgG levels demonstrated its significant ($P \leq 0.05$) association with egg weights in the selected line; whereas body weights at 20th week of age of the birds of the control line had association ($P \leq 0.05$) with the birds' serum IgG levels. Least squares means of egg weights at 28th and 40th weeks of age had some influences ($P \leq 0.05$) on the birds' serum IgG levels, whereas the estimate of egg weight at only 40th week of age was

found to be influenced ($P \leq 0.05$) by the birds' HA titre level. Hens of the selected line containing high HA titre and serum IgG levels laid heavier ($P \leq 0.05$) eggs at 40th week of age than those with medium or low levels of HA titre and serum IgG. Again pullets having high and medium serum IgG levels also laid heavier ($P \leq 0.05$) eggs at 28th week of age than those with low IgG level in the selected line. But birds of the control line containing high serum IgG level had higher ($P \leq 0.05$) body weights at 20th week of age than those having medium IgG.

2.3.5 Influence of microsatellites genotypes immunocompetence traits

Chatterjee *et al*. (2008b) employed five microsatellites (ADL0020, ADL0023, ADL0102, ADL0176, and MCW0007) in six crossbred populations of White leghorn chicken selected over ten generations of selection, and reported that none of the microsatellites were significantly associated with immune response against SRBC at 4-5week of age.

Chatterjee *et al*. (2010a) also explored nine microsatellite markers to study the genetic variability and its possible relationship with growth, egg production, and immunocompetence traits in six crossbred populations of White leghorn chicken selected over ten generations of selection and reported that none of the microsatellites were significantly associated with immune response against SRBC.

2.4 Objective 4: To analyze relative expression of important immunity related genes in various tissues of Rhode Island Red chicken by quantitative reverse transcription PCR (qRT-PCR)

Recent literature related to the proposed immunity related genes viz., IL-1beta, iNOS and TLR15 and their expression patterns have been reviewed.

Chicken IL-1β, which belongs to the IL-1 super-family of cytokines, was one of the first chicken cytokines described. Chicken IL-1β mediates an inflammatory response and increases antibody production, similar to its mammalian counterpart (Leutz *et al*., 1989; Sterneck *et al.,* 1992). Expression of IL-1β gene after parasitic infestation differed between chicken inbred lines disparate for the MHC (Kim *et al*., 2008a).

Inducible nitric oxide synthase (iNOS), produced by macrophages stimulated with cytokine and/or microbial components (Bogdan, 2001; Bogdan *et al*., 2000; MacMicking *et*

al., 1997) and Blanchette *et al*., (2003) observed that the Nitric oxide plays a powerful role in immune responses because of its antimicrobial and anti-tumor functions.

Toll-like receptors (TLRs) are a group of highly conserved molecules that initiate innate immune response to pathogens by recognizing structural motifs. In response to pathogen associated molecular patterns (PAMPs). TLRs induce the production of reactive oxygen and nitrogen intermediates (ROI and RNI), inflammatory cytokines and up regulates the expression of co-stimulatory molecules, subsequently initiating the adaptive immunity. The expression of TLR15 mRNA, a novel, avian-specific TLR, was highest in bone marrow, bursa, spleen, and cecum, and expression was increased in the cecum by infection with S. enteric serovar Typhimurium (Higgs *et al.,* 2006). Expression of TLR15 varies in heterophils from different chicken lines in response to stimulation with Salmonella enteric serovar Enteritidis, but not in non stimulated cells (Nerren *et al.,* 2009).

2.4.1 Differential expression of immune response genes in different sexes

Abasht *et al*. (2008) studied expression of TLR2, TLR4 and TLR5 genes in caecum and spleen of advanced intercross line chicks infected with Salmonella enteric serovar Enteritidis. Sex significantly affected expression of all three TLRs. Male showed higher expression level than female.

Kumar *et al*. (2011) studied effect of immunomodulators and genetic line on relative expression of innate immunity genes (IL1-β, IL-2, iNOS, TLR4 and TLR15) in spleen cells of broiler, layer and Fayoumi line. The expression of IL1-β was significantly affected by sex. Males exhibited higher IL1-β expression than females.

Kokate (2013) reported non-significant ($P>0.05$) effect of sex on mRNA expression of IL1-β, IFN-γ, iNOS gene in bursa and spleen tissue of Aseel, Kadaknath, and White leghorn chicken.

2.4.2 Differential expression of immune response genes in different tissues

Dil and Qureshi (2003) found that expression of the iNOS gene in chickens varies with genetic background.

Sundaresan *et al.* (2005) reported altered expression of iNOS gene in monocyte cell

culture after induction with Newcastle Disease virus.

Ahmed *et al.* (2007) postulated that the expression of immune related genes like IFN-γ and iNOS are under genetic control.

Kannaki *et al.* (2011) studied the expression profiling in Aseel, Kadaknath, Naked neck, Dwarf and WLH chicken breeds and reported that Aseel chicken expressed more TLR15 in liver, spleen, bone marrow and bursa than other breeds.

Kumar *et al*. (2011) studied effect of immunomodulators and genetic lines on relative expression of innate immunity genes in spleen cells of broiler, layer and Fayoumi line. The genes studied were IL1-β, IL-2, iNOS, TLR4 and TLR15. The mRNA expression did not differ significantly among diets, genetic lines for any genes studied. But, the expression of IL1-β was significantly affected by sex. Males exhibited higher IL1-β expression than females.

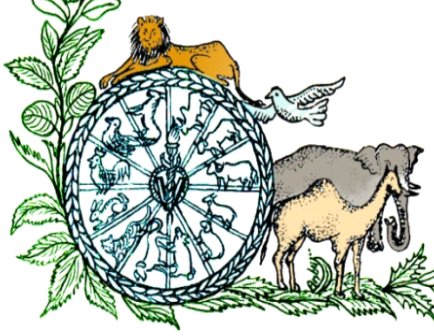
Patel *et al*. (2013) studied constitutive expression levels of TLR3, TLR4, TLR15 and TLR21 genes in PBMCs of native Indian poultry breeds, Aseel and Kadaknath. Expression of TLR3 (14.77 fold), TLR15 (7.18 fold) and TLR21 (3.50 fold) genes were significantly higher in Kadaknath than Aseel

Kokate (2013) studied the relative expression of four immunity related genes viz., IL-1beta, IFN- γ, iNOS and TLR-15 and reported that in bursa tissue, neither genotype nor sex had significant effect ($P > 0.05$) on the expression of these genes. The mRNA expression of iNOS gene in spleen tissue was highest in Kadaknath chicken followed by WLH and Aseel chicken. The mRNA expression of all the four genes varied significantly ($P \leq 0.01$) among tissues in Aseel, Kadaknath and white Leghorn chicken. The mRNA expression of IL1-β and TLR15 were highest in spleen whereas IFN-γ and iNOS were highest in thymus tissue of all the breeds studied. In all the three genotypes, the significant expression differences among genes were observed ($P \leq 0.01$) in spleen and thymus tissues. In all three genotypes, IFN-γ gene was more expressed in spleen and thymus tissue than any other immunity related genes in the study.

Jie *et al*. (2013) studied differential expression of TLR3, TLR5, TLR7, TLR15 and TLR21 genes in bursa, spleen and thymus tissues between MDV infected and non-infected

chickens. TLR3 and TLR15 genes were upregulated in bursa at 7 and 4 dpi, respectively. TLR3 and TLR15 genes were downregulated in infected spleen at 28 dpi. No differential expression of TLRs was observed between MDV infected and non-infected thymus. Upregulation of TLR3 and TLR15 in MDV infected bursa might be due to involvement of these TLRs in bursa in recognition of MD virus in early stage of infection. The down regulation of these same genes in spleen might be due to inhibition of expression of TLRs by tumor transformation phase. Thymus did not respond to MDV as much as the spleen and bursa.

Materials and Methods



3 MATERIALS AND METHODS

In the present investigation, 325 chicks of selected strain of Rhode Island Red (RIR^s) chicken, maintained at Experimental Layer Farm, Central Avian Research Institute, Izatnagar, Bareilly (India) were evaluated to assess the effects of various genetic and non-genetic factors on layer economic traits, growth traits and immunocompetence (IC) traits, viz., immune response to sheep RBCs, serum lysozyme levels and IgG concentration, and to determine inter-relationship and association amongst IC, growth and layer economic traits. It was also envisaged to assess genetic variability at ten egg production - associated microsatellite (MS) markers and determine the association of various MS genotypes with immunocompetence, growth and layer economic traits. It was also envisaged to estimate basal level expression profiles of three important immune response genes, viz., IL1-β, iNOS and TLR15 in three vital lymphoid tissues, thymus, spleen and bursa, determine sex or tissue differences and also to assess the influence of levels of various IC traits on relative gene expression of these genes. The details of materials used and methodologies employed including various statistical analyses are presented below in objective-wise manner:-

3.1 Objective 1: To analyze effects of various genetic and non-genetic factors on layer economic traits in Rhode Island Red chicken

3.1.1 Experimental population

A pure strain of Rhode Island Red (RIR), selected for part period egg production up to 40 weeks and being maintained as closed flock at Experimental Layer Farm, Central Avian Research Institute, Izatnagar, Bareilly (U.P.) India was used as experimental birds for this work. All the experimental birds were kept in similar environmental conditions and raised under similar management and nutritional conditions.

3.1.2 Brief history of experimental Stock

The Rhode Island Red (RIR) chicken is recognized as dual purpose, brown-egger chicken breed and considered suitable for development of multicolored strains for rural poultry production. The Central Avian Research Institute, Izatnagar (India) had imported about 1400 fertile eggs of Rhode Island Red chicken in 1980 from the USA, hatched out at institute's hatchery and maintained at the Institute's farm. The population was acclimatized and then subjected to selection on the basis of part-record egg production i.e. egg production up to 40 weeks of age, to bring genetic improvement in egg production over last 35 years covering 30 generations of selection and is being maintained as selected strain along with a random-bred control strain. The method of selection was combined family index in which the weighing factors for various components entering into the index were derived as per Osborne (1957a, 1957b). The combination index values for each individual pullet was computed by assigning appropriate weightage to individual's own egg production, average egg production of its full- and half-sisters. The cockerels were selected on the basis of combination of the average egg production of their full- and half-sisters. The main emphasis in selection was laid on part-year egg production (up to 40 weeks of age). In addition to egg production some independent culling levels were also practiced for egg weight (individual basis) and viability (family basis) in selection. For some generations, selection for fertility and hatchability based on independent culling levels (individual and dam family basis) for hatchability of total egg set was also incorporated.

3.1.3 Formulation of mating plan and regeneration of stock

Twenty five males, selected at random and 125 females, selected on the basis of part-period egg production, were used as parents. The mating plan involving selected parents was so made so as to assign 5 dams to one sire avoiding mating between full- and half-sibs, and mated through artificial insemination. Semen was collected from the each selected cock by gentle massaging at the back and groin region of the bird. The semen collected in a sterile funnel was diluted two times with normal saline. Then, 0.2 ml of diluted semen was inseminated into selected pullets using tuberculin syringe in individual laying cages after 40 weeks of age.

3.1.4 Incubation and hatching

Eggs were collected twice (morning and evening) in a day and marked properly just after collection for their pedigree record and then stored in cold chamber till setting. Eggs were collected for 10 days. Cracked and grossly abnormal eggs were discarded. Fumigation of eggs was done prior to setting. A total 568 eggs were collected and set sire-wise in the Automatic Force draft Incubator (Mfd. by Dayal Poultry Appliances, New Delhi, India) at Hatchery Unit CARI, Izatnagar. The fertility was checked by candling of eggs on 18th day of incubation and 416 fertile eggs were transferred to Hatcher in pedigree trays for hatching of chicks. On 21nd day, a total of 325 chicks were obtained in single-hatch.

3.1.5 Management practice and feeding

All chicks were wing-banded at hatchery itself for pedigree recording and dubbed (beak trimming) to prevent fight, and kept in clean electrically heated battery brooders up to four weeks with uniform and standard management practices with provision of feeder and waterer.

Electric bulb (60W and 100W) and having adjustable hover fitted with single infrared lamp (250W) was used for maintaining the shed temperature during brooding period. At four weeks of age, chicks were shifted to a new litter brooder house and kept there up to 16 weeks. Sexes were separated at 12 weeks of age. One hundred thirty-one pullets were shifted to individual laying cages and kept up to 64 weeks of age. One hundred seventy males were also kept into individual cages in the same house up to 64 weeks of age for recording of various growth traits. Floor space, lights and brooding temperature were provided to the birds as per the standard requirement. As far as the light regime is concerned, chicks were initially given continuous light for 24 h in for first 3 weeks. Then lights were reduced @ 2 hour per week till 8 weeks so as to provide light for about 14 hours and thereafter maintained throughout its growing and laying stage. *Ad libitum* Fresh water and feed were provided in the morning and evening daily. Chicks were fed on CARI-formulated chick mash with CP-20.65%, ME-2694.64 Kcal/Kg, Calcium-1.02%, Available Phosphorous-0.45%, Lysine-1.05% and Methionine-0.41% for 0-8 weeks of age, then on grower mash with CP-16.78%, ME-2536.00 Kcal/Kg, Cal-1.15%, P-0.40%, Lys-0.76% and Met-0.37% for 9-20 weeks of

age and then on layer ration with CP-18.18%, ME-2676.52 Kcal/Kg, Cal-3.61%, P-0.34%, Lys-0.83% and Met-0.36% for 20-64 weeks of age. Mortality and health were monitored regularly. Dead chicks were removed at its first notice. Proper health care was taken as per the standard health care procedures being followed at the Institute.

3.1.6 Vaccination schedule

Birds were vaccinated by Avian Medicine Section, of the Institute as per the vaccination schedule being followed at other farms, which are presented below:-

Table 3.1.1: Vaccination schedule for chicks, growers and layers

Age	Vaccine	Dose and Route
1 day	RD	Intra nasal
1 day	MD	Swab scratch
14 day	IBD	Intra nasal and Intra ocular, one drop each
28 Day	RD Booster	Intra nasal and Intra ocular, one drop each
35 Day	IBD Booster	Intra nasal and Intra ocular, one drop each
42 Day	Fowl Pox	Wing Prick
56 Day	RD (R_2B)	0.5 ml, intra muscular
18-19 weeks	EDS	0.5 ml, intra muscular
20-22 weeks	IBD (Killed)	0.5 ml, intra muscular

3.1.7 Traits recorded

The performance traits were sub-grouped into percent fertility and hatchability, growth performance traits, layer production traits, and percent mortality. The data were recorded at specified time points.

3.1.7.1 Percent fertility and hatchability

Percent fertility and percent hatchability was calculated on total egg set (TES) basis and fertile egg set (FES) basis as per the following formulae:

$$\textbf{\% Fertility} = \frac{\text{No. of fertile eggs}}{\text{Total no. of egg set}} \times 100$$

$$\textbf{\% Hatchability (TES)} = \frac{\text{No. of chicks hatched}}{\text{Total no. of egg set}} \times 100$$

$$\textbf{\% Hatchability (FES)} = \frac{\text{No. of chicks hatched}}{\text{Total no. of fertile egg set}} \times 100$$

3.1.7.2 Evaluation of growth performance traits

Body weights (BW) of experimental birds were recorded in grams at 16 (BW16), 20 (BW20), 40 (BW40) and 64 (BW64) weeks of age using electronic top pan digital weighing balances having capacities of 0.5g to 30 kg to the nearest of 10 g accuracy during morning hour before feeding.

3.1.7.3 Evaluation of layer economic traits

Following layer economic traits were recorded on individual basis as per standard procedure:

i. Age at first egg (AFE)

Age at sexual maturity for individual pullet was calculated as the number of days taken from hatching to the laying of first normal egg irrespective of its subsequent pattern of laying.

ii. Egg weights

Egg weight of each pullet was recorded as average of the three eggs laid on three consecutive days for each pullet at 28 (EW28), 40 (EW40) and 64 (EW64) weeks of age using electronic digital balance.

iii. Part period egg production up to 40 weeks of age

Egg production of each pullet was recorded daily up to 40 weeks of age (EP40) in individual laying cages.

iv. Annual egg production up to 64 weeks of age

Egg production of each pullet was recorded daily up to 64 (EP64) weeks of age in individual laying cages.

3.1.8 Percent mortality

Brooder, litter houses and laying cages were routinely checked on each morning for recording mortality of the previous day followed by its wing band number recording and post mortem examination. Mortality was expressed in percentage during 0-4 weeks, 5-8 weeks, 9-16 weeks, 17-40 weeks, 41-64 weeks and 0-64 weeks of age. Mortality % was calculated using following formula:

$$\textbf{\% Mortality} = \frac{\text{Total no. of mortality}}{\text{Total no. of chicks placed}} \times 100$$

3.1.9 Statistical analysis

The data generated on growth traits were subjected to least squares analysis of variance (Harvey 1990) by incorporating sire as random, sex as fixed effect in the statistical model:

$$\mathbf{Y_{ijk} = \mu + S_i + H_j + e_{ijk}}$$

Where,

Y_{ijk} = value of a trait measured on ijk^{th} individual

μ = Population mean,

S_i = Random effect of i^{th} sire

H_j = fixed effect of j^{th} sex

e_{ijk} = Random error associated with mean zero and variance $\sigma^2 e$.

Data generated on layer economic traits were also subjected to least squares analysis of variance (Harvey 1990) by incorporating sire as random effect in the statistical model presented below:

$$\mathbf{Y_{ij} = \mu + S_i + e_{ij}}$$

Where,

Y_{ij} = value of a trait measured on ij^{th} individual

μ = Population mean,

S_i = Random effect of i^{th} sire

e_{ij} = Random error associated with mean zero and variance $\sigma^2 e$.

Genetic and phenotypic parameters of growth and layer performance traits were estimated using paternal half-sib correlation method (Becker, 1975).

3.2 Objective 2: To determine allelic polymorphism at egg production – associated microsatellite loci in Rhode Island Red chicken and their association with layer economic traits.

3.2.1 Experimental birds

Seventy-six pullets of Rhode Island Red (RIR) chicken chosen more than four pullets per sire family as mentioned in **objective no. 1,** were used for microsatellite studies.

3.2.2 Collection and storage of blood

Approximately 0.5-1 ml of venous blood was collected from each of the experimental birds from jugular vein in a sterile 1.5 ml heparinized (20 IU/ml) centrifuge tube. After collection of blood, the tubes were tightly capped and mixed gently to facilitate thorough mixing of blood with the anticoagulant. All the blood samples were properly labeled and then kept immediately in icebox/ gel cool packs and were transported immediately to the laboratory. The samples were stored at -20°C till further use.

3.2.3 Isolation of Genomic DNA from blood

Genomic DNA was isolated from the venous blood samples by Phenol: Chloroform extraction method as described by Kagami *et al.* (1990) with slight modifications. The steps followed for the genomic DNA isolation were as follows:

i. The blood samples were taken out of -20°C and thawed by incubating in a water bath set at 37°C for 10 minutes.

ii. Approximately 100 µl of thawed/ fresh blood was transferred into 2.0 ml centrifuge tubes and 1.0 ml of PBS was added into it. The tubes were mixed gently upside down and centrifuged at 3,000 rpm for 5 minutes. The supernatant was discarded. This step was repeated again.

iii. One ml of lysis buffer (*Refer Annexure for composition*) was added into tube containing red blood cells pellet and mixed gently and kept at room temperature for 15-20 minute.

iv. Then 5.0 µl of proteinase K (final concentration: 200 µg/ml) and 50 µl of 10% SDS (final concentration: 0.5%) was added into the tube containing lysis buffer and mixed thoroughly by vortexing. The lysate was then incubated for overnight in a water bath at 37ºC.

v. In this mixture, equal volume of Tris-saturated phenol (*Refer Annexure for composition*) was added and mixed gently for 10 minutes and then centrifuged at 5,000 rpm for 5 minutes.

vi. The upper aqueous phase containing DNA was collected and transferred into a fresh 1.5 ml centrifuge tube without disturbing the interface which contained protein. This step was repeated once again.

vii. The aqueous phase was then extracted with equal volume of phenol: chloroform: isoamyl alcohol (*Refer Annexure for composition*) mixture. After gentle mixing, the contents were centrifuged at 5,000 rpm for 5 minutes and upper aqueous phase was carefully transferred into a fresh 1.5 ml centrifuge tube. This step was repeated once again.

viii. Lastly, the aqueous phase was extracted twice with equal volume of chloroform: isoamyl alcohol (*Refer Annexure for composition*) mixture after gentle mixing and centrifuged at 5,000 rpm for 5 minutes.

ix. Finally, the aqueous phase was collected in a fresh 1.5 ml centrifuge tube and added with 2-2.5 volume of chilled absolute alcohol and 25 µl of sodium chloride (2M) followed by gentle mixing and then it was kept at -20ºC until complete precipitation of DNA for further use.

3.2.4 Dissolution of precipitated DNA

i. Precipitated DNA samples were subjected to centrifugation at 10,000 rpm for 10 minutes and supernatant was discarded without disturbing the DNA pellet.

ii. The DNA pellets were subjected to washing twice with 70% ethanol by adding about 500 µl of 70% ethanol, gentle mixing and centrifugation at 10,000 rpm for 10 minutes. Then, the DNA pellet was air-dried for one hour to remove traces of ethanol and subsequently dissolved in 200µl nuclease-free water.

iii. The tubes containing DNA were kept in water bath at 60ºC for one hour to dissolve pelleted DNA properly in distilled water and to inhibit DNase activity, if any.

iv. Finally, the DNA solution was cooled and stored at -20ºC until further use. For long-term storage, the DNA was re-precipitated and stored in absolute alcohol at -20ºC.

3.2.5 Quantitation and purity determination of genomic DNA

Concentration and purity of genomic DNA was checked by spectrophotometer using NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies Inc., U.S.A.). Concentration of DNA was directly recorded in the unit of nanogram per microlitre with the help of absorbance at 260nm. The samples showing absorbance ratio between 1.7 and 1.9 (260/280)) were considered as satisfactory for their purity and used in subsequent experiments.

If A260/280 = 1.7 -1.9 then the DNA samples were considered as of satisfactory purity.

If A260/A280 < 1.7 then the DNA samples were considered to be contaminated with proteins and needs re-extraction.

If A260/A280 > 1.9 then the DNA samples were considered to be contaminated with RNA and needs RNase-treatment

The concentration of genomic DNA was calculated by O.D. method. Amount of DNA was calculated as:

DNA concentration (µg/ ml) = O.D.260 × 50 × dilution factor (1 O.D. value at 260 nm wavelength is equivalent to 50 µg dsDNA/ ml).

3.2.6 Quality assessment of genomic DNA

The Quality of extracted genomic DNA was assessed through 0.7% horizontal submarine Agarose gel electrophoresis as mentioned below-

i. The gel casting plate was sealed with adhesive tape and placed on a leveled table surface.

ii. The comb was properly set in the casting plate.

iii. Agarose (0.7% w/v) was boiled in 1X TBE buffer (*Refer Annexure for composition*). After boiling it was cooled to 55ºC and ethidium bromide (0.5µg/ml) was added. The gel was gently poured into the casting tray avoiding bubble formation and was allowed to solidify at room temperature.

iv. After solidification, the comb and adhesive tape were removed.

v. The gel casting tray was submerged in gel tank of electrophoresis unit having 1 X TBE buffer.

vi. DNA samples for loading were prepared by mixing 3µl of genomic DNA, 7µl of D.W. and 2µl of 6X Bromo Phenol Blue loading dye. Samples were carefully loaded in the wells.

vii. Electrophoresis was performed at 2-5 volts/cm for one hour and then gel was visualized under UV transilluminator.

The genomic DNA samples having good quality (Intact bands without smearing) were used in further experiments

3.2.7 Polymerase Chain Reaction (PCR) Assay

3.2.7.1 Preparation of PCR ready template DNA

The DNA concentrations of the isolated DNA samples were determined and PCR ready working solutions were prepared from the stock solution by diluting them to the final concentration of 50 ng/µl using $N_1V_1 = N_2V_2$. Where, N_1 = concentration of stock DNA sample, N_2 = concentration of PCR ready DNA sample (50 ng/µl), V_1 = volume of available stock DNA to be taken (in µl) and V_2 = volume of required DNA sample (50 µl).

Nuclease-free water was used for this purpose. PCR Ready DNA samples were mixed gently and stored at -20ºC and used within a month. After that, fresh PCR ready DNA samples were prepared.

3.2.7 Microsatellite primers

A total of 10 informative microsatellite markers having known association with egg production traits in various chicken breed were identified from database as reported by Chatterjee *et al* (2008a, b); Chatterjee *et al* (2010a) and Arya (2012). Forward and reverse primers were got synthesized from M/S Xcelris Genomics Labs Ltd., Ahmedabad (India) and screened for their use in the present study. The nucleotide sequences of the primers and corresponding optimized annealing temperature used in present study are given in **table 3.2.1.**

Table 3.2.1: **Details of the microsatellite primers sequences and their annealing temperature**

MS loci		Primer sequence	Ta (°C)	References
ADL0020	**F**	5-GCA CTC AAA AGA AAA CAA T-3	55°C	Chatterjee *et al*., 2008a
	R	5-TAG ATA AAA ATC CTT CCC TT-3		
ADL0023	**F**	5-CTT CTA TCC TGG GCT TCT GA-3	61°C	Chatterjee *et al*., 2008a
	R	5-CCT GGC TGT GTA TGT GTT GC-3		
ADL0102	**F**	5-TTC CAC CTT TCT TTT TTA TT-3	48°C	Chatterjee *et al*., 2008a
	R	5-GCT CCA CTC CCT TCT AAC CC-3		
ADL0176	**F**	5-TTG TGG ATT CTG GTG GTA GC-3	55°C	Chatterjee *et al.*, 2008a
	R	5-TTC TCC CGT AAC ACT CGT CA-3		
ADL0210	**F**	5-ACA GGA GGA TAG TCA CAC AT-3	52°C	Chatterjee *et al.*, 2010a
	R	5-GCC AAA AAG ATG AAT GAG TA-3		
MCW0007	**F**	5-AGC AAA GAA GTG TTC TCT GTT CAT-3	62°C	Chatterjee *et al.*, 2008a
	R	5-ACC CTG CAA ACT GGA AGG GTC TCA-3		
MCW0014	**F**	5-AAA ATA TTG GCT CTA GGA ACT GTC-3	60°C	Chatterjee *et al.*, 2010a
	R	5-ACC GGA AAT GAA GGT AAG ACT AGG C-3		
MCW0041	**F**	5-CCC ATG TGC TTG AAT AAC TTG GG-3	57°C	Chatterjee *et al.*, 2010a
	R	5-CCA GAT TCT CAA TAA CAA TGG CAG-3		
MCW0069	**F**	5-GCACTCGAGAAAACTTCCTGCG-3	55°C	Arya, 2012
	R	5-ATT GCT TCA GCA AGCA TGG GA GGA-3		
MCW0103	**F**	5-AAC TGC GTT GAG AGT GAA TGC-3	55°C	Arya, 2012
	R	5-TTT CCT AAC TGG ATG CTT CTG-3		

The synthesized primer pairs were received from the supplier in lyophilized form and were reconstituted with nuclease-free water as per manufacturer's instructions. A stock of 100 picomoles /µl was futher diluted in nuclease-free water and from this working primer solution of final concentration of 10.0 picomoles/ µl was prepared and used in PCR.

3.2.8 Reaction mixture

The reaction mixture was prepared in a 200 µl nuclease free PCR tubes (Axygen Scientific, Inc. USA) in a final volume of 25 µl in thermal cycler (PTC-200 DNA Engine® thermal cycler, Bio-Rad, USA). Quantity and concentration of various components used in PCR were as follows:-

5X GoTaq® Flexi buffer (Promega, Madison WI, U.S.A)	5.0 µl	
25 mM $MgCl_2$ (Promega, Madison WI, U.S.A)	1.5 µl*	
10 mM dNTP mix (Thermo Fisher Scientific Inc., U.S.A.)	0.5 µl	
10 pM forward primer (M/S Xcelris Genomics Labs Ltd.)	1.0 µl	
10 pM reverse primer (M/S Xcelris Genomics Labs Ltd.)	1.0 µl	
GoTaq® DNA polymerase (5U/µl) (Promega, Madison, U.S.A)	0.15 µl	(0.75U)
Nuclease free water	13.85 µl**	
Template DNA (50 ng/µl)	1.0 µl	
Total volume	**25.0 µl**	

**3 µl of 25 mM $MgCl_2$ and **12.35 NFW for ADL0020 MS Loci.*

3.2.8.1 Setting up of PCR reaction

One µl genomic DNA (50ng) was taken in 0.2ml labelled thin-walled PCR tubes and kept on ice. Subsequently, a master mix was prepared for the required number of reactions. The reaction components for the master mix were added in the following order:

Autoclaved triple distilled water
↓
5 X PCR assay buffer
↓
Forward and reverse primers
↓
dNTPs mix
↓
Finally, Taq DNA polymerase enzyme

All the above procedures were carried out on ice at 4ºC. Master mix was mixed properly by vortexing followed by spinning. Finally, 24.0µl of master mix was added to each PCR tube, containing 1.0µl of genomic DNA, followed by gentle mixing and spinning at 3000 rpm for 5-10 seconds to get the reactants at the bottom.

3.2.9 Microsatellite profiling

PCR amplification of egg production associated microsatellite loci were carried out using programmable thermal cycler (PTC-200, M. J. Research Inc., U.S.A.). All reactions were performed in 0.2 ml clear, thin walled PCR tube with clear flat caps (Axygen Scientific Inc., U.S.A).

3.2.10 PCR amplification programme

PCR cycling conditions were optimized for all the 10 microsatellite loci and are mentioned below:-

i. Initial denaturation at 95°C for 5 minutes

ii. 30 cycles of

a. Denaturation at 94°C for 1 minute,

b. Annealing (optimized for each microsatellite primer pair as mentioned above)

c. Extension at 72°C for 30 seconds.

iii. Final extension at 72°C for 5 minutes.

3.2.11 Checking of PCR products

The PCR products were stored at -20ºC until resolved on 2% agarose gel by horizontal gel electrophoresis (Bangalore Genei, India) for confirming their satisfactory amplification.

3.2.12 MetaPhor agarose gel electrophoresis (MAGE) of microsatellites alleles

Having confirmed the successful amplification of all the samples, the amplicons along with molecular size marker were run on 3.4% MetaPhor agarose gel electrophoresis to resolve microsatellite alleles for further genotyping as per the published procedure (Asif *et al*., 2008, Jagadeesan, 2015).

3.2.12.1 Resolution and documentation of microsatellite alleles

The amplified products of microsatellite markers were resolved on 3.4% MetaPhor agarose gel to resolve microsatellite alleles for genotyping purpose and the procedure used has been described below:

i. Initially, choose a 500 ml volume clean Erlenmeyer conical beaker (2-4 times the volume of the solution) added with 120 ml of chilled 1X TBE buffer and a magnetic stirrer bar.

ii. Slowly sprinkle the pre-measured 4.08 g of high resolution MetaPhor Agarose powder, while stirring the 1X TBE buffer in the conical flask, placed on the magnetic stirrer.

iii. MetaPhor suspension was continuously stirred for about 20-30 minutes to avoid formation of foam during boiling. To prevent the spillage the flask's mouth was covered with aluminium foil.

iv. Magnetic bar was removed and the flask was covered with aluminium foil. A few holes were made on the aluminium foil for ventilation before boiling. The MetaPhor suspension was boiled in microwave oven for about 2-3 minutes till it became a transparent solution.

v. After complete boiling, a few ml of boiling water was added to make up the initial volume of 120 ml by compensating evaporation losses, if any.

vi. MetaPhor solution was mixed thoroughly and kept at room temperature for 10-15 minutes to bring down the solution temperature to 50-60^{o}C.

vii. Ethidium bromide @0.3µg/ml was added and the solution was mixed thoroughly just prior to casting.

viii. Gel tray with comb was kept on flat surface and the MetaPhor solution was poured on the tray and allowed for solidification. The gel was then kept at 4^{o}C for 20-30 minutes to obtain optimal resolution and gel handling characteristics.

3.2.12.2 Resolving of microsatellite alleles in MetaPhor gel

The Solidified mataphor gel was submerged in horizontal agarose gel electrophoresis tank (Bangalore Genei, India) filled with chilled 1X TBE buffer on flat surface. Then, 8 µl PCR products per well were loaded carefully. In parallel, 20 bp and/or GeneRuler low range DNA ladder (Thermo scientific, U.S.A) was also loaded on the marker lane. Electrophoresis was carried out @ 6-8V/cm for 2 hour 30 minutes. The MetaPhor gel was visualized in Gel-doc system (Bio-Rad Laboratories Inc., U.S.A.) and microsatellite allelic patterns were photographed for further genotyping.

3.2.13 Determination of molecular sizes of alleles and recording of microsatellite alleles and genotypes

Quantity One® software (Bio-Rad Laboratories Inc., U.S.A.) through Gel Doc system was used to determine the molecular sizes (in bp) of all the alleles at ten studied microsatellites loci. Genotypes of all the birds were determined based on the presence of microsatellite alleles.

3.2.14 Statistical analysis of population genetics data

Data on genotypes of all experimental birds at ten microsatellites were compiled and analyzed using POPGENE® software (Yeh *et al*., 1999) for their population genetics parameters. The primary genotype data was subjected to co-dominant marker diploid data analysis to estimate observed and expected genotypic frequencies, Hardy-Weinberg (HW) equilibrium status, allele frequency, observed and effective number of alleles, percentage of polymorphic loci, observed and expected and heterozygosity and Shannon index.

3.2.14.1 Genetic Variability analysis

Average heterozygosity per microsatellite marker was calculated according to Nei (1978) using fallowing formula

$$H_i = \frac{2N}{2N-1}\left(1-\sum_{j=1}^{k} P_j^2\right)$$

Where P_j is the frequency of the j^{th} allele at i^{th} locus with k number of alleles in a population and N is the total number of individuals, assuming that the population was under Hardy-Weinberg equilibrium.

Polymorphic Information Content (PIC) at each microsatellite locus was calculated using following formula as described by Botstein *et al.* (1980):

$$\mathbf{PIC} = 1-\left(\sum_{i=1}^{n} P_i^2\right)-\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} 2P_i^2P_j^2$$

Where,

P_i and P_j are the frequencies of i^{th} and j^{th} alleles, respectively with n numbers of alleles in a population.

3.2.14 Association between microsatellites genotypes with layer economic traits

3.2.14.1 Samples Size

Data on the 76 birds studied in objectives **no. 1** and **2** were utilized for association studies

3.2.14.2 Microsatellite allele based Genotyping

Each individual pullet was genotyped with respect to each of the ten microsatellite loci using allelic data as mentioned above.

3.2.14.3 Statistical analysis

Performance data recorded on experimental birds under objectives no. **1** and **2** was analyzed for assessing association of microsatellites alleles with growth and layer economic were analyzed by least squares analysis of variance (LS ANOVA) incorporating sire as random and microsatellite genotype as fixed effects in the model using JMP 9.0.0 statistical program package (SAS, 2010). The analysis was done microsatellite-wise for all the ten microsatellites, as they were independent.

$$\mathbf{Y_{ijk} = \mu + S_i + M_j + e_{ijk}}$$

Where,

$Y_{ijk=}$ Observation at j^{th} MS genotype measured on ijk^{th} individual,

μ= Overall mean,

$S_{i=}$ Random effect of i^{th} sire,

$M_{j=}$ Fixed effect of j^{th} genotype of a particular microsatellite marker and

$e_{ijk=}$ Random error associated with mean zero and variance σ^2.

Critical difference (CD) test at 5% level of probability of significance was performed for assessing critical differences among the least squares means of microsatellite traits in different genotypes groups.

3.3 Objective 3: To estimate immunocompetence traits and their association with layer economic traits in Rhode Island Red chicken

3.3.1 Experimental animals

3.3.1.1 Birds

Three hundred and one Rhode Island Red chicks of combined sex as mentioned in **objective no.1** above were used for immunocompetence studies

3.3.1.2 Sheep

Healthy Muzaffarnagari breed of sheep, maintained at Experimental Nutrition Shed, Indian Veterinary Research Institute, Izatnagar, were used for collection of blood to prepare SRBC suspension, which was subsequently used in humoral immune response study.

3.3.2 Assaying of immunocompetence traits

The immunocompetence status of experimental birds was assessed by analyzing various components of immune system *viz.,* humoral immune response to sheep red blood cells (SRBC), serum lysozyme concentration and serum IgG concentration. The detailed methodology is presented below.

3.3.2.1 Humoral immune response to SRBC

The immune response to SRBC was assessed by Haemagglutination (HA) test as per Van der Zijpp and Leenstra (1980) as mentioned below:-

3.3.2.1.1 Preparation of sheep RBCs antigen

1. Approximately 10-15 ml of venous blood was collected from jugular vein of healthy sheep in a sterile heparinized (20 IU/ml) test tube.
2. Tube was tightly capped and mixed gently to facilitate thorough mixing of blood with the anticoagulant.
3. It was centrifuged at 1500 rpm for 10 minutes at 4ºC to settle down the RBCs.
4. The RBCs were then washed thrice with PBS (*Refer Annexure for composition*) by mixing and centrifuging to remove other serum components.

5. Finally, 1% (v/v) sheep RBCs suspension was prepared by mixing 1 ml of packed sheep RBCs and 99 ml of PBS, which was then used for injection in the experimental birds as an antigen. The suspension was stored at 4ºC.

3.3.2.1.2 Immunization of birds with Sheep RBCs

One ml of 1% sheep RBC suspension was injected into the jugular vein of each bird with tuberculin syringe. Jugular vein is the choice of injection as it leads to minimum bleeding in comparison to other veins like brachial vein etc.

3.3.2.1.3. Harvesting of immune sera

1. Approximately 1.5 ml of venous blood was collected from each birds in sterilized 2 ml centrifuge tubes on 5th day post immunization (5 dpi) and allowed to clot for 2 - 3 hours at 37 ºC.
2. The hyperimmune sera oozed out of the clot else the clot was broken
3. Sera samples were collected in 0.5 ml sterile tubes.
4. Samples in which the volume of serum was insufficient, the clots were broken gently and tubes were centrifuged at 1500 rpm for 1 - 2 min. to separate and collect serum.
5. Sera samples were stored at - 20ºC till further analysis

3.3.2.1.4. Estimation of antibody titre against sheep RBCs

The antibody response against Sheep RBCs was determined by using haemagglutination test (HA) as per Van der Zijpp and Leenstra (1980). The following steps were fallowed.

1. The HA test was performed in a fresh U shaped round bottom microtitre plates.
2. Firstly 50µl of the PBS was distributed in each well with the help of multichannel pippets.
3. Than 50 µl of serum was added in the first well of each row except the last row where 50 µl of PBS was added, which acted as control.
4. After thorough mixing, the sera were two-fold serially diluted by taking 50µl from first well of each row and transferring it in to next well, mixed gently but throughly. This process was continued till last column from where 50µl solution was discarded.

5. Equal volume i.e. 50µl of 1% SRBC suspension was then added in all the wells followed by thorough mixing by rocking plates on table surface.
6. The plates were covered with almunium foil and then incubated at 37ºC for 1 hr in a humid chamber.
7. The plates were read under bright light
8. The number of well with highest dilution (n), which demonstrated complete agglutination (button shaped clumping of RBCs indicating haemagglutination reaction), was recorded as titre and expressed as log2 n.

3.3.2.2. Estimation of serum lysozyme concentration

The serum lysozyme concentration was estimated using Lysoplate assay (Lie *et al*., 1986) method as described below:

3.3.2.2.1 Reagents and chemicals required

- Dibasic buffer 0.066 M, pH 6.3 (composition given in Appendix I)
- *Micrococcus lysodiekticus*
- Standard Lysozyme
- Agarose
- Agar

Procedure

1. The lysozyme standards were prepared by dissolving 2 mg of standard lysozyme (Genei®) in 1 ml of dibasic buffer.
2. Serial dilutions were prepared so as to get the final concentration of lysozyme as 40µg / ml, 20µg / ml, 10µg / ml, 5.0µg / ml and 2.5 µg / ml..
3. The agar lysoplate was set up on a perfect horizontal surface. The glass plate was cleaned and sterilized with spirit and air-dried. The size of the gel was determined on the basis of number of samples to be analyzed. The borders were prepared by placing glass strips on the edges of required area. All the four sides of the borders were sealed with 2% agar.

4. Volume for 0.5 cm thick gel was calculated by following formula: Volume (ml) = Length (cm) X Width (cm) X 0.5 cm
5. About 50 ml of 1% Agarose in dibasic buffer was sufficient for 10 X 10 cm^2 plate.
6. After boiling the Agarose in dibasic buffer it was cooled to 60ºC and the pre diluted *Micrococcus lysodieketicus* (Sigma®, USA) (50µg per ml of dibasic buffer), was added into it and mixed well.
7. Then the whole content was poured onto the bordered and sealed glass plate, allowed to spread uniformly and was left at room temperature for polymerization.
8. After polymerization of gel, the wells were punched at a distance of approximately 1.5 cm with the help of a gel punch.
9. Ten µl of serum sample was loaded in each well.
10. Lysozyme standards were also loaded in the wells at one side (4 - 5 dilutions).
11. The plate was incubated at 37ºC in humidity-controlled chamber for 24 h.

3.3.2.2.2 Staining and Destaining

- Plates were stained with 0.2% Coomassie Brilliant Blue (*Refer Annexure for composition*) for 6 h and excess stain was removed with destaining solution.
- The diameters of the lysed zone around standard as well as unknown samples were measured with the help of Digital Vernier Calipers.
- The plates were photograph for documentation

3.3.2.2.3 Determination of Lysozyme concentration

The concentrations (After $\log_2$ transformation) of standards were regressed on diameter of the lysed zones around these standards. The slope of the curve and intercept were determined. The lysozyme concentration in the unknown sera samples were estimated using following regression equation:

$$Y = bx + c$$

Where,

Y = Concentration of lysozyme in unknown sample

b = Slope of regression equation

c = Intercept of regression equation

x = Diameter of the lysed zone around sample.

3.3.2.3 Estimation of serum IgG concentration by Single Radial Immunodiffusion (SRID) Assay

Chicken serum IgG neutralizes the antichicken IgG. A 1.5% (w/v) agarose gel was used as solidifying base to assay IgG concentrations through Single Radial Immunodiffusion (SRID) assay (Mancini *et al.,* 1965) as per following procedure:

Procedure

1. Clean and sterilized glass plate was placed on smooth leveled horizontal surface.
2. The borders prepared with glass strips were sealed with 1 % agar.
3. Approximatly, 50ml of 0.1 M Tris - HCl was divided equally into two halves.
4. To the first half 0.75 g Agarose was added @ 3% (w/v) and boiled. To the second half 1.750 ml of anti-chicken IgG (Sigma®, USA) was added and after thorough mixing, it was kept at 50-55ºC in a water bath.
5. The temperature of first half was brought down to about 50ºC and second half was mixed. The whole content was then poured on to the glass plate.
6. The gel was allowed to solidify for 1 - 2 hour.
7. Then, wells were punched at a distance of 1.5 cm with the help of gel punch.
8. The standards of chicken IgG (IgY) (GenScript, USA) viz. 4mg/ml, 2mg/ml, 1mg/ml, 0.5mg/ml and 0.25 mg/ml, prepared by serial dilution of stock solution were loaded in the wells to plot standard curve.
9. Then 5µl of unknown sera was diluted to 10 times with 0.1 M Tris and from this 10 µl of each sample was loaded in the wells.
10. The plate was incubated at 37ºC for 24 h in humid chamber·

3.3.2.3.1 Determination of IgG concentration

The serum IgG concentrations in unknown samples were determined with the help of regression equation obtained by plotting $\log_2$ concentrations of IgG standards against diameter of the precipitation ring. The slope of the curve and intercept was determined. The IgG concentration in the unknown sera samples were determined by following regression equation:

$$Y = bx + c$$

Where,

Y = Concentration of unknown sample

b = Slope of regression equation

c = Intercept of regression equation

x = Diameter of the precipitation ring around unknown sample.

3.3.2.4 Statistical analysis:

The data generated on immunocompetence traits and their inter-relationship with growth traits were analyzed by Least-squares analysis of variance (Harvey 1990) taking sire as random and sex as fixed effect in the statistical models:-

$$\mathbf{Y_{ijk} = \mu + S_i + H_j + e_{ijk}}$$

Where,

Y = value of a trait measured on ijk^{th} individual

μ = Overall mean

S_i = Random effect of i^{th} sire

H_j = fixed effect of j^{th} sex

e_{ijk} = random error associated with mean 0 and variance σ^2

Data on pullets on immunocompetence traits and their inter-relationship with layer economic traits were also analyzed by Least-squares analysis of variance (Harvey 1990) by incorporating sire as random effect in the statistical models:-

$$\mathbf{Y_{ij} = \mu + S_i + e_{ij}}$$

Where,

Y_{ij} = Value of a trait measured on ij^{th} individual

μ = Overall mean

S_i = Random effect of i^{th} sire

e_{ij} = Random error associated with mean 0 and variance σ^2

Heritability, Genetic correlation, and phenotypic correlation were estimated using paternal half sib correlation method (Becker 1975)

3.3.2.5 Association of immunocompetence traits with growth and layer economic traits

The influence of immunocompetence traits on growth and layer traits were analyzed after classifying the birds in three groups on the basis of the population means ± standard deviation. For this purpose, HA titre, serum lysozyme and serum IgG concentrations were classified as 'high' group which had estimates above mean ± standard deviation, 'low' group included those below mean ± standard deviation and medium which had estimates between high and low groups. The levels of HA titres, serum lysozyme and serum IgG levels were taken as independent variable in statistical model.

Critical difference (CD) test at 5% level of probability of significance was performed for assessing critical differences among the least squares means under individual IC levels

3.3.2.6 Association of microsatellite genotypes with immunocompetence traits.

Individual experimental birds were genotyped with respect to each of the ten microsatellite loci used in objectives no. 2 for assessing influence of microsatellites genotypes with IC traits by least squares analysis of variance (LS ANOVA) incorporating microsatellite genotype as fixed and sire as random effect in the model using JMP 9.0.0 statistical program package (SAS, 2010).

3.4. Objective 4: To analyze relative expression of important immunity related genes in various tissues of Rhode Island Red chicken by quantitative reverse transcription PCR (qRT-PCR).

3.4.1 Experimental birds

Twelve Rhode Island Red (RIR) chicks, six of either sex, were selected based on HA titre with minimum three at each of the low (HA titre less than 8), medium (HA titre equal to 8) and high (HA titre more than 8) levels of humoral antibody response to sheep erythrocytes at the age of 6-8 weeks form 30th generations of strain selected on the basis of part-record egg production i.e. egg production up to 40 weeks of age and maintained at Central Avian Research Institute (CARI), Izatnagar under standard managemental conditions and used in the gene expression studies

3.4.2 Sample size, tissue collection and storage

Three tissues viz., bursa, spleen and thymus, weighing approximately 50-100 mg were aseptically collected **(Fig. 3.4.1)** from each of the experimental birds in 2.0 ml centrifuge tube containing ~1.0 ml RNAlater® (Ambion, U.S.A.). Tissues were cut into small pieces to ensure proper infusion of RNAlater® into it and cryopreserved after proper labeling at -80°C until used for RNA isolation.

3.4.3 Sterilization of labwares and inactivation of RNases

All the plastic ware and stainless steel based accessories related to RNA work were initially dipped in 0.1% diethyl pyrocarbonate (DEPC), incubated at 37°C for overnight to destroy RNases and subsequently sterilized by autoclaving at 121°C, 15 psi for 15 minutes. All the glassware used for RNA work were sterilized in hot air oven at 180°C for at least 5 hours to make them RNase free. The working surfaces were cleaned with RNase AWAY® (Molecular BioProducts Inc., U.S.A.) and subjected to UV light exposure for 30 minutes. Fresh Nitrile powder-free hand gloves (ALTALON®, China) were used during the course of experiment. Micropipettes (Finnpipette®, Thermo Fisher Scientific Inc., U.S.A.) were cleaned with RNaseZap® (Ambion, U.S.A.). before used. Throughout, only clear, racked, pre-sterilized filtered tips (Axygen Scientific Inc., U.S.A) were used.

3.4.4 Isolation of total RNA

Total RNA from each tissue sample was isolated using TRIzol® reagent (Invitrogen, U.S.A) and the step-wise procedure (Hongbao *et al*., 2008) was as follows: -

i. Approximately 20 mg of chopped tissue (Bursa, Spleen and thymus) was taken into 2 ml RNase-free micro-centrifuge tubes containing 1ml TRIzol reagent.
ii. The tissue was homogenized using tissue homogenizer (Polytron, Kinematica AG, Switzerland) at 20,000 rpm for about 30 seconds till a uniform suspension was formed.
iii. Homogenized tissue suspension was incubated at 15-30°C for 5 minutes to permit the complete dissociation of nucleoprotein complexes.
iv. 200 µl of chloroform (Molecular biology grade) was added per 1000 µl of TRIzol into the tube and was thoroughly mixed for 15 seconds.

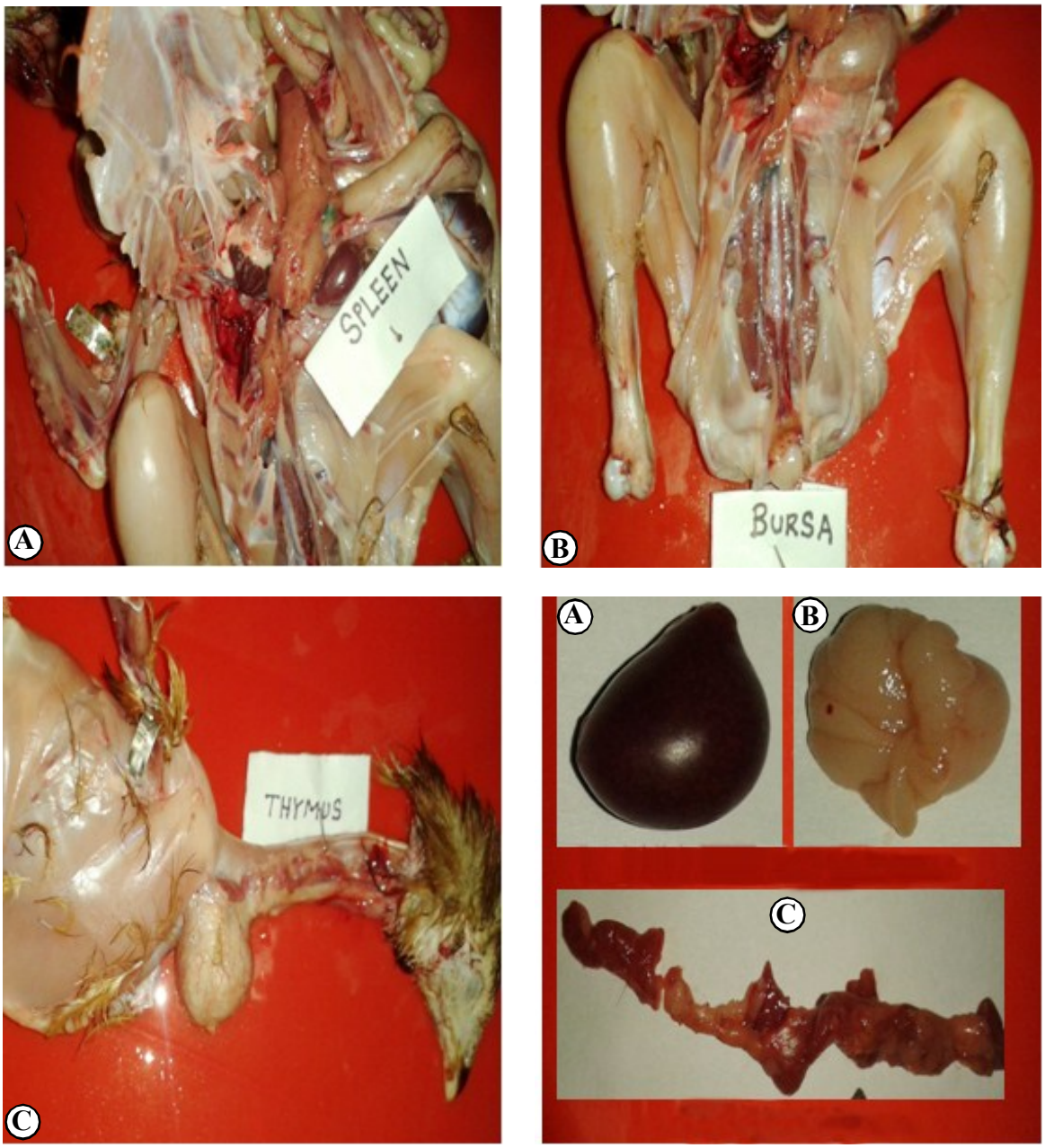


Fig. 3.4.1: Lymphoid tissues (A) spleen (B) bursa (C) thymus collected for gene expression studies in selected pure strain of RIR chicken

v. The tube was incubated at 15-30°C for 2-3 minutes and tissue suspension was centrifuged at 13,500 rpm for 15 min at 4°C in a micro-centrifuge (KUBOTA-3500, Japan).

vi. The top aqueous phase (containing RNA) was removed and transfer carefully into a fresh 1.5 ml RNase free micro-centrifuge tube without disturbing interphase and organic phase containing protein and DNA, respectively.

vii. Equal volume of isopropanol was added to the aqueous phase and incubated at room temperature for 20 minutes, then the tube was centrifuged at 10,000 rpm for 10 min at 4°C to precipitate RNA.

viii. The isopropanol supernatant was decanted carefully without disturbing RNA pellet that was stuck on side-bottom of the tube.

ix. The pellet was washed with 0.5ml of 75% ice-cold ethanol and the tube was centrifuged at 10,000 rpm for 10 min at 4°C.

x. The ethanol was decanted and RNA pellet was air-dried for 5-10 minutes in an RNase free environment.

xi. The air dried RNA pellet was re-suspended in 50 µl of nuclease-free water and stored at -20°C until further use.

3.4.5 Quantitation and purity determination of RNA

The purity and concentration of total RNA was determined spectrophotometrically taking absorbance at 260 and 280nm by NanoDrop Spectrophotometer instrument. (Thermo Fisher Scientific Inc., U.S.A.). Concentration of total RNA was recorded as nanogram per microliter. A (260/280) ratio of the samples was > 1.8 were considered to have satisfactory purity and used in subsequent analysis. The concentration of RNA was adjusted to 1 µg/ µl before proceeding for removal of DNA contamination.

3.4.6 Removal of DNA contamination of RNA samples

The RNA samples, which showed any contamination of genomic DNA, the contaminant DNA was removed by treating 5µl of each RNA sample with 5U RNase-free DNase (Biogene, CA, USA) at 37°C for 1 hour. The samples were subsequently incubated at 65°C for 10 minutes to inactivate DNase I enzyme. After DNase treatment, the concentration of RNA was

determined once again using NanoDrop Spectrophotometer and all the samples were adjusted to equal concentration of 500ng/µl. DNase treated RNA samples were properly labeled and stored at -20°C till further processing.

3.4.7 Synthesis of first strand cDNA

Required amount of DNase-treated total RNA from each sample was taken as template and first strand cDNA was prepared using Thermo Scientific Verso cDNA synthesis kit® (Thermo Fisher Scientific Inc., U.S.A.). The reaction was prepared in 20.0 µl reaction mixture in a 0.2 ml clear, thin walled PCR tube on ice using following reagents: -

S. No.	Verso Kit Reagents	Volume
1.	5X cDNA synthesis buffer	4.0 µl
2.	dNTP mix (5mM each)	2.0 µl
3.	Random hexamer primer	1.0 µl
4.	RT Enhancer	1.0 µl
5.	Verso reverse transcriptase	1.0 µl
6.	Template RNA (500 ng)	1.0 µl
7.	Nuclease free water	10.0 µl
	Total volume	**20.0 µl**

The tube containing 20.0 µl reaction mixtures was spun and incubated at 42°C for 30 minutes; followed by inactivation by incubating at 95°C for 2 minutes. The tube containing resultant first stand cDNA was carefully labeled, and stored at -20°C till further use. The concentration of cDNA of each sample was equalized to 25.0 ng/µl for subsequent usage in qRT-PCR.

3.4.8 Primers

Primer pairs of three immune response genes viz. IL1-β, iNOS and TLR15 and housekeeping or the reference gene (β-actin) were used for this investigation. IL1-β and β-actin primer pairs were selected from published literature. Two primer pair's iNOS and TLR15 were designed using PrimerQuest tool of integrated DNA technologies (IDT). The specificity of primers was checked by NCBI blast program. All the primers were got synthesized

from M/S Xcelris Genomics Labs Ltd., Ahmedabad (India). The nucleotide sequences of the primers, gene accession number, amplicon length and corresponding optimized annealing temperature were as follows (**Table 3.4.1**):

Table 3.4.1: Details of the primers for qRT-PCR

Target gene	Primer sequences	Ta (°C)	Amplicon size (bp)	Gene (Accession No.) or References
TLR15	**F** 5'- TGT GGT ATG TGA GAA TGG GC -3' **R** 5'- GCA TCG AAG GGC TTA TTT TCT G -3'	58°C	85	NM_001037835.1
iNOS	**F** 5'- GGC ATC TGT ATG TCT GTG GAG -3' **R** 5'- CTT CAT GGT ATC GCT TTT GGC -3'	59°C	147	NM_204961.1
IL1-β	**F** 5'- CGC TCA CAG TCC TTC GAC ATC -3' **R** 5'- CCG CTC ATC ACA CAC GAC ATG T -3'	56°C	230	Higgs *et al*., 2006
β-actin	**F** 5'- GGA AGT TAC TCG CCT CTG -3' **R** 5'- AAA GAC ACT TGT TGG GTT AC -3'	56°C or 58°C or 59°C	114	Higgs *et al*., 2006

The primer pairs were obtained in lyophilized form and were reconstituted with nuclease-free water. A stock of 100 picomoles / µl was prepared and from this working primer solution of 10.0 picomoles/ µl was prepared and used in PCR.

3.4.9 Quantitative Reverse Transcriptase Real time polymerase chain reaction (qRT-PCR)

Relative quantification of mRNA expression of three immune response genes viz. TLR15, iNOS and IL1-β in each of the twelve tissues viz. bursa, spleen and thymus in RIR selected strain was done by quantitative reverse transcriptase PCR (qRT-PCR) method using CFX 96® - Real Time PCR detection system (Bio-Rad Laboratories Inc., U.S.A.).

3.4.9.1 Reaction mixture

RT-PCR was carried out using DyNAmo ColorFlash SYBR Green qPCR Kit® (Thermo Fisher Scientific Inc., U.S.A.) in 20.0 µl reaction mixture, which was prepared in 0.2 ml clear, thin walled nuclease-free 8-tube strips with optically clear flat (Axygen Scientific Inc., U.S.A) containing following components:

1.	2X SYBR Green master mix with blue dye	10.0 µl
2.	10 pM forward primer	0.5 µl
3.	10 pM reverse primer	0.5 µl
4.	Nuclease free water	6.0 µl
5.	cDNA (75ng)	3.0 µl
	Total volume	**20.0 µl**

The reaction mixture for each sample was prepared in triplicate to avoid pipetting error. A negative control (NTC; no template control) in triplicate containing all the ingredients except cDNA was also set up to check any contamination. β-actin gene was used as reference gene. The SYBR green master mix was added in dark room to avoid possible exposure to light. The 8-tube strips containing reaction mixtures were placed in plastic PCR tube rack and covered with aluminium foil to protect them from light. The 8-tube strips containing reaction mixtures were spun before placing them in the real time PCR system. The strips were sometimes stored at -20°C temporarily, whenever needed.

3.4.9.2 RT-PCR Programme

Initially, RT-PCR condition for each gene of interest was optimized individually before set up of actual experiment. Real-time PCR cycling conditions used were as follows: initial denaturation at 95°C for 7 minutes followed by 40 cycles of denaturation at 95°C for 10 seconds, annealing at optimized temperature for 20 seconds and extension at 72°C for 20 seconds; followed by detection of fluorescent signal by the real time detection system to generate amplification curve. After completion of 40 cycles, each sample was subjected to 60-95°C @ +0.5°C increment for 10 seconds to generate dissociation curve or melt curve to identify whether amplification was specific or non-specific.

3.4.10 Retrieval and compilation of qRT-PCR data

After the completion of RT-PCR, threshold cycle (C_t) value and melting point temperature of each tube was carefully retrieved and reviewed for its corresponding amplification and dissociation curve to ensure appropriateness of specific amplifications. Subsequently, data were imported into MS-Excel file and saved for further statistical analysis.

3.4.11 Determination of 40-ΔCt

Among triplicate C_t values of the target gene, minimum two C_t values which showed high resemblance were chosen and averaged for subsequent analysis. ΔC_t value for each sample was calculated after subtracting average C_t value of housekeeping gene from average C_t value of target gene. For each sample, the ΔC_t value was subtracted from 40 (total cycle number) so as to obtain 40-ΔC_t. Higher 40-ΔC_t value was considered as higher expression (MacKinnon *et al.*, 2009).

3.4.12 Statistical analysis of qRT-PCR data for differential expression of immune response genes in two sexes in each tissue

Influence of sex on relative expression of mRNA (40-ΔCt) of all the three genes were analyzed by least squares analysis of variance (LS ANOVA) using JMP 9.0.0 statistical program package (SAS, 2010) by incorporating sex as fixed effects in the statistical model.

$$Y_{ij} = \mu + S_j + e_{ij}$$

Where,

Y_{ij} = 40-ΔC_t value of mRNA expression of genes under study in i^{th} sex

μ = overall mean

S_i = fixed effect of i^{th} sex (i = 1, 2)

e_{ij} = random error of i^{th} sex of j^{th} individual (mean '0'; variance 'σ^2')

3.4.13 Statistical analysis of qRT-PCR data for differential expression of immune response genes in different tissues

Differential expression of three target genes in the three tissues was analyzed by least squares analysis of variance (LS ANOVA) using JMP 9.0.0 statistical program package (SAS, 2010). Tissue was taken as fixed effect in the model.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where,

Y_{ij} = 40-ΔC_t value of mRNA expression of genes under study in i^{th} tissue on j^{th} individual

μ = overall mean

T_i = fixed effect of i^{th} tissue (i = 1-3)

e_{ij} = random error of i^{th} tissue of j^{th} individual (mean '0'; variance 'σ^2')

3.4.14 Influence of levels of IC traits on relative gene expression (40-ΔCt) in various lymphoid tissues

HA titre, serum lysozyme and serum IgG concentrations in the sampled birds were classified in to high, medium and low levels based on their means and standard deviations. The influence of IC traits on gene expression was determined by least square analysis using JMP 9.0.0 statistical program package (SAS, 2010) taking IC traits' levels as fixed effect in the following statistical model:

$$Y_{ij} = \mu + IC_i + e_{ij}$$

Where,

Y_{ij} = 40-ΔC_t value of mRNA expression of genes under study in i^{th} tissue of j^{th} individual

μ = overall mean

IC_i = effect of i^{th} level of IC trait (i= high, medium and low).

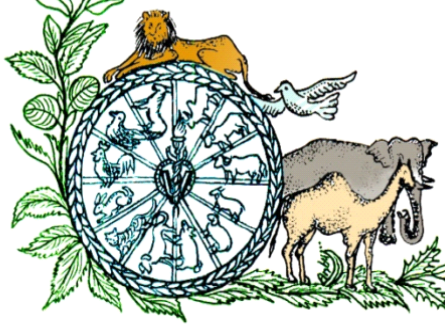
e_{ij} = random error of i^{th} level of IC trait of j^{th} individual (mean '0'; variance 'σ^2')

Averages of 40-ΔC_t in different tissues was compared by critical difference test.

3.4.15 Determination of fold expression of genes by $2^{(-\Delta\Delta Ct)}$ method

The data from qRT-PCR experiment, imported in excel, was analyzed for fold change expression analysis by $2^{(-\Delta Ct)}$ method (Livak and Schmittgen, 2001). The ΔC_t value for each sample was calculated, as described earlier. Further, ΔC_t value for each sample was calculated after subtracting ΔC_t value of calibrator from ΔC_t value of target gene. As per Livak and Schmittgen (2001) calibrator could be any one tissue among different tissues which are under study. In this study, the calibrator tissue was not chosen arbitrarily, but based on the ΔC_t value. The tissue which was showing highest ΔC_t value was chosen as the calibrator tissue in each breed. Finally, the fold expression was determined using the formula $2^{(-\Delta\Delta Ct)}$.

Results



4 RESULTS

Data on growth, production and reproduction performances of selected strain of Rhode Island Red chicken was analyzed. The polymorphism was assessed at a few egg production-associated microsatellites (MS) and association between various MS genotypes and layer economic traits were determined. Genetic variability was estimated in some important immunocompetence (IC) traits and their associations with growth and layer economic traits were determined. The basal mRNA expressions of immune response genes in lymphoid tissues were evaluated. The results were compiled, tabulated and presented with the help of graphs wherever necessary in an objective-wise manner.

4.1 Objective 1: To analyze effects of various genetic and non-genetic factors on layer economic traits in Rhode Island Red chicken

Rhode Island Red (RIR), dual-purpose chicken breed is preferred for rearing in backyard and rural systems. Periodic evaluation of its performance is necessary to exploit its production performance. The pedigreed chicks of selected strain of RIR chicken were evaluated for growth, production and reproduction performance traits along with their inheritance pattern.

Data on growth traits of combined sex at different weeks of age were analyzed by least squares ANOVA taking sire as random and sex as fixed effects in the statistical model. The data on layer economic traits such as age of first egg, egg weight at 28, 40 and 64 weeks of age and egg production up to 40 and 64 weeks of age were also analyzed by least square ANOVA taking sire as random effect in the model.

4.1.1 Percent fertility and hatchability

A total 568 eggs of selected strain of RIR were set sire-wise in incubator and 416 fertile eggs were transferred for hatching under standard incubation and hatching conditions. Three hundred twenty-five good chicks were obtained in a single hatch. The overall percent fertility was estimated as 73.24% and hatchability on fertile eggs set (FES) and on total eggs set (TES) basis was 78.13% and 57.22%, respectively.

4.1.2 Body weights at different weeks of age

The body weights at 16 (BW16), 20 (BW20), 40 (BW40) and 64 (BW64) weeks of age were recorded in selected strain of RIR chicken and analyzed by Least squares ANOVA.

Least squares analysis of variance of body weights at various ages are presented in **table 4.1.1**, which revealed significant ($P \leq 0.01$) effect of sire and highly significant effect of sex ($P \leq 0.001$) on body weights at all ages. The factor-wise least squares means along with standard error of various growth traits are presented in **table 4.1.2**.

The overall least-squares means of body weight at 16 (BW16), 20 (BW20), 40 (BW40) and 64 weeks of age on combined sex basis were 1362.6±21.4g, 1791.6± 24.6g, 2184.6±26.2 and 2433.8±34.2g, respectively. The corresponding mean body weights in males were 1492.9±24.0g, 2040.3±28.1g, 2624.4±31.3g and 2784.5±41.3g and in females 1232.4±25.5g, 1542.9±30.2g, 1744.8±33.7g and 2083.1±42.9g. The differences between sexes were significant at all ages and the males demonstrated higher body weights than the females.

Table 4.1.1: Least squares analysis of variance of various growth traits in selected pure strain of Rhode Island Red chicken

Source of variation	df	Mean sum of squares			
		BW16	BW20	BW40	BW64
Sire	24	121007.5***	159602.9***	169779.6**	252012.3**
Sex	1	4629264.9***	16804705.1***	48031907.8***	26381405.6***
Error/ Remainder	272	42089.1	67247.2 (271)	92241.1 (246)	129794.2 (213)

*df = Degrees of freedom; **P≤0.01, ***P≤0.001; Figures within parentheses denote degrees of freedom.*

Table 4.1.2: Least squares mean ± standard errors of various growth traits in selected pure strain of Rhode Island Red chicken

Factors	N	Least squares means± standard errors (in gram)			
		BW16	BW20	BW40	BW64
Overall	298	1362.6± 21.4	1791.6 ± 24.5 (297)	2184.6 ± 26.2 (272)	2433.8 ± 34.2 (239)
Sex					
Male	168	1492.9[a]±24.0	2040.3[a]± 28.1 (168)	2624.4[a] ± 31.3 (151)	2784.5[a] ± 41.3 (127)
Female	130	1232.4[b]±25.5	1542.9[b]± 30.2 (129)	1744.8[b] ± 33.7 (121)	2083.1[b] ± 42.9 (112)

N= Number of observations; Means within a factor having different superscripts in a column differ significantly (P≤0.05); Figures within parentheses denote number of observation.

4.1.3 Layer economic traits

The RIR[s] were evaluated for various layer economic traits. The least-squares analysis of variance of various layer economic traits viz., age at first egg (AFE), egg weight at 28 (EW28), 40 (EW40) and 64 (EW64) weeks of age and egg production up to 40 (EP40) and 64 (EP64) weeks of age and their least squares means ± standard errors have been presented in **table 4.1.3** and **4.1.4**, respectively.

4.1.3.1 Age at first egg (AFE)

Sire had no significant effect on age at first egg. The overall least-squares mean of AFE was 134.5 ± 0.9 days in RIR[s] chicken.

4.1.3.2 Egg weight at different weeks of age

The analysis revealed non-significant effect of sire on egg weights at different ages in RIR[s] chicken. Overall least squares means of egg weights at 28 (EW28), 40 (EW40) and 64 (EW64) weeks of age were 44.8±0.3g, 47.7±0.4g and 51.5±0.7g, respectively.

4.1.3.3 Egg production

Least squares analysis of variance revealed significant (p≤0.01) effect of sire on egg production up to 64 weeks of age in RIR[s] chicken. Least squares means of part-period eggs production up to 40 weeks (EP40) and annual egg production up to 64 weeks (EP64) were 118.3±1.2 and 214.5±4.8 eggs, respectively.

Table 4.1.3: Least squares analysis of variance of various layer economic traits in selected pure strain of Rhode Island Red chicken

Source of variation	Degrees of freedom	Mean Sum of squares					
		AFE	EW28	EW40	EP40	EW64	EP64
Sire	24	95.2	14.0	13.6	169.1	39.2	2030.4**
Remainder	103	95.4	11.5 (98)	16.4 (88)	165.2 (92)	25.8 (63)	977.6 (74)

***P≤0.01, Figures within parentheses denote degrees of freedom (df).*

Table 4.1.4: Least squares means ± standard error of various layer economic traits in selected pure strain of Rhode Island Red chicken

Factors	N	Least squares means± SE					
		AFE (days)	EW28 (g)	EW40 (g)	EP40 (eggs)	EW64 (g)	EP64 (eggs)
Overall	128	134.5 ± 0.9 (128)	44.8 ± 0.3 (123)	47.7 ± 0.4 (113)	118.3 ± 1.2 (117)	51.5 ± 0.7 (88)	214.5 ± 4 .8 (99)

N= Number of observations; Figures within parentheses denote number of observations.

4.1.4 Genetic parameters

Genetic parameters, viz., heritability, genetic and phenotypic correlations amongst body weights and various layer economic traits have been presented in **table 4.1.5.**

4.1.4.1 Heritability (h^2)

4.1.4.1.1 Body weight

The heritability (h^2) estimates of body weights at different weeks of age in RIRs chicken, estimated from sire variance component are presented in **table 4.1.5.** The heritability estimates for BW16, BW20, BW40 and BW64 were 0.55± 0.22, 0.42± 0.19, 0.29± 0.18 and 0.36± 0.21, respectively. The heritability estimates of body weights at different age were positive and high in magnitude and were associated with low standard errors thereby suggesting them to be reliable and precise.

Table 4.1.5: Heritability (at diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations of various growth and layer economic traits in selected pure strain of RIR chicken

Traits	AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
AFE	0.01±0.30	≤-1	≤-1	NE	NE	≤-1	≤1	NE	≤-1	NE
	(108)	(108)	(108)			(108)	(108)		(108)	
BW16	-0.33	0.55±0.22	≤1	0.99± 0.14	0.92 ±0.16	NE	NE	≤1	0.16±0.64	≤-1
	(116)	(298)	(297)	(272)	(230)			(78)		(78)
BW20	0.02	0.79	0.42±0.19	0.99± 0.10	0.81±0.18	NE	NE	NE	NE	NE
	(112)	(297)	(297)	(272)	(230)					
BW40	-0.03	0.56	0.70	0.29±0.18	0.99±0.09	NE	NE	NE	NE	NE
	(103)	(272)	(272)	(272)	(230)					
BW64	-0.06	0.53	0.59	0.75	0.36± 0.21	NE	NE	NE	NE	NE
	(230)	(230)	(230)	(230)	(230)					
EW28	0.14	0.48	0.49	0.40	0.46	0.17±0.30	≤-1	NE	≤1	NE
	(112)	(78)	(82)	(78)	(78)	(123)	(108)		(108)	
EW40	-0.06	0.48	0.43	0.38	0.41	0.63	0.09±0.32	0.51±0.49	≤1	NE
	(103)	(78)	(82)	(78)	(78)	(108)	(103)	(78)	(108)	
EW64	0.10	0.49	0.44	0.45	0.43	0.60	0.67	0.52±0.45	NE	-0.31 ±0.49
	(81)	(78)	(82)	(78)	(78)	(82)	(82)	(88)		(81)
EP40	-0.48	0.10	-0.06	-0.08	-0.02	-0.02	-0.03	0.01	0.02±0.28	0.89±0.15
	(108)	(78)	(99)	(78)	(78)	(108)	(81)	(78)	(117)	(99)
EP64	-0.04	0.03	0.02	0.06	0.05	0.13	0.09	0.18	0.63	0.86±0.43
	(81)	(78)	(99)	(78)	(78)	(81)	(81)	(81)	(99)	(99)

Figures within parentheses denote number of observations; NE., not estimable.

4.1.4.1.2 Layer economic traits

The heritability (h^2) estimates for various layer economic traits viz., AFE, EW28, EW40, EW64, EP40 and EP64 along with standard errors are presented in **table 4.1.5.** The heritability estimate of AFE was very low 0.01±0.30 and having high standard error. The estimates of heritabilities were low for EW28 (0.17±0.30), EW40 (0.09±0.32) and EP40 (0.02±0.28) and high for EW64 (0.52±0.45) and EP64 (0.86±0.43) with relatively high standard errors, hence not precise.

4.1.4.2 Genetic and phenotypic correlations amongst body weights and layer economic traits

The Genetic and phenotypic correlations of body weights and layer economic traits are presented in **table 4.1.5.**

4.1.4.2.1Genetic correlation (r_G)

Genetic correlations of AFE with BW16, BW20, EW28 and EP40 were negative and less than unity, but positive and more than unity with EW40. The r_G between AFE and BW40, BW64, EW64 and EP64 could not be estimated.

The genetic correlation of BW16 with BW20 and EW64 was positive but more than unity, highly positive with BW40 and BW64 but low with EP40 and could not be estimated with EW28 and EW40. The r_G of BW20 was highly positive with BW40 and BW64 but could not be estimated with any other trait. Similarly, BW40 had highly positive correlation with BW64. The r_G of BW40 and BW64 with other traits could not be estimated. The r_G of EW28 was negative and less than unity with EW40 but positive and more than unity with EP40, Other r_G were not estimable. EW40 had highly positive genetic correlation with EW64 and more than unity with EP40, but could not be estimated with EP64. The r_G between EW64 and EP64 was moderately negative, however, EP40 had highly positive r_G with EP64 (0.89±0.15).

4.1.4.2.2 Phenotypic correlation (r_P)

The phenotypic correlations of AFE with all the traits were very low and close to zero except with BW20 and EW64 where they were low and positive. The r_P among all body

weights and egg weights were high and positive. However, r_p between body weights at various ages was close to zero except between BW16 and EP40, where it was lowly positive. Similarly, egg weights had highly positive r_p amongst them but had very low r_p with EP40 and EP64, although r_p was lowly positive between EW64 and EP64. The EP40 had highly positive phenotypic correlation with EP64 (0.63).

4.1.5 Mortality percentage

Percent mortality recorded in combined sexes of selected strain of RIR was 1.54%, 0.31%, 2.60%, 6.35% and 7.86% during different period from 0 to 4, 5 to 8, 9 to 16, 17 to 40 and 41 to 64 weeks of age. The overall mortality from day zero up to 64 weeks of age was 17.57%.

4.2 Objective 2: To determine allelic polymorphism at egg production – associated microsatellite loci in Rhode Island Red chicken and their association with layer economic traits.

Allelic polymorphism at 10 egg-production associated microsatellite loci were analyzed in random representative sample of 76 RIR selected strain using MetaPhor Agarose gel electrophoresis. Various numbers of allele were recorded and analyzed for their molecular sizes with the help of Quantity One® software (Bio-Rad Laboratories Inc., U.S.A.). Based on the allelic pattern, the individual were genotyped. The allelic and genotypic data were analyzed using POPGENE® software (Yeh *et al.,* 1999) for various population genetics estimates. Influences of microsatellite genotype on layer economic traits were analyzed using JMP 9.0.0 (SAS) statistical software package.

4.2.1. Allelic profile and annealing temperature of microsatellite loci

The optimized annealing temperature for amplification of various microsatellite loci are presented in **table 4.2.1**. Allelic patterns at egg associated microsatellite loci are presented in **figures 4.2.1, 4.2.2, 4.2.3, 4.2.4, 4.2.5, 4.2.6, 4.2.7, 4.2.8, 4.2.9** and **4.2.10**.

Table 4.2.1: Optimized annealing temperatures, chromosomal location and amplified products size range of various microsatellites in selected strain of RIR chicken

Sl. No.	Microsatellite Loci	Chromosome location	Optimum Annealing Temperature (T_a) in °C	Products size range (bp)
1	ADL0020	1	55	102-126
2	ADL0023	5	61	176-202
3	ADL0102	30	55	110-122
4	ADL0176	2	55	202-220
5	ADL0210	E 30	52	124-132
6	MCW0007	1	62	262-302
7	MCW0014	6	60	176-192
8	MCW0041	2	57	164
9	MCW0069	26	55	172-232
10	MCW0103	3	55	236-298

4.2.1.1. Number of alleles, allelic sizes and allelic frequencies

Number of alleles, their sizes and frequencies at ten microsatellite loci are presented in **table 4.2.2**.

4.2.1.1.1 Microsatellite ADL0020

Four alleles were observed at ADL0020 microsatellite locus with the size range of 102 – 126 bp (**Fig. 4.2.1**). The sizes of different alleles noticed were 126 bp (A), 118 bp (B), 110 bp (C) and 102 bp (D). Allele C with a frequency of 0.783 was most prevalent, whereas, allele A, with frequencies of 0.013 was least prevalent in the population analyzed. The frequencies of B and C allele were 0.053, and 0.151, respectively.

4.2.1.1.2 Microsatellite ADL0023

ADL0023 locus reveled presence of three alleles in the selected RIR population studied (**Fig. 4.2.2**). The different alleles noticed were 202 bp (A), 188 bp (B) and 176 bp (C). The frequencies of A, B and C allele were 0.105, 0.158, and 0.737, respectively.

Table 4.2.2: Number of alleles, their molecular sizes and frequencies at various microsatellites loci in selected strain of RIR chicken

MS loci	No. of alleles	Allele code	Allele size (bp)	Allele frequency
ADL0020	4	A	126	0.013
		B	118	0.053
		C	110	0.783
		D	102	0.151
ADL0023	3	A	202	0.105
		B	188	0.158
		C	176	0.737
ADL0102	3	A	120	0.289
		B	112	0.408
		C	110	0.303
ADL0176	4	A	220	0.053
		B	214	0.243
		C	206	0.474
		D	202	0.230
ADL0210	2	A	132	0.053
		B	124	0.947
MCW007	3	A	302	0.105
		B	275	0.586
		C	262	0.309
MCW0014	2	A	192	0.138
		B	176	0.862
MCW0041	1	A	164	1.000
MCW0069	6	A	232	0.026
		B	221	0.059
		C	210	0.198
		D	194	0.138
		E	184	0.408
		F	172	0.171
MCW0103	2	A	298	0.263
		B	276	0.737
Total	**30**			
Mean±SE	**3.50 ± 0.29**			

4.2.1.1.3 Microsatellite ADL0102

Only three alleles were observed at ADL0102 microsatellite locus with the size range of 110 - 122 bp (**Fig. 4.2.3.**). The sizes of different alleles noticed were 122 bp (A), 114 bp (B), and 110bp (C). The frequencies of A, B and C allele were 0.289, 0.408 and 0.303, respectively.

4.2.1.1.4 Microsatellite ADL0176

Only four alleles were noticed at ADL0176 microsatellite locus (**Fig. 4.2.4**.). The sizes of alleles were 220 bp (A), 214 bp (B), 206 bp (C) and 202 bp (D). The frequencies of A, B, C and D allele were 0.053, 0.243, 0.474 and 0.230, respectively.

4.2.1.1.5 Microsatellite ADL0210

At ADL0210 microsatellite Marker, two different alleles i.e. 132 bp (A) and 124 bp (B) were observed (**Fig. 4.2.5.**). The frequencies of A and B alleles were 0.053 and 0.947 respectively.

4.2.1.1.6 Microsatellite MCW0007

MCW0007 locus reveled presence of three alleles in the selected RIR population studied (**Fig. 4.2.6**). The different alleles noticed were 302 bp (A), 275 bp (B) and 262 bp (C). The frequencies of A, B and C allele were 0.105, 0.586 and 0.309, respectively.

4.2.1.1.7 Microsatellite MCW0014

Only two alleles i.e. 192bp (A) and 176 bp (B) were noticed at MCW0014 microsatellite locus (**Fig. 4.2.7**), with frequency of A and B allele as 0.138 and 0.862, respectively.

4.2.1.1.8 Microsatellite MCW0041

At MCW0041 microsatellite Marker, no polymorphism could be detected as only 1 allele i.e. 164 bp was observed in selected RIR population studied (**Fig. 4.2.8**.) and also confirmed by repeating the analysis.

4.2.1.1.9 Microsatellite MCW0069

Six alleles were observed at MCW0069 microsatellite locus with the size range of 172 – 232 bp (**Fig. 4.2.9.**). The sizes of different alleles noticed were 232 bp (A), 221 bp (B), 210 bp (C) 194 bp (D), 184 bp (E) and 172 bp (F). The frequencies of A, B, C, D, E and F alleles were 0.026, 0.059, 0.198, 0.138, 0.408 and 0.171, respectively.

4.2.1.1.10 Microsatellite MCW0103

MCW0103 microsatellite locus reveled presence of two alleles in the selected RIR population studied (**Fig. 4.2.10.**). The different alleles were of 298bp (A) and 276 bp (B) size. The frequencies of A and B alleles were 0.263 and 0.737, respectively.

4.2.2. Population genetic analysis of microsatellite data

Data on number of alleles and genotypes of all experimental birds on all the ten microsatellites was analyzed using POPGENE® software (Yeh *et al.*, 1999).

4.2.2.1. Average heterozygosity and polymorphic information content (PIC)

Based on allelic frequencies at each locus, the average heterozygosity as per Nei (1978) and PIC as per Botstein *et al.* (1980) were calculated and the results are presented in **table 4.2.3.**

Table 4.2.3: Average heterozygosity and polymorphic information content (PIC) statistics at various microsatellite loci in RIR chickens

MS loci	Nei's H (%)	PIC
ADL0020	0.3612	0.3294
ADL0023	0.4211	0.3814
ADL0102	0.6582	0.5845
ADL0176	0.6606	0.6020
ADL0210	0.0997	0.0947
MCW0007	0.5505	0.4752
MCW0014	0.2381	0.2098
MCW0041	0.0000	0.0000
MCW0069	0.7421	0.1451
MCW0103	0.3878	0.3126
Mean ± SE	**0.4119± 0.2475**	**0.313±0.064**

H = Average Nei's heterozygosity; PIC: Polymorphic Information Content.

The mean ± SE of Nei's heterozygosity and PIC value of ten microsatellite loci was 0.4119± 0.2475 and 0.313±0.064, ranging from 0.0997 (ADL0210) to 0.7421 (MCW0069) and 0.0947 (ADL0210) to 0.6020 (ADL0176), respectively. Nei's heterozygosity of MS

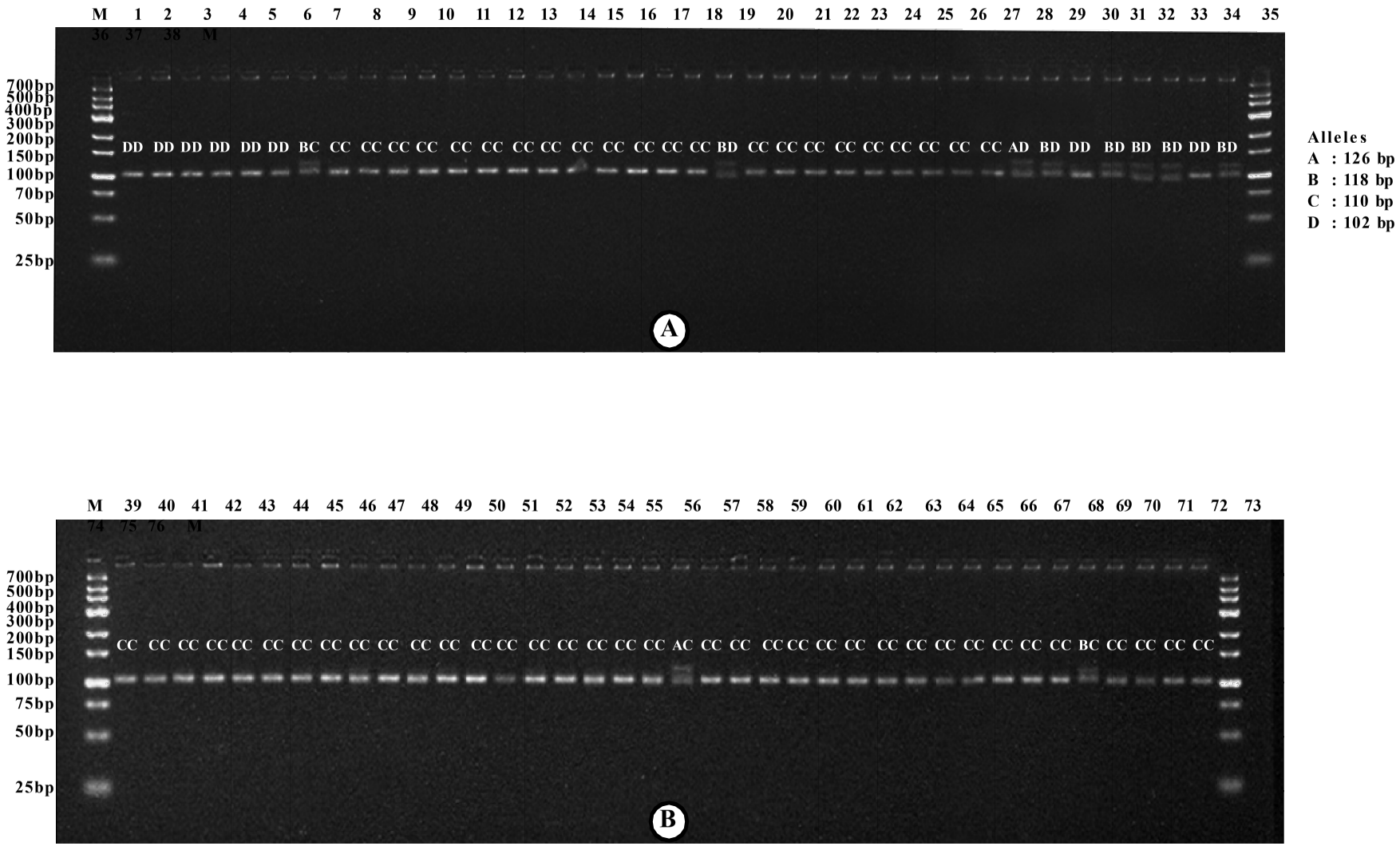


Fig. 4.2.1: Allelic profiling of ADL0020 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

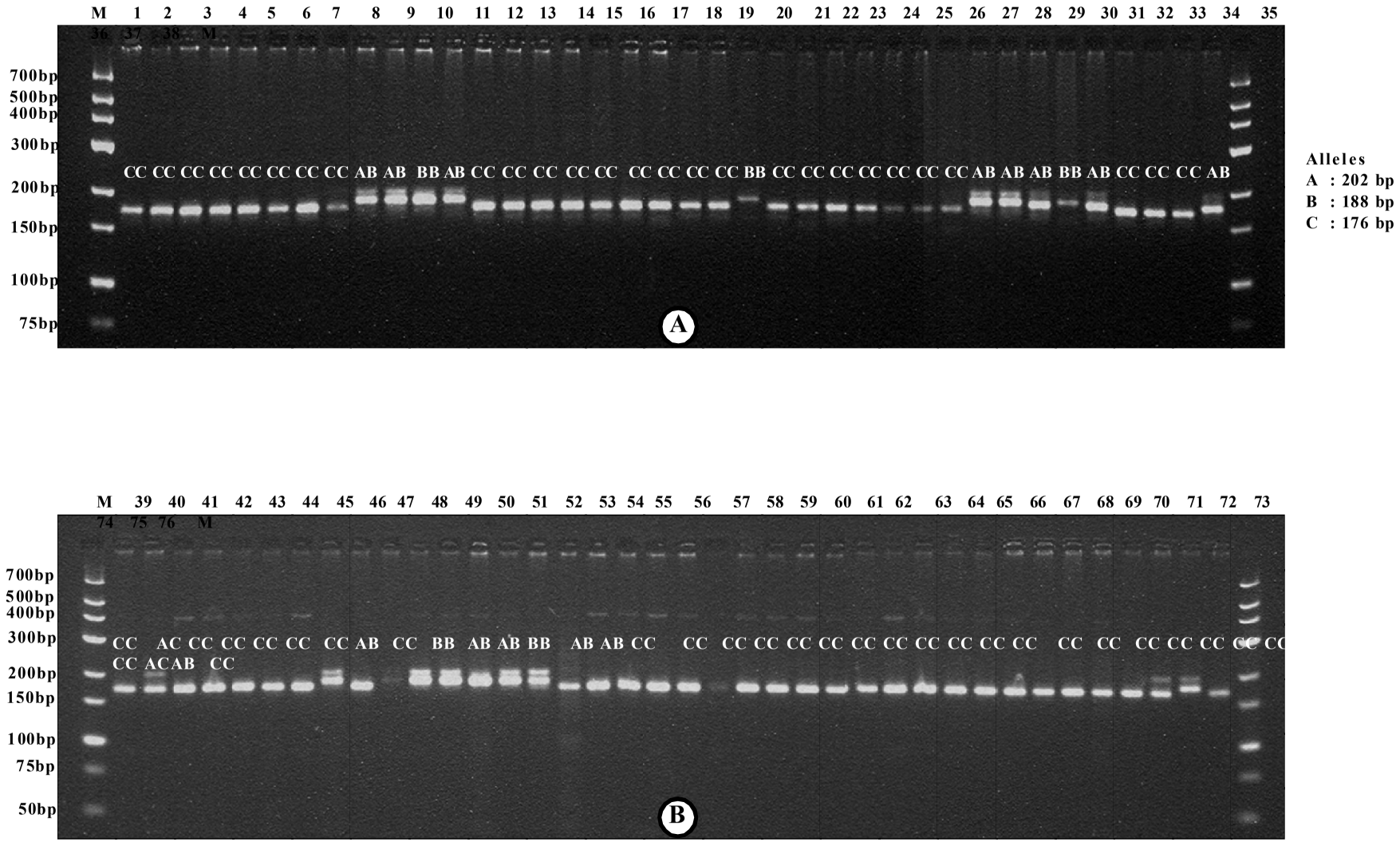


Fig. 4.2.2: Allelic profiling of ADL0023 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

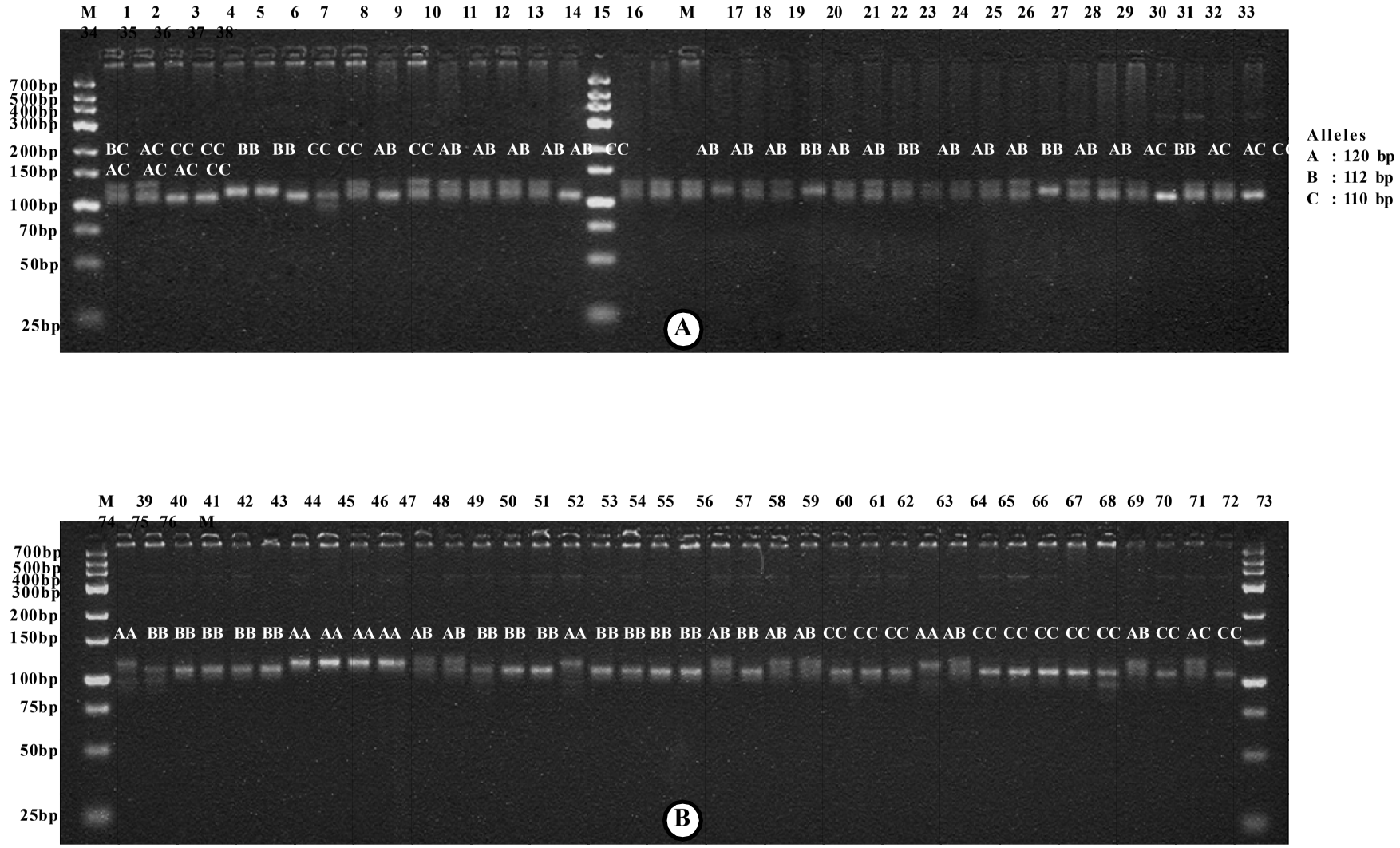


Fig. 4.2.3: Allelic profiling of ADL0102 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

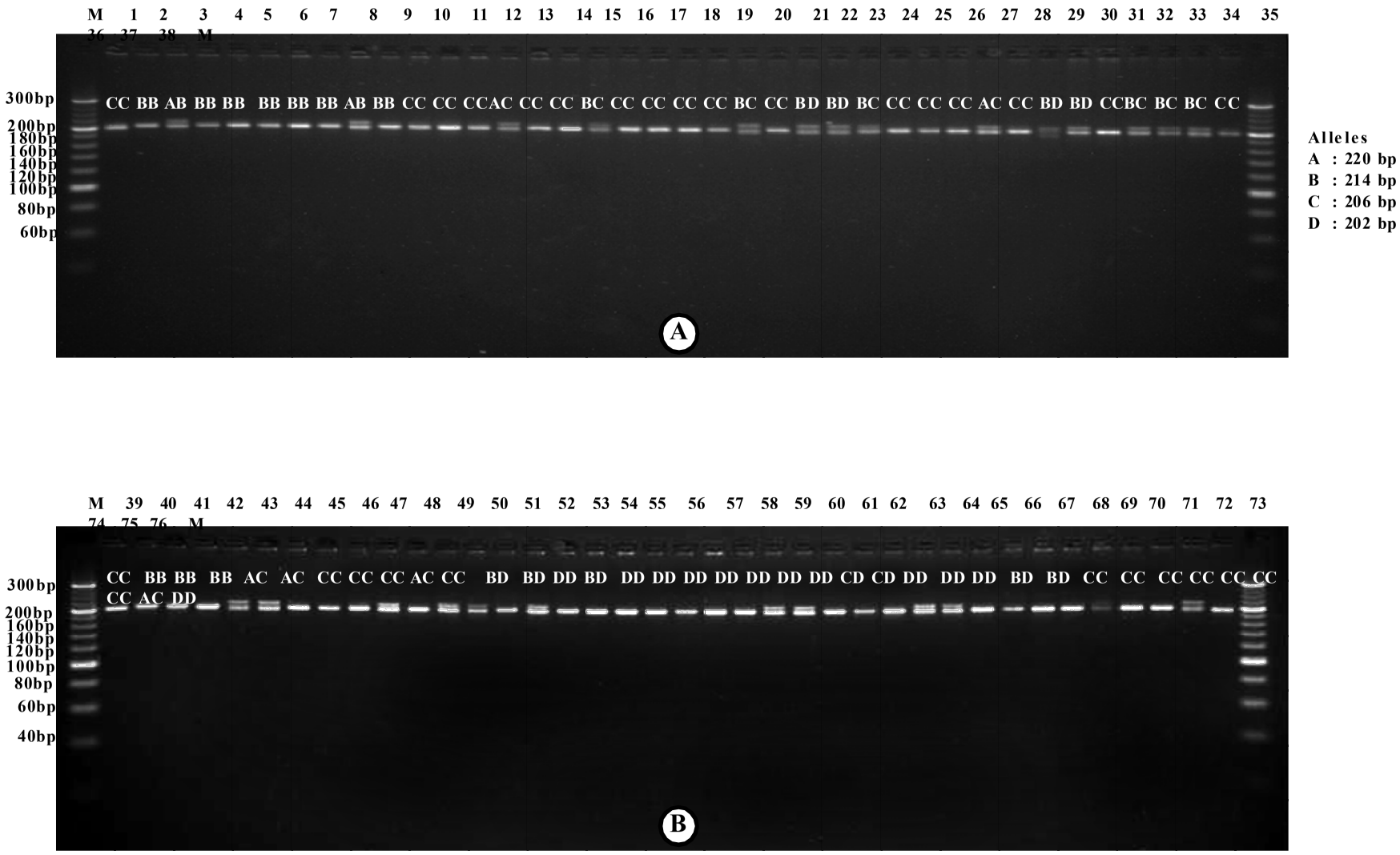


Fig. 4.2.4: Allelic profiling of ADL0176 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: 20bp DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

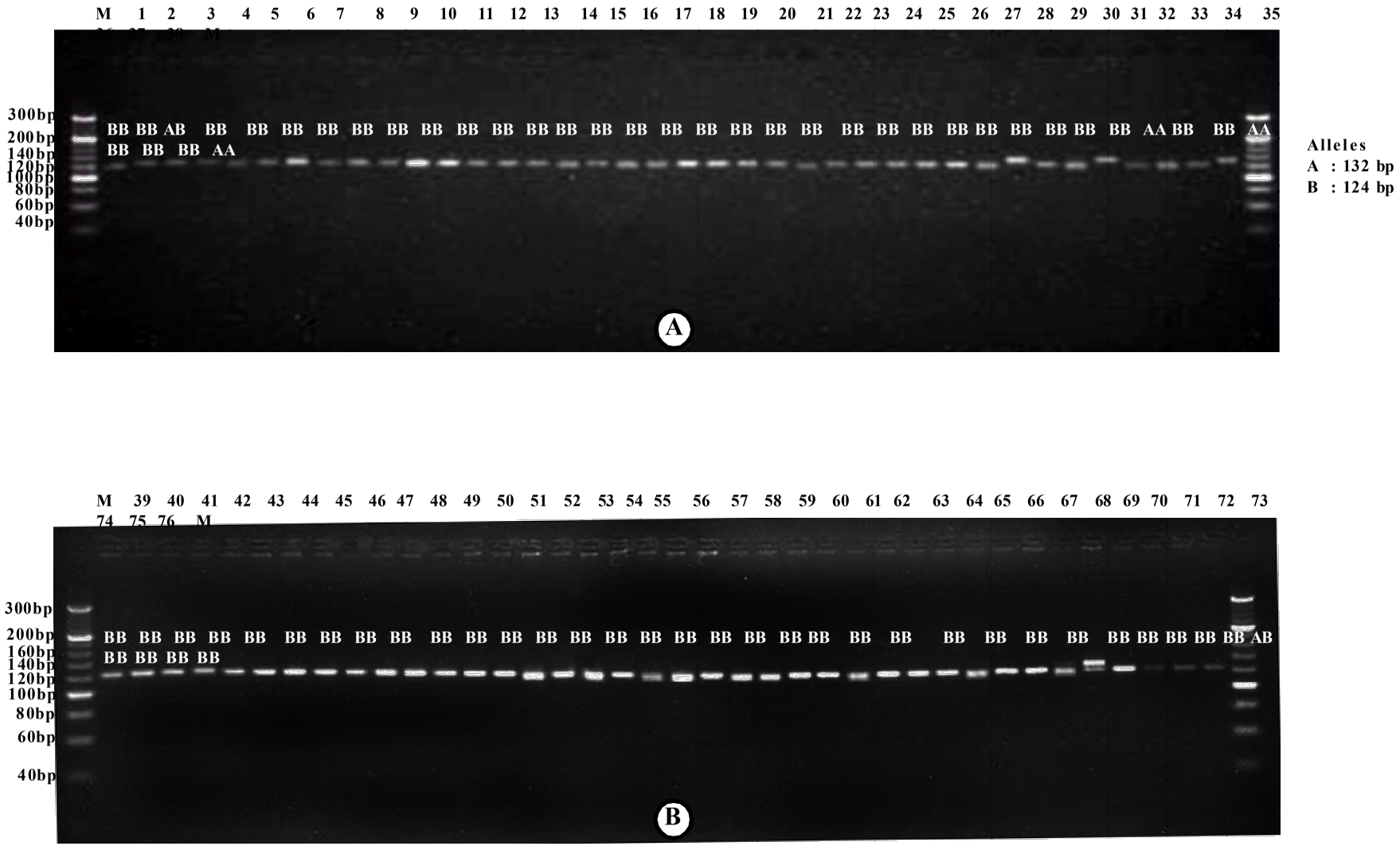


Fig. 4.2.5: Allelic profiling of ADL0210 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: 20bp DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

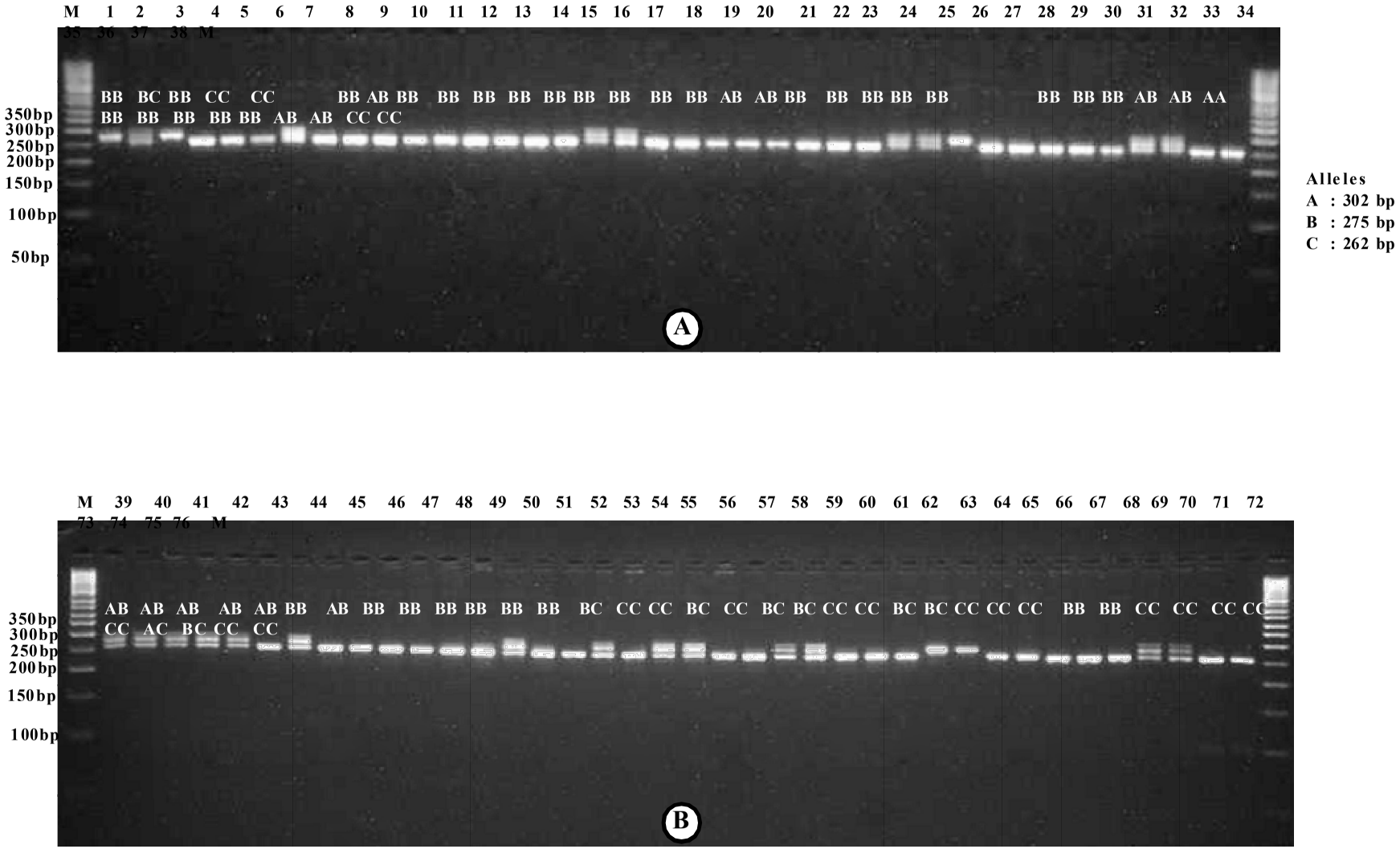


Fig. 4.2.6: Allelic profiling of MCW0007 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: 50bp DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

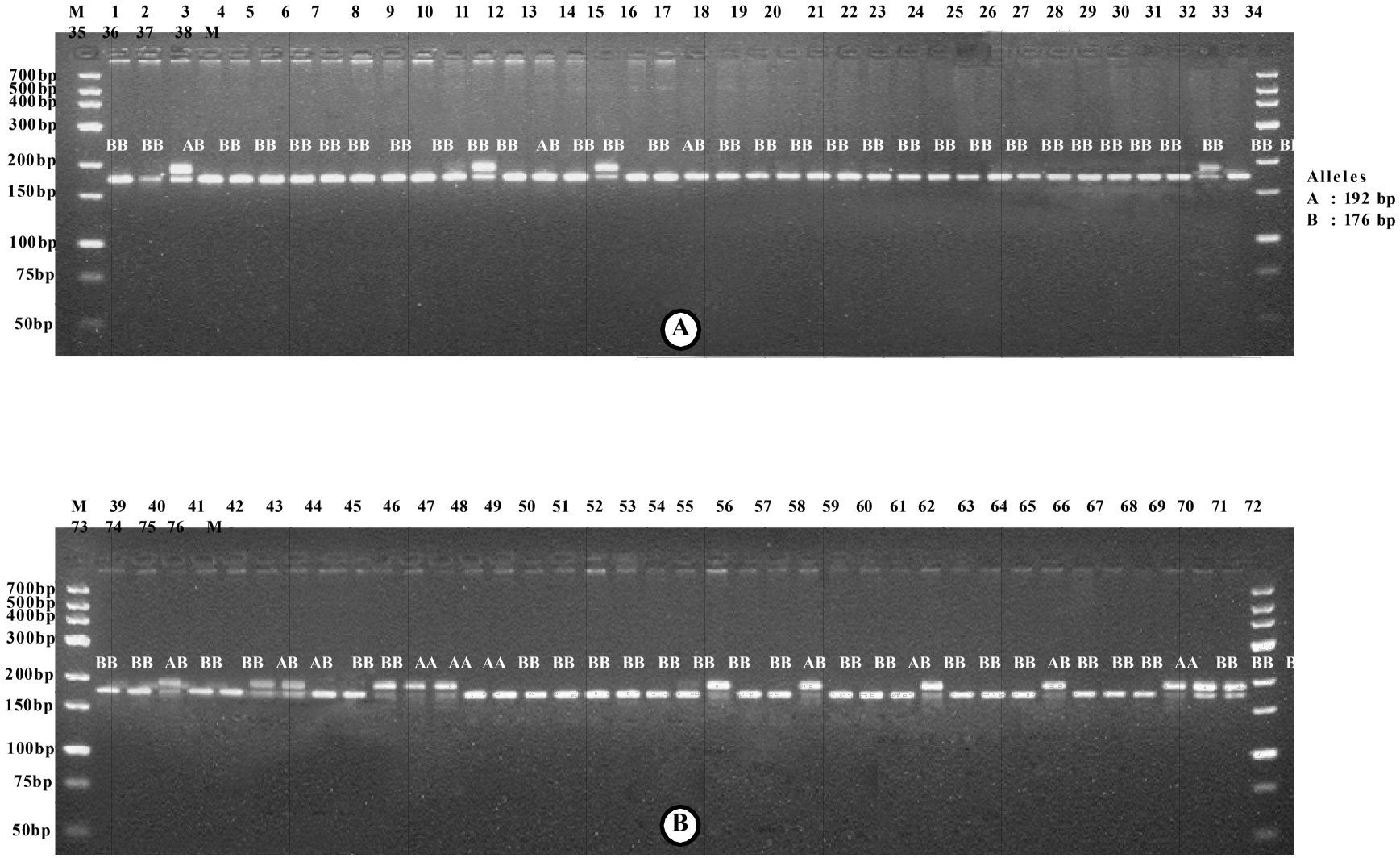


Fig. 4.2.7: Allelic profiling of MCW0014 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

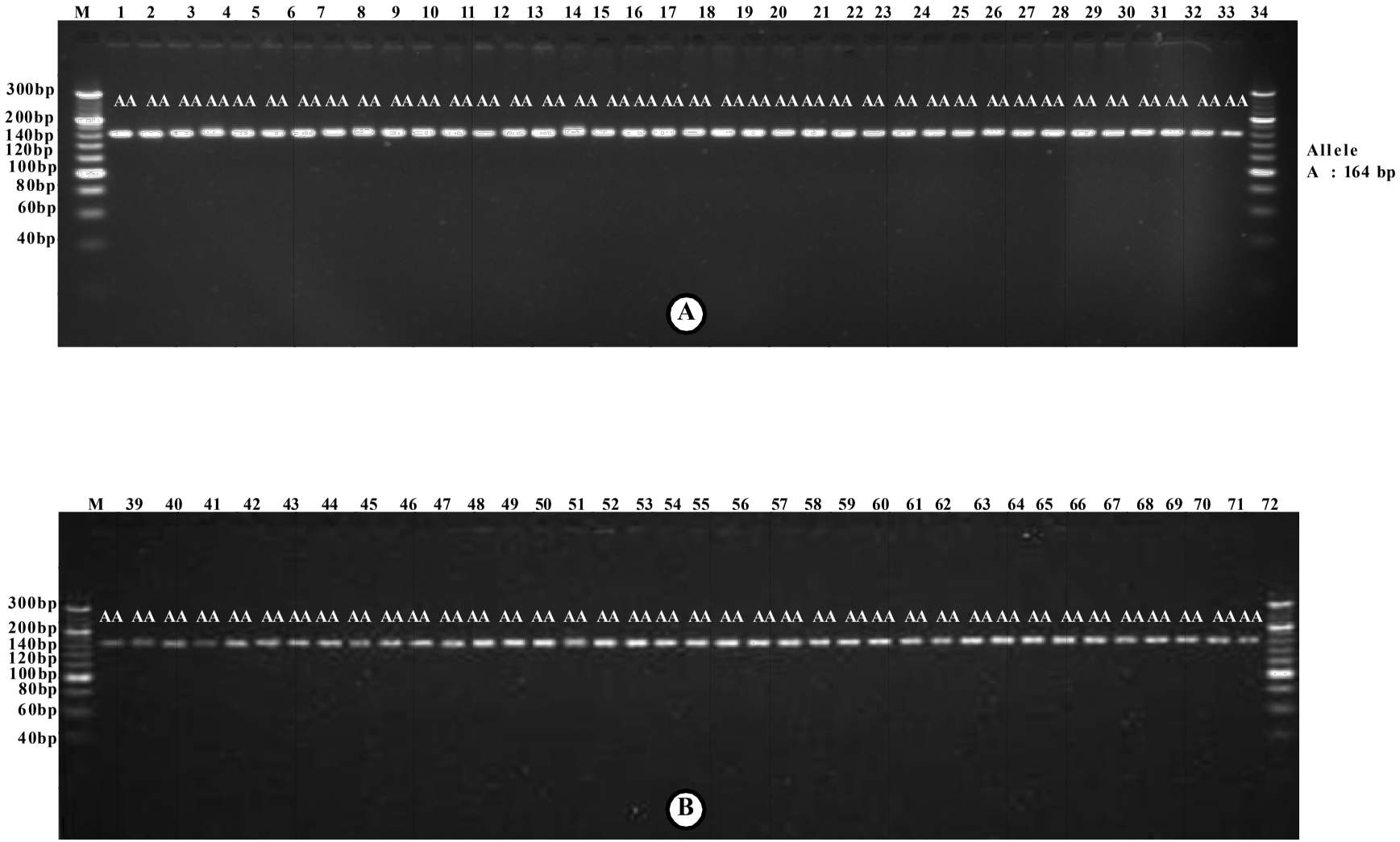


Fig. 4.2.8: Allelic profiling of MCW0041 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

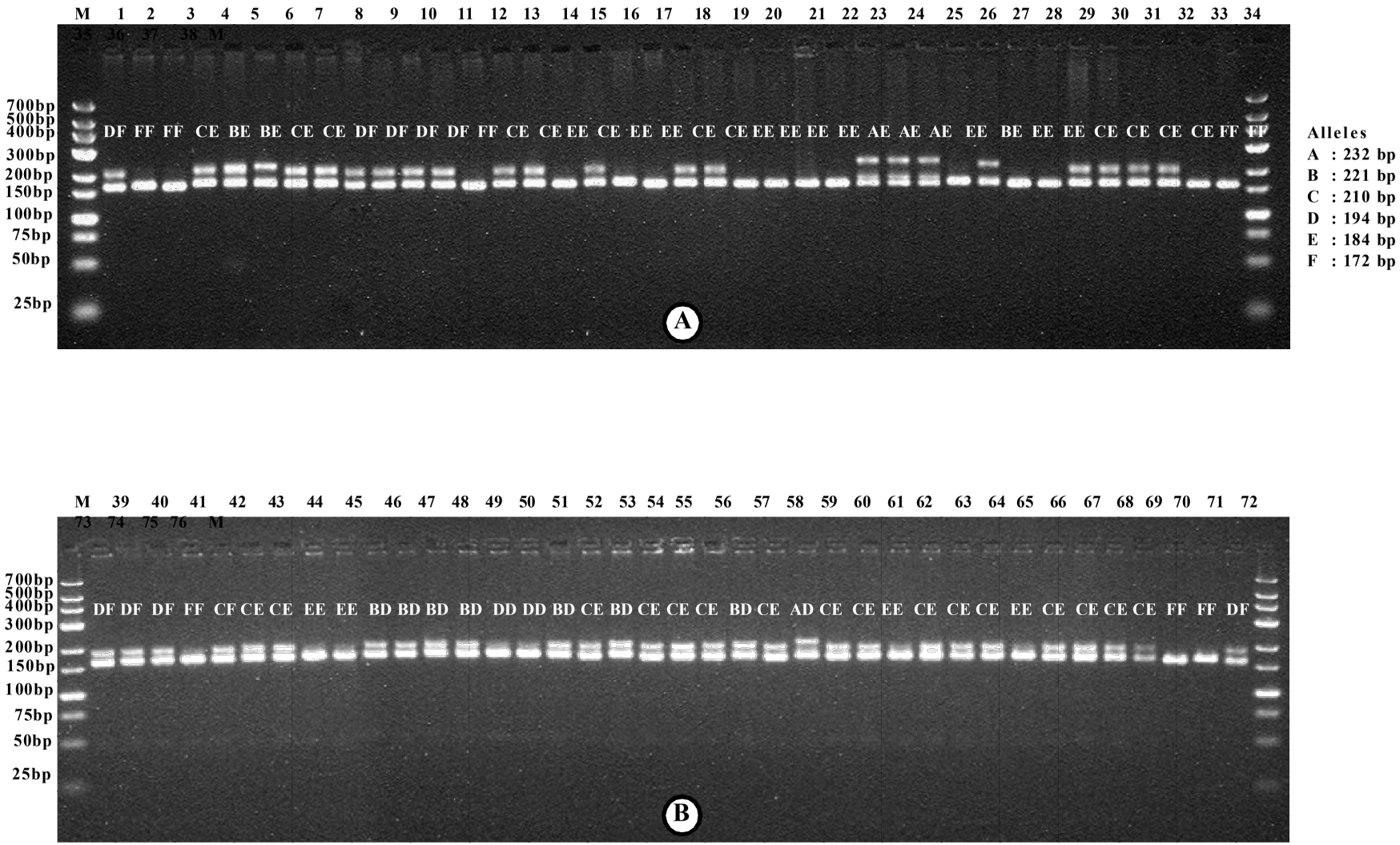


Fig. 4.2.9: Allelic profiling of MCW0069 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

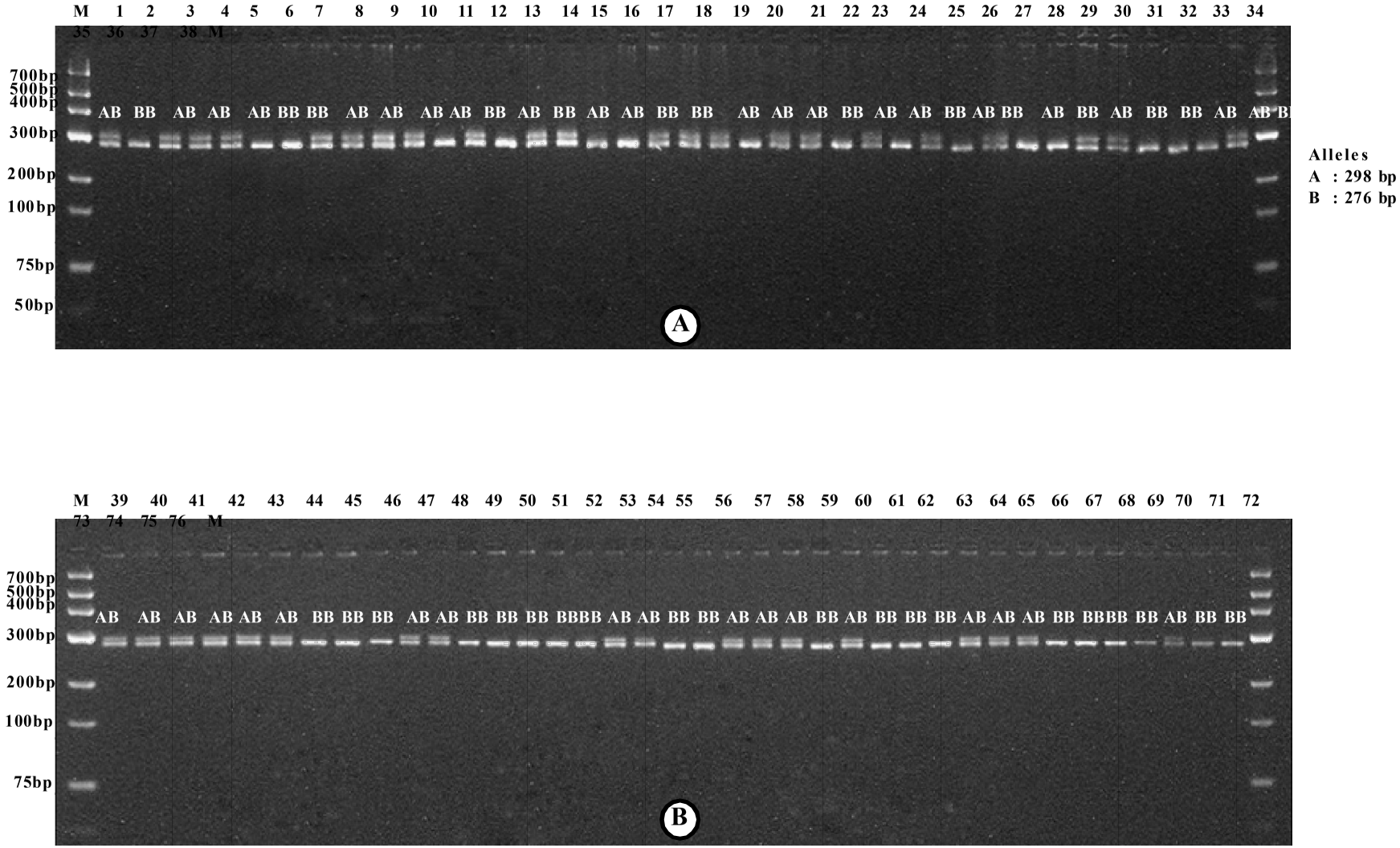


Fig. 4.2.10: Allelic profiling of MCW0103 microsatellite locus in RIR chicken.
Lanes (From left to right) - M: low range DNA ladder; (A) 1-38 and (B) 39-76 Females. Alleles with their sizes have been shown on the right side; size of the molecular marker on the left side and the genotypes above the amplicons in each lane

Loci was intermediate to high and it was more than 50% at ADL0102, ADL0176, MCW0007, and MCW0069 loci, respectively. PIC values were low to intermediate and it was more than 50% for ADL0102 and ADL0176 loci respectively.

4.2.2.2. Genic variation

Genetic variance at each locus is indicated by not only number of alleles and PIC but also by effective number of alleles and Shannon's index (I). The observed (N_a) and effective number (N_e) of alleles and the Shannon's index for each MS loci are presented in **table 4.2.4.**

Table 4.2.4: Number of observed and expected alleles and genic variation at various microsatellite loci in RIR chickens

MS loci	N_a	N_e	I
ADL0020	4	1.5655	0.6893
ADL0023	3	1.7273	0.7534
ADL0102	3	2.9260	1.0864
ADL0176	4	2.9462	1.1910
ADL0210	2	1.1108	0.2062
MCW0007	3	2.2245	0.9133
MCW0014	2	1.3126	0.4016
MCW0041	1	1.0000	0.0000
MCW0069	6	3.8778	1.5246
MCW0103	2	1.6335	0.5763
Mean ± SE	**3.0000±1.4142**	**2.0324± 0.9416**	**0.7342± 0.4649**

N_a = Observed number of alleles; N_e = Effective number of alleles and I = Shannon's index.

The mean ± SE of observed and effective number of alleles and Shannon's index were 3.0000±1.4142, 2.0324± 0.9416 and 0.7342± 0.4649. Effective number of allele ranged from 1.1108 (ADL0210) to 3.8778 (MCW0069). The difference between observed and expected number of allele was high for ADL0020 MS locus. Effective number of alleles at each locus was less than the observed number of alleles indicating the prevalence of heterozygosity at each locus.

4.2.2.3. Hardy-Weinberg equilibrium

The population under study was investigated for it being in Hardy-Weinberg equilibrium through Chi-square and G square likelihood ratio test. The results of observed and expected heterozygosities, chi-square test and likelihood ratio test are presented in **table 4.2.5**.

Table 4.2.5: Observed and expected heterozygosities, statistics of Chi-square ($\chi 2$) and likelihood ratio (G-square) test for Hardy-Weinberg equilibrium at various microsatellite loci in RIR chickens

MS loci	H_o	H_e	df	Chi square	G square
ADL0020	0.1316	0.3636	6	68.642***	66.660***
ADL0023	0.2105	0.4238	3	88.571***	82.570**
ADL0102	0.4079	0.6626	3	46.305***	52.272***
ADL0176	0.3289	0.6650	6	56.104***	59.279***
ADL0210	0.0263	0.1004	1	46.991***	17.091***
MCW0007	0.2895	0.5541	3	40.692***	41.228***
MCW0014	0.1711	0.2397	1	6.511**	5.014*
MCW0041	0.0000	0.0000	-	-	-
MCW0069	0.6842	0.7470	15	110.640***	114.805***
MCW0103	0.5263	0.3904	1	9.411**	14.248***
Mean ±SE	**0.2776 ±0.211**	**0.4147 ±0.2491**			

*H_o = Observed heterozygosity; H_e = Expected heterozygosity; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$*

The mean ± SE of observed and expected heterozygosity were 0.2776±0.211and 0.4147±0.2491, respectively. The results of Chi-square test and likelihood ratio test revealed that the population under study was in Hardy-Weinberg disequilibrium at all loci.

4.2.2.4 Association of microsatellite genotype with growth and layer economic traits.

Out of ten microsatellites studied, only nine microsatellites revealed polymorphism. All the experimental birds were genotyped for each of these nine polymorphic microsatellite loci.

Least squares analysis of variance was carried out to determine the effect of MS genotypes on layer economic traits by least squares analysis of variance wherein MS genotype was taken as independent factor. Out of nine polymorphic microsatellites, only five microsatellites

were found to have significant effect on growth and or layer economic traits. The microsatellite loci-wise LS analysis of variance and LS means of various economic traits are given below: -

4.2.2.4 .1 Association of ADL0020 locus with growth and layer economic traits

Least squares ANOVA and ADL0020 MS genotype- wise least square means are presented in **table 4.2.6** and **4.2.7.** Least squares analysis of variance revealed significant effect of sire on AFE, BW16 and EP40. ADL0020 MS-genotype had significant ($P \leq 0.05$) effect on BW16, BW20, BW40, BW64, EP40, EP64 and also on EW64 ($P \leq 0.08$).

The pullets with AD genotype recorded highest **BW16** (1887.09±189.63g) which did not differ significantly with pullets having BD genotype (1530.38±106.71g), it was followed by pullets with BC (1220.63 ±124.24g), DD (1217.09 ±145.09g), CC (1200.63 ±29.90g) and AC (1110.63 ±175.25g) genotypes, which did not differ among themselves.

In case of **BW20**, pullets with AD genotype at ADL0020 locus revealed highest value, 2024.24±247.04g, although it was statistically non-significantly different than pullets with BD (1906.93 ±139.02g) and BC (1836.52±161.86g) genotypes. The pullets with BC and DD (1599.24±189.01) genotypes did not differ significantly ($P \leq 0.05$) but had significantly more BW20 than birds with CC (1490.83 ±38.95g) and AC (1260.83±228.3g) genotypes, although the difference between CC and AC genotypes was not significant.

Birds with AD genotype revealed highest **BW40** (2820.83±332.80g) which was followed by statistically not different BD (2131.75±187.33g), DD (2072.83b±254.65g) and BC (2016.78±218.08g) genotypes and then by statistically not different BC and CC (1690.19±52.72g) genotypes. The AC genotype revealed lowest BW40 (1268.19±307.57g), which although was not statistically different than CC genotyped birds.

The birds with AD genotype at ADL0020 revealed significantly ($P \leq 0.05$) higher **BW64** (3414.29±408.87g) than the birds with any other genotype. Birds having BD (2681.29±230.58g), DD (2382.29±313.05g) and BC (2365.43±268.24g) genotypes did not differ significantly for BW64. Birds with BC genotype and CC (2013.29±66.91g) and AC (1720.62±394.98g) genotypes were statistically not significantly different.

The pullets with AD genotype at ADL0020 demonstrated highest **EW64**, 67.06±6.26g, than those with other genotypes (P≤0.08), viz., BC (54.31±3.78g), AC (51.98±5.79g), CC (51.04±1.09g), DD (50.57±5.09g) and BD (49.83±5.28g) genotypes, which did not differ significantly among themselves.

The egg production up to 40 weeks (**EP40**) was highest in birds with CC (122.7±3.14 eggs), AC (111.9±18.41eggs) and BC (100.4±13.05eggs) genotypes at ADL0020; the genotypes did not differ significantly. Pullets with AC, BC, DD, (80.29±15.24 eggs), BD (62.40±11.21 eggs) and AD (61.29±19.92 eggs) genotypes did not differ significantly (P≤0.01) for EP40.

The egg production up to 64 weeks of age (**EP64**) was highest in the birds with CC (214.17±9.07 eggs) genotype at ADL0020, which however did not differ significantly with AC (197.77±53.14 eggs), BC (186.67±37.68 eggs), DD (167.18±44.00 eggs) and AD (136.18±57.50 eggs) genotypes. The difference in EP64 between AD and BD (70.58±32.36 eggs) genotypes was not significant.

4.2.2.4.2. Association of ADL0023 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.8** and **4.2.9**. Least squares analysis of variance revealed non- significant (P>0.05) effect of sire on all the traits. ADL0023 MS-genotype had significant effect on AFE and EP40.

The pullets with CC genotype at ADL0023 MS locus revealed lowest **age at first egg** viz., 132.72±1.25 days whish was statistically not different than those pullets having AB (139.05±2.74 days) or BB (141.44±3.90 days) genotypes. AC genotyped birds with 155.09±6.29 days AFE revealed the highest AFE which was statistically not different than BB genotyped pullets.

The AB genotype at ADL0023 locus demonstrated the highest **EP40** with 116.92±6.21 eggs which however did not differ statistically with CC (115.57±2.82 eggs) and BB (105.21±8.83 eggs) genotypes. The pullets with AC genotype demonstrated the lowest EP40 with 72.20±14.25 eggs.

Table 4.2.6 Least squares analysis of variance for effect of ADL0020 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0293*	0.0032*	0.3596	0.7276	0.2481	0.1511	0.6107	0.1869	0.0175*	0.2884
Genotype	5	0.3802	0.0042*	0.0145*	0.0186*	0.0182*	0.1482	0.3599	0.0742	0.0005*	0.0045*

df: degree of freedom

Table 4.2.7: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite ADL0020 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AC	1	132.43	1110.63	1260.83	1268.19	1720.62	41.86	44.94	51.98	111.9	197.77
		±9.76	±175.25^{b}	±228.3^{c}	±307.57^{d}	±394.98^{c}	±3.78	±4.96	±5.79^{b}	±18.41^{ab}	±53.14^{a}
AD	1	128.87	1887.09	2024.24	2820.83	3414.29	52.08	#	67.06	61.29	136.18
		±10.56	±189.63^{a}	±247.04^{a}	±332.80^{a}	±408.87^{a}	±4.50		±6.26^{a}	±19.92^{b}	±57.50^{ab}
BC	2	150.5	1220.63	1836.52	2016.78	2365.43	46.47	49.32	54.31^{b}	100.4	186.67
		±6.92	±124.24^{b}	±161.86^{ab}	±218.08^{bc}	±268.24^{bc}	±2.69	±3.45	±3.78	±13.05^{ab}	±37.68^{a}
BD	6	130.64	1530.38	1906.93	2131.75	2681.29	50.22	52.20	49.83	62.40	70.58
		±5.94	±106.71^{a}	±139.02^{a}	±187.33^{b}	±230.58^{b}	±3.28(5)	±2.97(5)	±5.28(2)^{b}	±11.21^{b}	±32.36^{b}
CC	58	136.03	1200.63	1490.83	1690.19	2013.29	44.03	47.50	51.04	122.7	214.17
		±1.67	±29.90^{b}	±38.95^{c}	±52.72(57)^{cd}	±66.91(1)^{c}	±0.73	±0.84(55)	±1.09(43)^{b}	±3.14^{a}	±9.07^{a}
DD	8	128.37	1217.09	1599.24	2072.83	2382.29	44.06	46.30	50.57	80.29	167.18
		±8.08	±145.09^{b}	±189.01^{bc}	±254.65^{b}	±313.05^{b}	±3.65	±4.03	±5.09(6)^{b}	±15.24^{b}	±44.00^{a}

Notes: Geno: Genotype; N= number of observations; Different superscripts in a column represent statistically different means at P≤0.01 for BW16, EP40 and EP64; P≤0.05 for BW20, BW64, BW40 and P≤0.08 for EW64. Figures in parentheses represent the number of observations. # indicates nil observation.

Table 4.2.8: Least squares analysis of variance for effect of ADL0023 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.2536	0.0513	0.4088	0.9202	0.6906	0.1648	0.5550	0.0583	0.8546	0.6370
Genotype	3	0.0018*	0.6656	0.8224	0.7881	0.1974	0.4079	0.5268	0.3760	0.0207*	0.6817

df: degree of freedom

Table 4.2.9: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite ADL0023 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AB	14	139.05 ±2.74[a]	1236.51 ±60.56	1548.13 ±77.39	1742.75 ±103.75	2070.41 ±132.97(12)	45.86 ±1.20	49.79 ±1.55(13)	53.47 ±2.06(9)	116.92 ±6.21[a]	192.51 ±18.35
AC	2	155.09 ±6.29[b]	1055.33 ±139.04	1393.85 ±177.69	1543.23 ±238.21	1617.35 ±291.82	41.01 ±2.75	46.47 ±3.49	45.41 ±4.33	72.20 ±14.25[b]	167.60 ±42.14
BB	5	141.44 ±3.90[ab]	1224.96 ±86.14	1599.09 ±110.08	1777.15 ±164.83(4)	1878.56 ±199.35(4)	44.41 ±1.70	47.32 ±2.42(4)	50.40 ±2.80(4)	105.21 ±8.83[a]	179.66 ±26.11
CC	55	132.72 ±1.25[a]	1232.61 ±27.54	1535.54 ±35.20	1785.19 ±47.23	2157.94 ±58.73(51)	44.23 ±0.55(54)	47.21 ±0.70(54)	51.41 ±0.93(40)	115.57 ±2.82[a]	204.68 ±8.35

Notes: Geno: Genotype; N= number of observations; Different superscripts in a column represent statistically different means at P≤0.01 for AFE and P≤0.05 for EP40. Figures in parentheses represent the number of observations.

4.2.2.4.3 Association of ADL0102 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.10** and **4.2.11**. Least squares analysis of variance revealed significant effect of sire on AFE, BW16 and EW28 ($P \leq 0.05$). ADL0102 MS-genotype did not have significant ($P > 0.05$) effect on any of the body weights and layer economic traits.

4.2.2.4.4 Association of ADL0176 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.12** and **4.2.13**. This analysis of variance revealed significant effect of sire on BW16 only ($P \leq 0.05$). MS-genotype at ADL0176 had no significant ($P > 0.05$) effect on body weights and layer economic traits.

4.2.2.4.5 Association of ADL0210 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.14** and **4.2.15**. Least squares analysis of variance revealed significant effect of sire AFE, BW16 and EW64 ($P \leq 0.05$). MS-genotype at ADL0210 MS locus had significant effect on AFE, BW16, BW20, BW40, BW64, EW28, EW40 and EW64.

The lowest **AFE** (134.80±1.04 days) was observed in BB genotypes followed by AA (135.80±6.02 days) and then AB (159.55±9.54 days) genotypes. The difference between BB and AA was not significant.

The highest **BW16** was observed in birds with AA genotype (1500.73±119.34g) followed by AB (1398.23±189.15g) and then by BB (1215.73±20.67g) genotypes. The differences in BW16 between AA and AB and AB and BB were not significant.

In case of body weight at 20 weeks of age (**BW20**), the AB genotyped birds revealed highest BW16 (2242.97±230.24g) followed by AA (1771.30±145.26g) and BB (1517.97±25.16g) genotypes. However, the difference between AB and AA was not significant.

AB genotype at ADL0210 revealed 2518.61±315.47g **BW40** which was statistically not significantly different than that of AA genotypes with 2127.61±199.08g but both genotypes

revealed significantly (P≤ 0.05) higher BW40 than pullets of BB genotype with 1744.61±34.87g BW40.

Similarly, AB genotyped birds had 2999.85±389.43g **BW64** which was statistically not significantly different than that of AA genotype with 2561.19±246.07g but both genotypes revealed significantly (P≤ 0.05) higher BW64 than pullets of BB genotype with 2076.85±45.91g BW64.

Likewise, the pullets with AB genotype demonstrated **EW28** as 54.88±3.59g which was statistically not significantly different than that of AA genotyped birds having EW28 as 49.23±2.26g. But, both AB and AA genotypes revealed significantly (P≤ 0.05) higher EW28 as compared to BB genotyped pullets with 44.11±0.39g EW28.

The AB genotyped pullets revealed **EW40** as 57.71±4.55g which did not differ significantly from AA genotyped pullets who demonstrated 56.38±3.33g EW40. However, both AB and AA genotypes revealed significantly (P≤0.05) higher EW40 in comparison to BB genotyped birds with 47.32±0.51g EW40.

The AA genotyped pullets demonstrated 67.56±4.73g **EW64** which did not differ significantly from AB genotyped pullets producing 62.82±4.81g EW64. However, both AB and AA genotypes revealed significantly (P≤0.05) higher EW64 in comparison to BB genotype showing 50.87±0.63g EW64.

4.2.2.4.6 Association of MCW0007 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.16** and **4.2.17**. Least squares analysis of variance revealed significant (P≤0.01) effect of sire on AFE and BW16 only. MCW0007 MS-genotype had significant effect on BW40 only.

Out of the six genotypes observed, the pullets of AC genotype demonstrated highest **BW40** with 2497.32±325.31g, followed by genotypes CC (1871.43±84.55g) and BB (1783.00±63.51g). The difference in BW40 between CC and BB genotypes was not significant. Pullets with BB, AB (1646.89±104.35), BC (1543.11±120.41) and AA (1347.40±301.81) did not differ significantly (P≤0.05) for BW40.

Table 4.2.10: Least squares analysis of variance for effect of ADL0102 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0162*	0.0326*	0.5154	0.6547	0.4172	0.0402*	0.7687	0.1047	0.8673	0.5216
Genotype	5	0.1190	0.7612	0.9499	0.2013	0.5041	0.2346	0.7665	0.3743	0.9803	0.8349

df: degree of freedom

Table 4.2.11: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite ADL0102 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AA	7	141.37	1248.78	1575.21	2119.92	2489.71	46.41	49.31	55.59	112.57	212.21
		±4.45	±92.64	±119.19	±153.79(6)	±234.80(4)	±1.79	±2.38(6)	±2.82	±10.30	±28.18
AB	23	130.06	1241.63	1545.49	1780.34	2160.69	44.75	48.40	51.44	111.48	189.08
		±2.28	±47.61	±61.25	±78.10	±99.68	±0.92(22)	±1.21(22)	±1.57(5)	±5.29	±14.48
AC	7	138.88	1092.59	1460.72	1507.24	1853.88	41.56	44.94	44.26	113.69	209.94
		±4.39	±91.55	±117.79	±150.24	±191.65	±1.77	±2.47	±3.79(18)	±10.18	±27.85
BB	19	138.85	1263.38	1554.99	1794.34	2033.82	45.97	48.47	52.52	115.66	213.69
		±2.61	±54.46	±70.06	±89.54	±122.73(15)	±1.05(17)	±1.51(11)	±1.81(6)	±6.06	±16.56
BC	1	138.45	1287.46	1325.98	1844.29	2180.99	39.16	42.66	51.24	101.61	165.61
		±9.48	±197.49	±254.07	±323.99	±412.25	±3.81	±4.99	±5.87(14)	±21.96	±60.07
DD	19	133.33	1203.97	1529.60	1665.25	2057.71	42.93	46.69	50.22	115.02	189.87
		±2.56	±53.32	±68.60	±87.54	±111.97	±1.03	±1.39	±1.89	±5.93	±16.22

Notes: Geno: Genotype; N= number of observations; Figures in parentheses represent the number of observations.

Table 4.2.12: Least squares analysis of variance for effect of ADL0176 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0694	0.0208*	0.2811	0.9299	0.7867	0.7320	0.9650	0.5920	0.7644	0.6491
Genotype	7	0.4771	0.2099	0.7816	0.7416	0.8899	0.9026	0.9331	0.9701	0.8764	0.8399

df: degree of freedom

Table 4.2.13: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite ADL0176 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AB	2	138.63	1159.19	1562.01	1791.42	2220.09	44.75	48.83	50.29	110.78	207.03
		±7.37	±142.15	±189.50	±253.67	±322.06	±3.01	±3.83	±4.64	±16.45	±45.36
AC	6	140.36	1208.21	1481.16	1605.98	1989.22	43.03	47.26	49.55	105.56	181.84
		±4.12	±79.43	±105.89	±155.91(5)	±202.64(5)	±1.68	±2.35(3)	±3.51(3)	±9.19	±25.35
BB	10	140.94	1155.83	1558.50	1663.15	1988.62	44.92	47.98	49.73	103.68	179.41
		±4.39	±84.69	±112.90	±151.53	±203.12(9)	±1.79	±2.30	±3.40(5)	±9.80	±27.02
BC	6	132.57	1072.09	1352.98	1709.21	1975.00	43.99	47.18	50.77	110.75	178.91
		±4.34	±83.71	±111.59	±149.39	±189.99	±1.77	±2.28	±2.91(5)	±9.69	±26.71
BD	9	137.82	1160.40	1576.22	1880.10	2142.84	43.72	46.78	50.84	113.75	222.26
		±3.89	±75.03	±100.03	±133.94	±171.97(8)	±1.59	±2.06	±2.52(8)	±8.68	±23.94
CC	29	134.73	1273.76	1524.48	1808.19	1822.64	45.40	49.23	52.47	111.89	185.74
		±2.28	±44.03	±58.69	±78.90	±104.80(27)	±0.94(28)	±1.24(27)	±1.54(23)	±5.10	±14.05
CD	2	130.46	1091.86	1462.48	1516.47	1895.49	44.87	46.52	53.96	125.98	215.05
		±8.22	±158.61	±211.44	±283.05	±356.66	±3.36	±4.27	±6.42(1)	±18.36	±50.61
DD	12	128.89	1309.49	1619.99	1797.38	2111.25	43.13	44.90	50.50	128.80	243.25
		±4.91	±94.76	±126.33	±169.23	±216.18(10)	±2.01	±2.56(11)	±3.17(8)	±10.97	±30.24

Notes: Geno: Genotype; N= number of observations; Figures in parentheses represent the number of observations.

Table 4.2.14: Least squares analysis of variance for effect of ADL0210 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0114*	0.0351*	0.3643	0.9516	0.6560	0.2238	0.5272	0.0276*	0.7461	0.6536
Genotype	2	0.0433*	0.0462*	0.0031*	0.0121*	0.0136*	0.0020*	0.0037*	0.0005*	0.6869	0.5958

df: degree of freedom

Table 4.2.15: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite ADL0210 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AA	3	135.80	1500.73	1771.30	2127.61	2561.19	49.23	56.38	67.56	113.47	165.25
		±6.02[a]	±119.34[a]	±145.26[ab]	±199.08[a]	±246.07[a]	±2.26[a]	±3.33(2)[a]	±4.73(1)[a]	±13.65	±37.67
AB	1	159.55	1398.23	2242.97	2518.61	2999.85	54.88	57.71	62.82	94.97	175.91
		±9.54[b]	±189.15[ab]	±230.24[a]	±315.47[a]	±389.43[a]	±3.59[a]	±4.55[a]	±4.81[a]	±21.64	±59.61
BB	72	134.80	1215.73	1517.97	1744.61	2076.85	44.11	47.32	50.87	113.97	200.91
		±1.04[a]	±20.67[b]	±25.16[b]	±34.87(71)[b]	±45.91(65)[b]	±0.39(71)[b]	±0.51(69)[b]	±0.63(53)[b]	±2.36	±6.52

Notes: Geno: Genotype; N= number of observations; Different superscripts in a column represent statistically different means at P≤0.01 for BW20, EW28, EW40, EW64 and P≤0.05 for AFE, BW16, BW40, BW64. Figures in parentheses represent the number of observations.

Table 4.2.16: Least squares analysis of variance for effect of MCW0007 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0092*	0.0099*	0.1566	0.6888	0.3402	0.1583	0.7547	0.0679	0.5991	0.8851
Genotype	4	0.1685	0.0848	0.1078	0.0155*	0.1678	0.1391	0.4731	0.5311	0.4548	0.5337

df: degree of freedom

Table 4.2.17: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite MCW0007 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AA	1	136.70	1226.39	1523.51	1347.40	1787.99	43.35	#	44.04	86.02	159.31
		±9.42	±183.64	±234.69	±301.81^{c}	±393.06	±3.71		±5.61	±20.96	±58.25
AB	13	135.24	1198.38	1420.63	1646.89	2057.47	42.96	46.31	48.66	103.06	177.47
		±3.26	±63.48	±81.13	±104.35^{c}	±137.71(11)	±1.28	±1.67	±2.35	±7.25	±20.14
AC	1	112.55	1765.71	2162.19	2497.32	2877.24	53.96	52.35	59.70	134.84	295.94
		±10.15	±197.92	±252.94	±325.31^{a}	±424.27	±4.00	±5.21	±6.12	±22.59	±62.78
BB	34	137.88	1193.59	1504.67	1783.00	2023.80	43.84	47.51	50.76	109.60	195.84
		±1.97	±38.41	±49.09	±63.51(33)^{bc}	±84.34(32)	±0.78(33)	±1.03(32)	±1.41(26)	±4.38	±12.18
BC	8	137.34	1158.48	1486.34	1543.11	1912.30	43.55	45.93	51.97	119.63	194.65
		±3.75	±73.17	±93.51	±120.41^{c}	±168.62(6)	±1.48	±1.98(7)	±3.09(4)	±8.35	±23.21
CC	19	132.15	1279.33	1625.11	1871.43	2272.71	45.83	49.01	53.15	122.45	213.59
		±2.63	±51.31	±65.58	±84.55^{b}	±115.62(18)	±1.04	±1.36	±1.95(15)	±5.86	±16.28

Notes: Geno: Genotype; N= number of observations; Different superscripts in a column represent statistically different means at Pd"0.05 for BW40. Figures in parentheses represent the number of observations. # indicates nil observation.

Table 4.2.18: Least squares analysis of variance for effect of MCW0014 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0759	0.0549	0.2362	0.5468	0.5264	0.1172	0.6473	0.0422*	0.7891	0.5700
Genotype	2	0.3883	0.0531	0.2467	0.0316*	0.2050	0.1044	0.7849	0.1569	0.9211	0.1909

df: degree of freedom

Table 4.2.19: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite MCW0014 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AA	4	128.94 ±5.10	1218.26 ±97.78[ab]	1401.89 ±127.41	1283.94 ±182.31(3)[b]	1714.30 ±236.91(3)	40.51 ±1.97[b]	45.80 ±2.84(3)	43.58 ±4.00(2)	112.96 ±11.21	157.57 ±30.24
AB	13	137.19 ±2.69	1111.66 ±51.55[b]	1470.04 ±67.18	1805.10 ±87.19[a]	2071.57 ±116.01(12)	44.07 ±1.04[a]	47.60 ±1.36	51.94 ±1.69(11)	115.85 ±5.91	220.25 ±15.94
BB	59	135.31 ±1.27	1256.86 ±24.32[a]	1566.37 ±31.68	1793.49 ±41.14[a]	2149.88 ±57.21(54)	44.88 ±0.49(58)[a]	47.83 ±0.65(56)	51.58 ±0.86(42)	113.14 ±2.79	197.96 ±7.22

otes: Geno: Genotype; N= number of observations; Different superscripts in a column represent statistically different means at P≤0.05 for BW40; P≤0.06 for BW16 and P≤0.11 for EW28. Figures in parentheses represent the number of observations.

Table 4.2.20: Least squares analysis of variance for effect of MCW0069 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.1866	0.0514	0.2415	0.7687	0.7717	0.2418	0.7393	0.1668	0.7983	0.5564
Genotype	9	0.5840	0.2853	0.2441	0.6089	0.1300	0.7451	0.8198	0.6886	0.6755	0.3953

df: degree of freedom

Table 4.2.21: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite MCW0069 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AD	1	131.51 ±9.80	1106.95 ±188.62	1515.74 ±238.50	1537.15 ±331.87	2054.95 ±387.31	46.11 ±3.93	50.65 ±5.01	55.02 ±5.95	121.76 ±21.42	211.84 ±57.82
AE	3	126.43 ±7.15	1350.47 ±137.64	1383.86 ±174.03	1779.23 ±242.69	2607.39 ±285.93	40.61 ±2.87	42.72 ±3.83	51.70 ±4.40	125.51 ±15.63	203.46 ±42.19
BD	7	137.10 ±5.09	1310.46 ±97.93	1687.09 ±123.82	1918.05 ±191.03(6)	1934.03 ±241.61(6)	46.33 ±2.04	50.86 ±2.96(6)	50.59 ±4.07(4)	113.36 ±11.12	216.74 ±30.02
BE	2	140.25 ±6.93	980.06 ±133.26	1182.34 ±168.49	1396.59 ±234.49	1645.40 ±274.53	42.36 ±2.78	46.61 ±3.56	49.48 ±4.18	118.50 ±15.13	233.49 ±40.85
CE	29	133.99 ±1.99	1221.84 ±38.20	1560.11 ±48.30	1801.54 ±67.28	2118.51 ±83.11(27)	44.58 ±0.80(28)	48.00 ±1.04(28)	51.73 ±1.47(20)	109.19 ±4.34	195.64 ±11.71
CF	1	127.95 ±10.17	1434.97 ±195.62	1580.65 ±247.34	1716.69 ±344.16	2666.51 ±413.51	42.21 ±4.08	47.96 ±5.20	#	75.36 ±22.22	67.55 ±59.97
DD	2	141.10 ±9.46	1243.80 ±181.95	1703.75 ±230.06	1713.38 ±330.57	1216.03 ±469.39(1)	43.37 ±3.79	46.17 ±5.03	42.43 ±6.47	133.19 ±20.66	289.57 ±55.78
DF	9	131.96 ±4.20	1180.52 ±80.75	1379.11 ±102.10	1610.82 ±142.11	1876.93 ±179.64(7)	43.34 ±1.69	46.12 ±2.16	49.71 ±2.90(7)	113.06 ±9.17	180.53 ±24.75
EE	14	136.09 ±3.21	1322.47 ±61.85	1643.86 ±78.21	1911.16 ±110.06	2361.12 ±135.59(13)	45.30 ±1.30	47.69 ±1.77(12)	54.76 ±2.37(11)	119.64 ±7.02	216.86 ±18.96
EF	8	140.86 ±3.86	1071.47 ±74.18	1395.81 ±93.79	1601.46 ±130.58	2134.09 ±154.56	43.86 ±1.55	47.27 ±2.00	51.19 ±2.88(5)	114.42 ±8.42	171.51 ±22.74

Notes: Geno: Genotype; N= number of observations; Figures in parentheses represent the number of observations. # indicates nil observation

Table 4.2.22: Least squares analysis of variance for effect of MCW0103 microsatellite genotypes on growth and layer economic traits in RIR chicken

Source of variation	df	P values									
		AFE	BW16	BW20	BW40	BW64	EW28	EW40	EW64	EP40	EP64
Sire	11	0.0554	0.0222*	0.2744	0.8280	0.5819	0.1696	0.6278	0.0877	0.7763	0.6650
Genotype	1	0.8285	0.5543	0.9544	0.4234	0.5830	0.1906	0.9420	0.3374	0.9111	0.4214

df: degree of freedom

Table 4.2.23: Least square means ± standard errors of growth and layer economic traits for different genotypes at microsatellite MCW0103 in RIR chicken

Geno	N	Least squares means± standard errors									
		AFE (days)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
AB	40	135.00 ±1.56	1213.28 ±30.77	1535.44 ±39.22	1800.33 ±53.82(39)	2078.41 ±68.88(36)	43.83 ±0.61(39)	47.72 ±0.81(38)	50.55 ±1.07(26)	113.36 ±3.38	193.99 ±9.30
BB	36	135.50 ±1.58	1240.52 ±31.31	1538.79 ±39.91	1736.54 ±53.29	2134.02 ±70.24(33)	45.04 ±0.62(36)	47.63 ±0.81(34)	52.03 ±1.05(29)	113.93 ±3.44	205.20 ±9.46

Notes: Geno: Genotype; N= number of observations; Figures in parentheses represent the number of observations.

4.2.2.4.7 Association of MCW0014 locus with growth and layer economic traits

Least squares ANOVA and MS genotype- wise least square means are presented in **table 4.2.18** and **4.2.19**. Least squares analysis of variance revealed significant effect of sire on EW64 only. MCW0014 MS-genotype had significant effect on BW16 ($P \leq 0.06$), BW40 ($P \leq 0.05$) and EW28 ($P \leq 0.11$).

The highest **BW16** was observed in pullets having BB genotype (1256.86±24.32g) which was statistically not significantly different than pullets with AA genotype (1218.26±97.78g). However, AA genotyped pullets did not differ significantly from AB genotyped pullets demonstrating BW16 as 1111.66±51.55g.

The highest **BW40** was observed in pullets having AB genotype (1805.10±87.19g), which was statistically not significantly different than pullets with BB genotype (1793.49±41.14g). Both, AB and BB genotyped pullets revealed significantly ($P \leq 0.05$) higher BW40 in comparison to AA genotyped pullets (1283.94±182.31g).

The **EW28** was observed to be highest in BB genotyped pullets showing mean as 44.88±0.49g which was statistically not different than that of AB genotyped pullets (44.07±1.04). Both, BB and AB genotypes revealed significantly ($P \leq 0.11$) higher EW28 in comparison to AA genotyped pullets (40.51±1.97g).

4.2.2.4.8 Association of MCW0069 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.20** and **4.2.21**. The analysis revealed significant ($P \leq 0.06$) effect of sire on BW16 only. The effect of MCW0069 MS-genotypes was non-significant ($P > 0.05$) on all the body weights and layer economic traits analyzed.

4.2.2.4.9 Association of MCW0103 locus with growth and layer economic traits

Least squares ANOVA and MS genotype-wise least square means are presented in **table 4.2.22** and **4.2.23**. Least squares analysis of variance revealed significant ($P \leq 0.05$) effect of sire on BW16 only. The effect of MCW0103 MS-genotypes was non-significant ($P > 0.05$) on all the body weights and layer economic traits analyzed.

4.3 Objective 3: To estimate immunocompetence traits and their association with layer economic traits in Rhode Island Red chicken

Evaluatation of various immunological traits is prerequisite for their incorporation into breeding programs to simultaneous genetic improvement of the immunocompetence and performance**.** Sera samples were collected from 253 RIR chicks as mentioned in objective no.1 for their assessment of immunocompetence (IC) traits. The Immunocompetence status were assessed by estimating *in vivo* antibody response against sheep Red Blood Cells (SRBC), serum lysozyme activity and serum IgG level. The data on immunocompetence traits was analyzed by mixed model least squares analysis of variance taking sire as random and sex as fixed effect in the model. The genetic parameters were estimated using paternal half-sib method.

The Least squares (LS) analysis of variance and factor wise least squares means for various IC traits are presented in **table 4.3.1** and **4.3.2**, respectively. Heritability, genetic and phenotypic correlations are presented in **table 4.3.5**.

4.3.1 Antibody titre against sheep RBCs

The antibody response against SRBC was estimated through Haemagglutination (HA) test (**Fig. 4.3.1**) on 5th day post immunization (dpi) in both sexes. Least square analysis of variance revealed that sire effect was non-significant (P>0.05) on serum HA titre (**Table 4.3.1**). The least square mean of HA titres reveled wide variability which ranged from 2-17. Average least square mean HA titre was 9.39 ± 0.22 and 9.33 ± 0.34 in males and females, respectively. The overall average HA titre was 9.35 ± 0.29 (**Table 4.3.2**).

4.3.2 Serum lysozyme concentrations

The serum lysozyme concentration was estimated using lysoplate assay method **(Fig. 4.3.2**) and its value was expressed in µg/ml. Least square analysis of variance is given in **table 4.3.1** which revealed non-significant (P>0.05) effect of sire on serum lysozyme concentration. The factor wise least square mean are presented in **table 4.3.2**. Serum lysozyme estimates ranged from 1.41 to 10.82 µg/ml. Average Least-squares mean of serum lysozyme level was 4.77±0.15 µg/ml and 4.51±0.16 µg/ml in male and females, respectively. The overall average was 4.64±0.11 µg/ml.

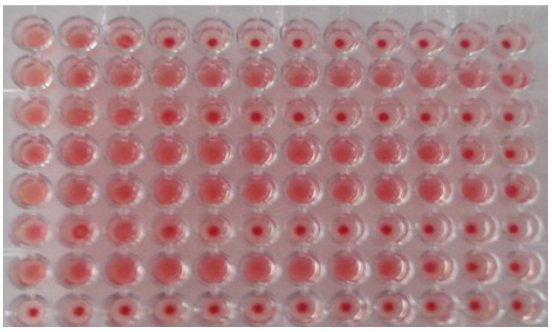


Fig. 4.3.1: Estimation of antibody response to SRBC in Rhode Island Red chicken using Heamagglutation test

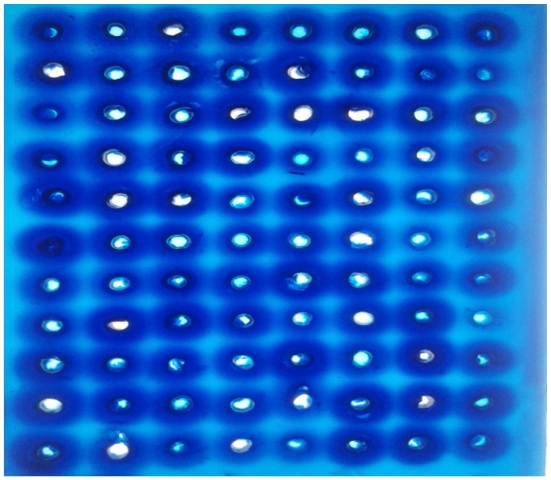


Fig. 4.3.2: Estimation of serum lysozyme concentration in Rhode Island Red chicken using Lysoplate assay

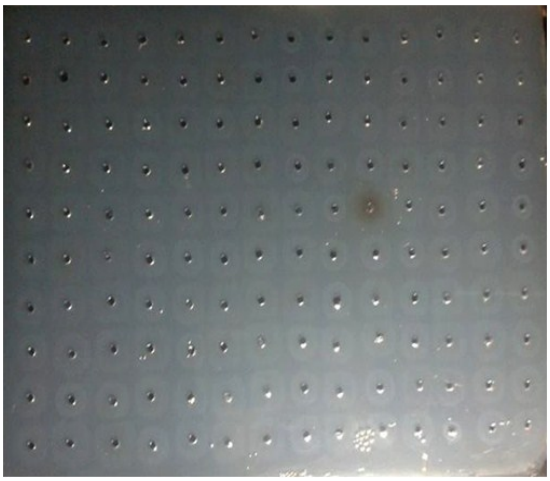


Fig. 4.3.3: Estimation of serum IgG concentration in RIR chicken using Single Radial immunodiffusion (SRID) Assay

4.3.3 Serum IgG concentration

Serum IgG concentration was estimated using Single Radial Immunodiffusion (SRID) assay method **(Fig. 4.3.3)**. The Least squares ANOVA revealed significant ($P \leq 0.01$) effect of sire on serum IgG level (**table 4.3.1)** and least square means of various factors are given in **table 4.3.2**. Overall least squares mean of serum IgG level was 8.61 ±0.34mg/ml. It was slightly higher in females (8.67 ±0.45 mg/ml) than males (8.56 ±0.42) although the difference was statistically non-significant.

4.3.4 Influence of sex on IC traits

The influence of sex on HA titre, serum lysozyme and serum IgG concentration was statistically non-significant ($P>0.05$), although males had higher antibody titre and serum lysozyme level than females and IgG concentration was somewhat higher in females (8.67±0.45 mg/ml) than males (8.56±0.42mg/ml).

Table 4.3.1: Least squares analysis of variance of immunocompetence traits in RIR chicken

Source of variation	Degrees of freedom	Mean Sum of squares		
		HA	Lysozyme	IgG
Sire	24	5.97	2.17	27.73*
Sex	1	0.03	3.74	0.68
Error/ Remainder	227	12.07	2.85	15.26

*($P \leq 0.01$)

Table 4.3.2: Factor-wise least squares means along with standard error of immunocompetence traits in RIR chicken

Source of variation	No. of observation	Least squares mean±SE		
		HA	Lysozyme (µg/ml)	IgG (mg/ml)
Overall	253	9.35 ± 0.29	4.64±0.11	8.61 ±0.34
Male	143	9.39 ± 0.22	4.77±0.15	8.56 ±0.42
Female	110	9.33 ± 0.34	4.51±0.16	8.67 ±0.45

4.3.5 Genetic and phenotypic parameters of immunocompetence and growth traits in RIR chicken

Data on various IC traits and body weights recorded at 16, 20, 40 and 64 weeks of ages in selected pure strain of RIR were analyzed using mixed model least-squares analysis of variance. The Least Square analysis of variance for IC and body weight traits is presented in **table 4.3.3** and their corresponding factor-wise least square means are presented in **table 4.3.4.** Least Square ANOVA revealed significant effect of sire on serum IgG ($P \leq 0.01$) level and body weights at BW16, BW20, BW40 and BW64 weeks of age. Sex also had highly significant ($P \leq 0.001$) effect on all body weights. Male demonstrated significantly more body weights than females at all ages.

4.3.5.1 Heritability

The genetic parameters IC traits and their inter-relationship with body weights are presented in **table 4.3.5.**

The heritability estimates of serum IgG level was high (0.302±0.188) and positive. Heritability for HA titre and serum lysozyme level could not be estimated

The genetic correlation (r_G) among IC traits could not be estimated for want of estimates of heritability of HA titre and serum lysozyme level.

The Phenotypic correlations (r_p) among IC traits were low in magnitude. The HA titre had negative phenotypic correlation with serum lysozyme and IgG level. But serum lysozyme levels had low and positive phenotypic correlated with serum IgG level (0.005).

The heritability estimates for BW16, BW20, BW40 and BW64 in RIR chicken on equal number of observations were 0.516± 0.22, 0.468 ± 0.219, 0.302±0.188 and 0.344±0.214, respectively.

4.3.5.2 Genetic and phenotypic correlations

Serum IgG concentration had positive and low genetic correlation with BW16 (0.162 ± 0.395) moderate with BW20 (0.302±0.392), although associated with high standard error, and positively and high genetic correlation with BW40 (0.928±0.459) and BW64 (0.796± 0.507). Genetic correlation of HA titre and serum lysozyme with body weight could not be estimated. Genetic correlations among body weights were highly positive.

Table 4.3.3: Least squares analysis of variance for immunocompetence and growth traits in selected strain of Rhode Island Red chicken

Source of variation	df	Mean sum of squares						
		HA	Lyso	IgG	BW16	BW20	BW40	BW64
Sire	24	5.95	2.17	27.73**	107391.43***	145532.16***	151230.73*	238481.56**
Sex	1	0.03	3.74	0.68	4427467.30***	16261409.01***	43935045.83***	25308924.52***
Error	227	12.07	2.85	15.26	43281.58	62599.35	92551.79	131476.35 (193)

*Lyso: Serum lysozyme level; df = Degrees of freedom; *P≤0.03;**P≤0.01, ***P≤0.001; Figures within parentheses denote degrees of freedom.*

Table 4.3.4: Least squares means of growth and immunological traits in selected strain of Rhode Island Red chicken

Factors	N	Least squares means± standard errors						
		HA	Lysozyme (µg/ml)	IgG (mg/ml)	BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)
Overall	253	9.35±0.29	4.64±0.11	8.61± 0.34	1364.41±21.96	1800.48± 25.51	2185.21± 25.58	2440.19± 34.68 (219)
Sex								
Male	143	9.39 ± 0.22	4.77±0.15	8.56± 0.42	1504.56±25.08[a]	2069.07± 29.37[a]	2626.70± 31.19[a]	2804.00± 42.52[a] (117)
Female	110	9.33±0.34	4.51±0.16	8.67± 0.45	1224.26±26.83[b]	1531.89± 31.53[b]	1743.73± 34.09[b]	2076.38± 44.42[b] (102)

N= Number of observations; Means within a factor having different superscripts in a column differ significantly (P≤0.05); Figures within parentheses denote number of observation.

Table 4.3.5: Heritability (at diagonal), genetic (above diagonal) and phenotypic (below diagonal) correlations among various immunocompetence and growth traits in selected strain of Rhode Island Red chicken

Traits	HA	Lysozyme	IgG	BW16	BW20	BW40	BW64
HA	NE	NE	NE	NE	NE	NE	NE
Lysozyme	-0.097	NE	NE	NE	NE	NE	NE
IgG	-0.088	0.005 (219)	0.302±0.188	0.162±0.395	0.302±0.392	0.928±0.459	0.796± 0.507
BW16	0.033	0.049	0.015	0.516± 0.22	1.050 ±0.049	1.086±0.179	0.972±0.168
BW20	0.029	0.049	0.064	0.786	0.468 ± 0.219	1.047±0.131	0.829±0.193
BW40	-0.033	0.108	-0.005	0.552	0.691	0.302±0.188	1.011±0.117
BW64	-0.037	0.026	-0.002	0.521	0.581	0.752	0.344±0.214
	(219)	(219)	(219)	(219)	(219)	(219)	(219)

Figures within parentheses denote number of observation; NE., not estimable

HA titre had negative phenotypic correlation with serum lysozyme, serum IgG level, BW40 and BW64 and positive with BW16 and BW20. Serum lysozyme concentration had positive and low phenotypic correlation with IgG, BW16, BW20, BW40 and BW64. HA titre and Serum IgG concentration had positive and low phenotypic correlation with early body weights such as BW16 and BW20 and very low and negative correlations with BW40 and BW64.

4.3.6 Immunocompetence and layer economic traits and their inter-relationship in RIR chicken

Data on IC and layer economic traits, viz., HA, lysozyme, IgG and age sexual first egg (AFE), body weight at 16 (BW16) weeks of age, egg weight at 28 (EW28), 40 (EW40) and 64 (EW64) weeks of age and egg number up to 40 (EP40) and 64 (EP64) weeks of age, individually recorded on females were analyzed using mixed model least-squares (LS) analysis of variance.

The Least Square analysis of variance for immunocompetence and layer economic traits are presented in **table 4.3.6** and the least square means (factor-wise) are presented in **table 4.3.7.** The heritabilities and correlations amongst IC and layer economic traits are presented in **table 4.3.8.**

Least Square ANOVA revealed significant effect of sire on body weight at 16 weeks, egg weight at 64 weeks of age and egg production upto 40 weeks of age.

4.3.6.1 Heritability

Heritability estimates were low for serum IgG level (0.109±0.48) and high for serum lysozyme level (0.414±0.38). The estimates of heritability were moderate to high for BW16, EW40, EW64, EP40 and EP64.

4.3.6.2 Genetic and phenotypic correlations

Serum lysozyme level had highly negative genetic correlation with serum IgG level, EP40 and EP64; low to moderately negative with EW28 and EW64, positive and high correlation with BW16 and EW40 and could not be estimated with AFE. Serum IgG concentration had high and positive correlation with body and egg weights at different ages,

but highly negative genetic correlation with EP40 and EP64. The genetic correlation of HA titre could not be estimated, which may be attributed to sampling variation due to small sample size.

Hemagglutination titre had very low and negative phenotypic correlations with lysozyme, IgG levels and EP40 and moderately positive correlation with EW64. The correlations with all other traits were very low and positive.

Serum lysozyme level had low positive phenotypic correlation with serum IgG level, BW16, EW40 and EP40; highly positive r_p with EW64 and EP64, negative and low phenotypic correlation with AFE and EW28.

Serum IgG concentration had also very low phenotypic correlation with all the traits which were not different than zero.

4.3.7 Influence of levels of immunocompetance traits on body weights in RIR chickens

In order to assess the influence of IC traits on growth traits, the levels of IC traits were classified into three groups based on descriptive analysis into low (birds eliciting lower estimates than mean minus one standard deviation), medium (values between mean + standard deviation and mean minus standard deviation) and high (values higher than mean plus one standard deviation) groups.

The IC traits groups were taken as fixed effect in the statistical model used for least squares analysis of variance along with other relevant factors.

Least square analysis of variance of influence of immunocompetence traits on body weights and factor-wise least square means are presented in **table 4.3.9** and **4.3.10** respectively.

Least squares analysis of variance elucidated significant effect of serum IgG levels on BW40 ($P \leq 0.06$) and BW64 ($P \leq 0.05$). Influence of HA titre and serum lysozyme levels were statistically non-significant ($P > 0.05$) on all the body weights.

Critical difference test signified LS means-differences in the traits immunocompetence levels. It was observed that birds with low serum IgG concentration had significantly highest

Table 4.3.6: Least squares analysis of immunocompetence and layer economic traits in selected strain of Rhode Island Red chicken

Source of variation	df	Mean Sum of squares									
		HA	Lysozyme	IgG	AFE	BW16	EW28	EW40	EW64	EP40	EP64
Sire	22	9.62	3.18	16.80	64.33	42841.23*	10.81	16.35	42.00*	306.60**	1131.90
Remainder	77	11.30	2.13	17.03	88.40	25738.40	11.47	15.42	21.80(49)	148.20(49)	872.51(49)

***P≤0.01; *P≤0.05. Figures within parentheses denote degrees of freedom (df).*

Table 4.3.7: Least squares means ± standard error of immunocompetence and layer economic traits in selected strain of Rhode Island Red chicken

Factor	N	Least squares mean ± SE									
		HA	Lysozyme (µg/ml)	IgG (mg/ml)	AFE (days)	BW16 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (eggs)	EP64 (eggs)
Overall	100	9.33 ±0.34	4.42 ±0.18	8.72 ± 0.41	133.68 ±0.90	1220.60 ±21.57	44.73 ±0.34	47.62 ±0.41	51.89 ± 0.81(72)	118.11 ±1.21(72)	219.39 ±4.09 (72)

N= Number of observations; Figures within parentheses denote number of observations.

Table 4.3.8: Heritability (at diagonal) and genetic (above diagonal) and phenotypic (below diagonal) correlations of immunocompetence and layer economic traits in selected strain of Rhode Island Red chicken

Traits	HA	Lysozyme	IgG	AFE	BW16	EW28	EW40	EW64	EP40	EP64
HA	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Lysozyme	-0.056	0.414±0.38	-1.672 ± 3.83	NE	0.334±0.61	-0.269±1.19	0.786±1.43	-0.031± 0.67	-0.808± 0.74	-1.670±1.45
	(100)	(100)	(72)		(100)	(72)	(72)	(72)	(72)	(72)
IgG	-0.131	0.022	0.109±0.48	NE	0.404±1.32	2.176±5.45	4.306±10.74	1.266±3.05	-0.909± 2.19	-1.186±2.95
	(100)	(100)	(72)		(72)	(72)	(72)	(72)	(72)	(72)
AFE	0.090	-0.124	-0.003	NE	NE	NE	NE	NE	NE	NE
	(100)	(100)	(100)							
BW16	0.056	0.066	0.050	-0.360	0.535± 0.40	1.749±1.40	2.053±1.99	1.120± 0.23	-0.068± 0.41	0.137± 0.64
	(100)	(100)	(100)	(100)	(100)	(72)	(72)	(72)	(72)	(72)
EW28	0.048	-0.028	-0.002	0.125	0.392	0.245±0.49	0.849± 0.55	1.581± 1.09	1.234±1.25	2.379± 2.81
	(100)	(100)	(100)	(100)	(100)	(72)	(72)	(72)	(72)	(72)
EW40	0.089	0.033	-0.025	0.077	0.401	0.735	0.211± 0.49	1.050±0.65	0.741±1.16	1.423±2.14
	(100)	(100)	(100)	(100)	(100)	(100)	(72)	(72)	(72)	(72)
EW64	0.136	0.667	-0.092	0.114	0.518	0.665	0.718	0.922± 0.53	0.412± 0.45	0.972±0.70
	(72)	(72)	(72)	(72)	(72)	(72)	(72)	(72)	(72)	(72)
EP40	-0.055	0.108	0.025	-0.448	0.101	0.053	-0.079	0.013	1.027± 0.53	0.668±0.42
	(100)	(100)	(72)	(100)	(100)	(100)	(100)	(72)	(72)	(72)
EP64	0.001	1.446	0.080	-0.135	0.037	0.062	0.013	0.252	0.604	0.351± 0.50
	(72)	(72)	(72)	(72)	(72)	(72)	(72)	(72)	(72)	(72)

Figures within parentheses denote number of observations; NE., not estimable.

Table 4.3.9: Least squares analysis of variance of various growth traits in selected strain of RIR chicken

Source ofvariation	df	Least squares analysis of variance			
		BW16	BW20	BW40	BW64
Influence of level of HA on Body weights					
Sire	24	10664.1**	146096.6**	151370.5*	228128.9*
Sex	1	4330070.4**	16097589.8**	43018215.1	24906677.2**
HA	2	15273.7	18934.1	38013.8	17378.9
Error	225	43530.5	62987.5	93036.6	132671.1(191)
Influence of level of serum lysozyme on Body weights					
Sire	24	106435.1**	142463.7**	148346.1*	238863.8*
Sex	1	4324709.7**	15682631.9**	42168119.5**	24736168.0**
Lysozyme	2	1518.5	7742.3	109417.6	9228.2
Error	225	43652.8	63086.9	92401.9	132756.4(191)
Influence of level of serum IgG on Body weights					
Sire	24	103687.7**	140135.7**	149525.1*	213664.4*
Sex	1	4427843.1**	16294745.5**	43857170.8**	25097373.3**
IgG	2	6726.5	66668.5	264248.9#	431404.4*
Error	225	43606.5	62563.2	91025.6	128804.0

*#P≤0.06, *P≤0.05, **P≤0.01, Figures within parentheses denote degrees of freedom (df).*

Table 4.3.10: Factor-wise least squares mean of various growth traits in selected strain of RIR chicken.

Factor	N	Least squares means			
		BW16 (g)	BW20 (g)	BW40 (g)	BW64 (g)
Influence of level of HA on Body weights					
Overall	253	1368.94 ± 30.43	1794.65 ± 35.55	2183.75 ± 35.56	2450.38 ± 47.74(219)
Male	143	1508.86 ± 32.26	2064.44 ± 37.81	2624.77 ± 38.85	2813.05 ± 53.15(117)
Female	110	1229.02 ± 34.72	1524.87 ± 40.83	1742.72 ± 43.14	2087.71 ± 55.99(102)
HA titre					
Low	49	1348.55 ± 39.15	1774.06 ± 46.27	2217.76 ± 50.60	2465.74 ± 67.68(40)
Medium	180	1364.90 ± 30.13	1807.14 ± 35.17	2180.77 ± 35.01	2432.24 ± 46.26(159)
High	24	1393.37 ± 50.15	1802.76 ± 59.69	2152.71 ± 68.27	2453.16 ± 89.965(20)
Influence of level of lysozyme on Body weights					
Overall	253	1364.93 ± 29.03	1807.66 ± 33.49	2184.77 ± 33.60	2445.69 ± 45.36(219)
Male	143	1505.36 ± 31.04	2075.07 ±35.99	2623.25 ± 37.21	2808.33 ± 50.66(117)
Female	110	1224.51 ± 3.47	1540.26 ± 39.02	1746.28 ± 41.43	2083.04 ±5 4.41(102)
Serum lysozyme level					
Low	35	1372.22 ± 43.11	1812.39 ± 50.88	2125.51 ± 57.25	2435.44 ± 72.30(34)
Medium	182	1364.01 ± 28.48	1795.51 ± 32.80	2185.81 ± 32.59	2436.53 ± 45.36(155)
High	36	1358.57 ± 43.20	1815.09 ± 50.99	2242.98 ± 57.40	2465.10 ± 76.93(30)
Influence of level of IgG on Body weights					
Overall	253	1371.06 ± 28.68	1821.75 ± 33.27	2218.48 ± 33.84	2494.12 ± 42.94(218)
Male	143	1511.26 ± 31.34	2090.71 ± 36.54	2659.73 ± 38.44[a]	2858.11 ± 49.81(117)[a]
Female	110	1230.85 ± 2.42	1552.79 ± 37.87	1777.24 ± 40.27[b]	2130.12 ± 50.66(101)[b]
Serum IgG level					
Low	35	1379.52 ± 43.29	1832.50 ± 51.13	2303.89 ± 57.78[a]	2588.41 ± 74.36(30)[a]
Medium	184	1359.77 ± 28.29	1785.72 ± 32.78	2161.85 ± 33.13[b]	2400.99 ± 42.85(156)[b]
High	34	1373.88 ± 43.82	1847.03 ± 51.78	2189.71 ± 58.62[b]	2492.95 ± 72.33 (32)[ab]

N= Number of observations; Means within a factor having different superscripts in a column differ significantly (P≤0.05); Figures within parentheses denote number of observations.

Table 4.3.11: Least squares analysis of variance of influence of immunocompetence traits on various layer economic traits in selected strain of RIR chicken

Source ofvariation	df	Least squares analysis of variance						
		AFE	BW16	EW28	EW40	EW64	EP40	EP64
***Influence of level of HA on* layer economic traits**								
Sire	22	61.7	42112.1	9.61	15.0	37.1	169.9	1154.4
HA	2	48.1	3270.4	7.8	18.9	14.8	45.5	331.7
Error	75	81.3	26337.5	11.6	15.3	22.1(47)	203.3	895.5(47)
***Influence of level of* serum lysozyme *on* layer economic traits**								
Sire	22	62.9	45107.4*	10.5	15.6	40.4*	172.3	1062.1
Lysozyme	2	7.9	27840.2	6.3	2.9	2.4	304.9	2769.9*
Error	75	82.3	25682.3	11.6	15.7	22.6(47)	196.3	791.8(47)
***Influence of level of* serum IgG *on* layer economic traits**								
Sire	22	59.7	42638.8*	10.7	16.9	43.9*	175.9	1172.1
IgG	2	22.8	32041.7	2.8	13.3	41.4	90.5	1056.1
Error	75	81.9	25570.3	11.7	15.5	20.9(47)	202.1	864.7(47)

**P≤0.05; df = Degrees of freedom; Figures within parentheses denote degrees of freedom.*

Table 4.3.12: Least squares means of influence of immunocompetence traits on various layer economic traits in selected strain of RIR chicken

Factor	N	Least squares means						
		AFE (no.)	BW16 (g)	EW28 (g)	EW40 (g)	EW64 (g)	EP40 (no.)	EP64 (no.)
Influence of level of HA								
Overall	100	133.61 ±1.33	1212.59 ± 31.72	45.20 ±0.50	48.16 ±0.57	52.00 ±1.25(72)	119.95 ±2.11	213.44 ±6.80 (72)
Low	17	131.40 ±2.37	1202.02 ±47.43	44.85 ±0.89	47.09 ±1.03	50.48 ±1.70(15)	120.33 ±3.75	216.71 ±9.98(15)
Medium	75	134.01 ±1.37	1225.97 ±32.32	44.54 ±0.51	47.50 ±0.59	52.18 ±1.55(53)	118.14 ± 2.18	221.45 ±8.95(53)
High	8	135.42 ±3.39	1209.77 ±64.53	46.22 ±1.28	49.90 ±1.47	53.34 ±2.98(4)	121.39 ±5.37	202.15 ±18.50(4)
Influence of level of serum lysozyme								
Overall	100	133.60 ±1.41	1220.18 ±34.80	45.06 ± 0.53	47.89 ± 0.62	51.55 ±1.32(72)	119.98 ±2.18	223.20 ± 6.61 (72)
Low	16	132.62 ±2.69	1281.42 ±53.37	45.58 ± 1.01	48.13 ± 1.18	51.19 ± 2.17(8)	113.48 ±4.16	197.09 ±12.18[c] (8)
Medium	77	133.84 ±1.32	1212.91 ±33.60	44.52 ±0.49	47.47 ± 0.58	52.03 ±1.33(59)	119.09 ±2.03	219.57 ± 6.67[b] (59)
High	7	134.34 ±3.89	1166.21 ±72.84	45.09 ±1.46	48.07 ±1.70	51.42 ±2.77(5)	127.37 ± 6.01	252.95 ± 15.82[a] (5)
Influence of level of serum IgG								
Overall	100	134.28 ±1.17	243.32 ±28.09	44.91 ±0.44	48.02 ±0.54	52.38 ±1.10(72)	117.59 ±1.85	221.30 ±5.49 (72)
Low	14	135.14 ±2.58	1261.51 ±49.31	44.85 ±0.97	48.98 ± 1.13	55.02 ± 1.89(9)	116.99 ± 4.05	213.61 ± 11.28 (9)
Medium	71	133.22 ±1.21	1203.08 ±28.56	44.58 ±0.45	47.32 ±0.56	51.69 ±1.16(51)	119.69 ±1.90	217.18 ± 5.96 (51)
High	15	134.48 ±2.47	1265.36 ±47.54	45.29 ±0.93	47.75 ±1.09	50.42 ±1.69(12)	116.09 ± 3.88	233.11 ± 9.86 (12)

N= Number of observations; Means within a factor having different superscripts in a column differ significantly ($P \leq 0.05$); Figures within parentheses denote number of observations.

BW40 (P≤0.06) than those having non-significantly different medium and high levels of serum IgG. Similarly, birds with low IgG level significantly (P≤0.05) demonstrated high BW64 than those having non-significantly different medium and high levels of serum IgG (**Table 4.3.10**).

4.3.8 Influence of levels of immunocompetance traits on layer economic traits in RIR chicken

In order to assess the influence of IC traits on layer economic traits, the levels of IC traits were grouped as low, medium and high and taken as fixed effect in the statistical model used for least squares analysis of variance along with other relevant factors for analyzing he layer production data

Least square ANOVA and factor-wise least square means are presented in **table 4.3.11** and **4.3.12**, respectively.

Least squares analysis of variance revealed that serum lysozyme levels had significant (P≤0.05) effect on egg production up to 64 weeks of age (EP64) in RIR chicken.

It was observed that birds with high serum lysozyme concentration produced highest number of eggs up to 64 weeks of age fallowed by birds with medium and then low levels (P≤0.05).

4.3.9 Association of microsatellite genotype with immunocompetence traits

Nine out of 10 MS loci revealed polymorphism. All the experimental birds were genotyped for each of these nine polymorphic microsatellite loci. Least squares analysis of variance was carried out to assess the effect of MS genotype on immunocompetance traits by least squares analysis of variance wherein MS genotype was taken as independent factor. Least squares ANOVA and MS genotype-wise least square means of various immunocompetence traits are presented below from **table 4.3.13 to 4.3.30.**

Out of nine polymorphic microsatellites, none of the microsatellites was found to have significant effect on any of the immunocompetence traits. Least squares analysis of variance revealed, sire also had non-significant (P>0.05) effect on all IC traits for all microsatellite loci. Thus, MS-genotype at all microsatellite loci had non-significant (P>0.05) effect on all IC traits.

Table 4.3.13: Least squares analysis of variance for effect of ADL0020 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	Mean Sum of squares		
		HA	Lysozyme	IgG
Sire	11	0.7360	0.3892	0.4041
Genotype	5	0.6004	0.1042	0.7593

df: degree of freedom

Table 4.3.14: Least square means ± standard errors of immunocompetence economic traits for different genotypes at microsatellite ADL0020 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AC	1	10.51±3.63	4.90±1.30	8.17±4.33
AD	1	13.84±3.94	6.94±1.42	2.19±4.69
BC	2	10.36±2.57	3.36±0.92	6.20±3.07
BD	5	9.13±2.25	3.36±0.83 (4)	6.58±2.68
CC	56	8.71±0.63	4.38±0.23 (55)	8.86±0.75
DD	7	12.84±3.02	3.39±1.09	6.60±3.60

Notes: Figures in parentheses represent the number of observations.

Table 4.3.15: Least squares analysis of variance for effect of ADL0023 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	Mean Sum of squares		
		HA	Lysozyme	IgG
Sire	11	0.6315	0.1127	0.4575
Genotype	3	0.2272	0.2700	0.9978

df: degree of freedom

Table 4.3.16: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite ADL0023 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AB	12	9.42±1.14	4.74±0.44 (11)	8.29±1.40 (11)
AC	2	9.80±2.56	5.06±0.97	8.56±3.14
BB	5	12.40±1.56	3.53±0.59	7.99±1.91
CC	53	8.91±0.52	4.13±0.20	8.39±0.64

Notes: Figures in parentheses represent the number of observations.

Table 4.3.17: Least squares analysis of variance for effect of ADL0102 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	Mean Sum of squares		
		HA	Lysozyme	IgG
Sire	11	0.8798	0.1894	0.0970
Genotype	5	0.7778	0.8278	0.1620

df: degree of freedom

Table 4.3.18: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite ADL0102 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AA	7	8.40±1.74	4.06±0.66	7.38±1.96
AB	22	9.52±0.95	4.71±0.36	6.58±1.07
AC	6	7.30±1.80	3.84±0.70	13.12±2.03
BB	19	10.09±1.10	4.14±0.42 (17)	8.63±1.24 (17)
BC	1	7.57±3.74	3.86±1.42	5.51±4.22
DD	19	9.45±1.01	4.09±0.39 (18)	8.84±1.14

Notes: Figures in parentheses represent the number of observations.

Table 4.3.19: Least squares analysis of variance for effect of ADL0176 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	Mean Sum of squares		
		HA	Lysozyme	IgG
Sire	11	0.9198	0.5808	0.3624
Genotype	7	0.8858	0.6947	0.3312

df: degree of freedom

Table 4.3.20: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite ADL0176 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AB	2	8.05±2.81	5.70±1.05	14.25±3.18
AC	6	8.35±1.58	3.87±0.59	10.67±1.78
BB	9	10.94±1.71	3.93±0.64	10.24±1.93
BC	6	9.43±1.68	3.77±0.63	5.50±1.90
BD	7	8.91±1.63	4.52±0.61	8.03±1.84
CC	28	9.78±0.93	3.96±0.35 (27)	8.39±1.05
CD	2	9.62±3.16	5.27±1.18	5.50±3.57
DD	12	7.93±1.96	4.83±0.73	6.41±2.21

Notes: Figures in parentheses represent the number of observations.

Table 4.3.21: Least squares analysis of variance for effect of ADL0210 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	P values		
		HA	Lysozyme	IgG
Sire	11	0.7327	0.1049	0.4686
Genotype	2	0.9101	0.4121	0.2551

df: degree of freedom

Table 4.3.22: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite ADL0210 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AA	3	10.18±2.39	5.51±1.02(2)	4.30±2.76
AB	1	9.99±3.68	3.54±1.37	5.43±4.25
BB	68	9.24±0.42	4.21±0.16	8.53±0.49

Notes: Figures in parentheses represent the number of observations.

Table 4.3.23: Least squares analysis of variance for effect of MCW0007 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	P values		
		HA	Lysozyme	IgG
Sire	11	0.7377	0.1927	0.7059
Genotype	4	0.9110	0.6477	0.2773

df: degree of freedom

Table 4.3.24: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite MCW0007 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AA	1	11.58±3.63	3.50±1.35	12.80±4.14
AB	13	8.80±1.27	4.19±0.47	10.67±1.44
BB	32	9.69±0.80	4.57±0.30 (31)	9.13±0.91
BC	8	9.07±1.45	4.16±0.54	7.75±1.66
CC	18	9.01±1.06	3.85±0.40	6.09±1.21

Notes: Figures in parentheses represent the number of observations.

Table 4.3.25: Least squares analysis of variance for effect of MCW0014 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	P values		
		HA	Lysozyme	IgG
Sire	11	0.6951	0.1869	0.2757
Genotype	2	0.5101	0.9052	0.4823

df: degree of freedom

Table 4.3.26: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite MCW0014 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AA	4	7.56±1.89	4.13±0.72	9.21±2.23
AB	13	8.83±1.01	4.39±0.39	9.49±1.19
BB	55	9.60±0.50	4.20±0.19 (54)	7.92±0.60

Notes: Figures in parentheses represent the number of observations.

Table 4.3.27: Least squares analysis of variance for effect of MCW0069 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	P values		
		HA	Lysozyme	IgG
Sire	11	0.7571	0.0754	0.4323
Genotype	9	0.9549	0.3858	0.5805

df: degree of freedom

Table 4.3.28: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite MCW0069 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AD	1	8.69±3.75	4.14±1.33	6.37±4.25
AE	3	9.97±2.77	5.38±0.99	12.11±3.15
BD	7	7.86±1.96	3.49±0.70	9.55±2.22
BE	1	7.29±3.70	4.58±1.33	13.77±4.20
CE	28	9.08±0.80	4.14±0.29(27)	7.70±0.91
CF	1	8.01±3.88	2.40±1.38	14.75±4.41
DD	1	7.19±4.36	3.35±1.55	10.39±4.95
DF	9	10.32±1.63	5.03±0.58	7.76±1.85
EE	13	10.71±1.32	4.80±0.47	7.85±1.50
EF	8	8.98±1.53	3.78±0.55	8.07±1.74

Notes: Figures in parentheses represent the number of observations.

Table 4.3.29: Least squares analysis of variance for effect of MCW0103 microsatellite genotypes on immunocompetence traits in RIR chicken

Source of variation	df	P values HA	Lysozyme	IgG
Sire	11	0.7152	0.1602	0.5037
Genotype	1	0.7286	0.2263	0.1820

df: degree of freedom

Table 4.3.30: Least square means ± standard errors of immunocompetence traits for different genotypes at microsatellite MCW0103 in RIR chicken

Genotype	No. of observation	Least squares mean±SE		
		HA titre	Lysozyme (µg/ml)	IgG (mg/ml)
AB	40	9.14±0.58	4.04±0.22 (39)	8.99±0.67
BB	32	9.45±0.62	4.44±0.23	7.62±0.72

Notes: Figures in parentheses represent the number of observations.

4.4. Objective 4: To analyze relative expression of important immunity related genes in various tissues of Rhode Island Red chicken by quantitative reverse transcription PCR (qRT-PCR).

The relative mRNA expression of three immunity related genes viz. IL1-β, iNOS and TLR15 were studied in three tissues (bursa, spleen and thymus) collected from selected strain of RIR chicken was done by quantitative reverse transcriptase PCR (qRT-PCR) method. The RT-PCR amplification curves and melting peak curves for each gene in each tissue are presented in **figures (4.4.1, 4.4.2. and 4.4.3.).** The data from real time experiment was transferred to excel and compiled for further analysis. Out of the triplicate samples, average of the minimum two C_t values were averaged for subsequent analysis. β-actin gene was used as housekeeping gene. ΔC_t value for each sample was calculated after subtracting average C_t value of housekeeping gene from average C_t value of target gene. For each sample, the ΔC_t value was subtracted from 40 (total cycle number) so as to obtain 40-ΔC_t. Higher 40-ΔC_t value was considered as higher expression (MacKinnon *et al.*, 2009).

The data on 40-ΔC_t estimates regarding expression of mRNA of all the three genes were analyzed by using JMP 9.0.0 of SAS (Copyright© 2010 SAS Institute Inc., U.S.A) for determining the differences among tissues and between sexes. Firstly, tissue-wise analysis was carried using a fixed model consisting of sex as fixed effect; secondly, tissue-wise analysis consisting of tissue as fixed effect. Thirdly, the 40-ΔC_t for each gene was also analysed in sampled birds having high, medium or low level IC traits. Subsequently, least squares means (LSM) were compared within each main factor by Tukey-Kramer HSD test (Tukey 1953; Kramer 1956, 1957). Results from all these analyses are presented below:

4.4.1 Differential mRNA expression of immune related genes in different sexes

Analysis of data on relative mRNA expression of three immune response genes, estimated as 40-ΔC_t, were carried out in tissue-wise manner**.**

4.4.1.1 Gene expression in bursa

The LS ANOVA for assessing the influence of sex on the mRNA expression of three immune response genes viz., IL1-β, iNOS and TLR15 in the bursa tissue is presented in **table 4.4.1** and the factor-wise least squares means ± standard error of 40-ΔC_t values are presented in **table 4.4.2**. Graphical representation of relative least squares means ± error of 40-ΔC_t values has also been presented in **figure 4.4.4**

Table 4.4.1: Least squares analysis of variance of 40-ΔC_t values of mRNA expression levels of immune response genes in bursa

SV	df	IL1-β		iNOS		TLR15	
		MSS	p value	MSS	p value	MSS	p value
Sex	1	5.603	0.1785	0.864	0.5420	0.0574	0.8973
Remainder		10	26.756		21.684		32.741

MSS: mean sum of squares; df **:** *degree of freedom*

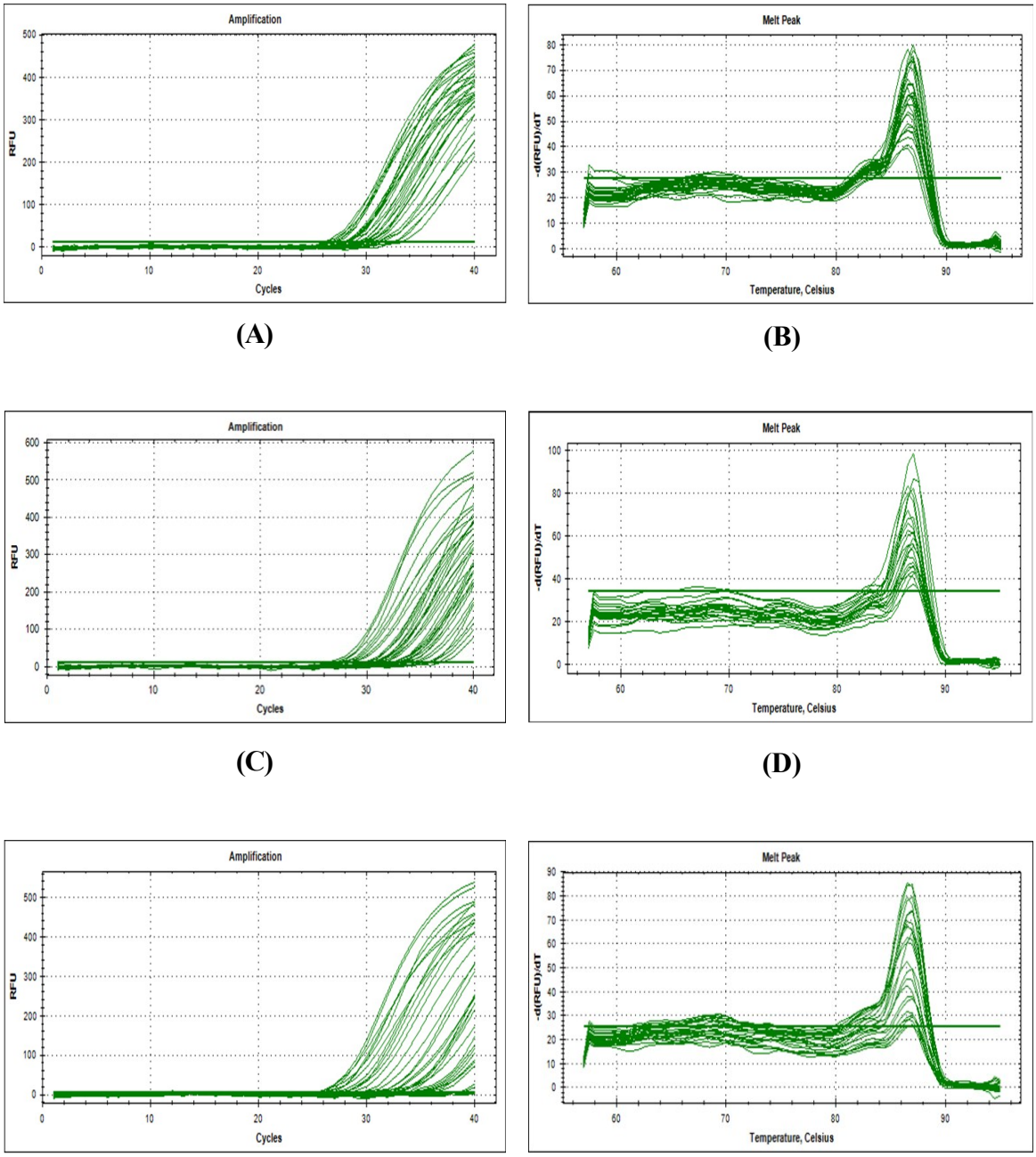
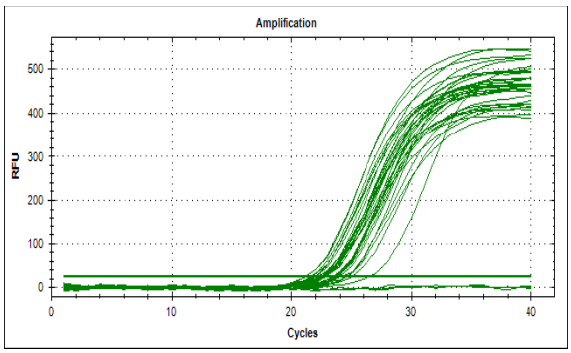
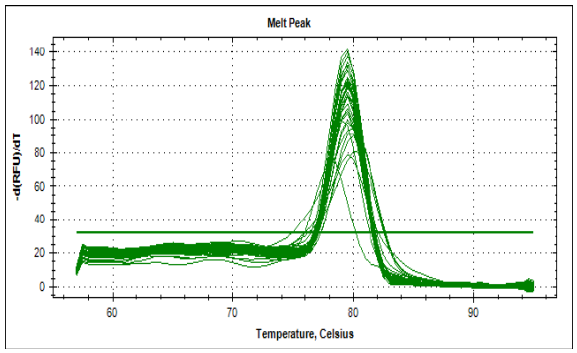
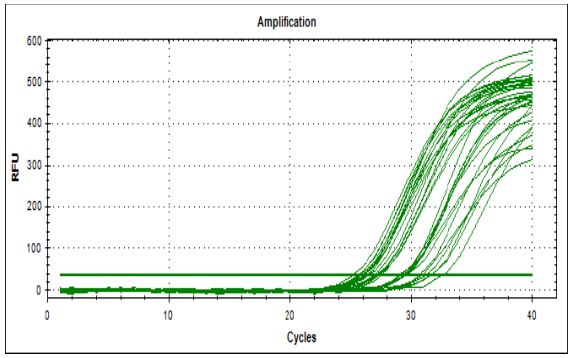


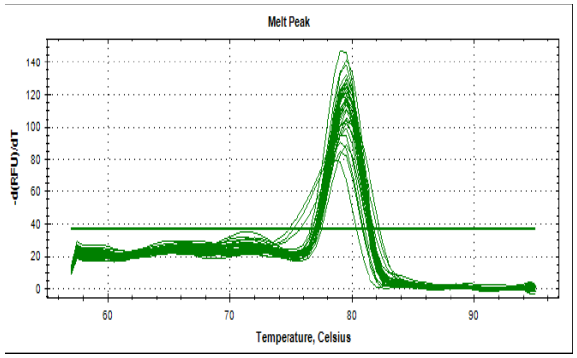
Fig. 4.4.1: Amplification (A, C, E) and dissociation (B, D, F) curves of IL1-β gene mRNA during qRT-PCR in bursa, spleen and thymus tissues, respectively in selected pure strain of RIR chicken



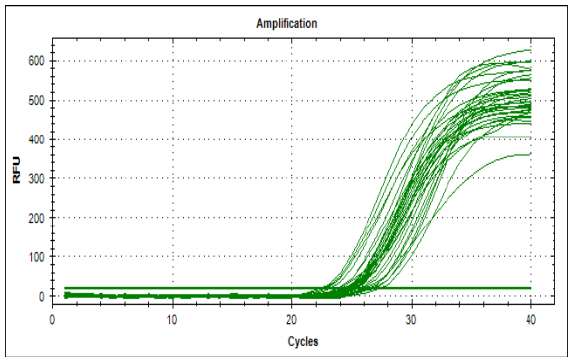


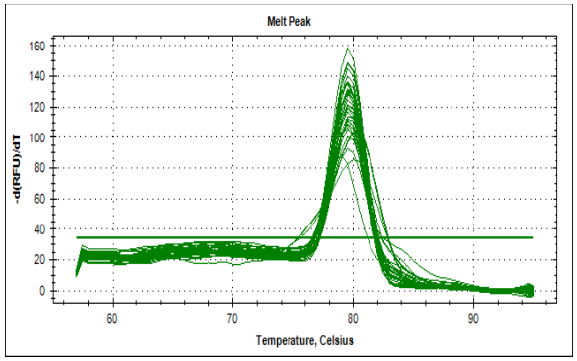
(A) **(B)**





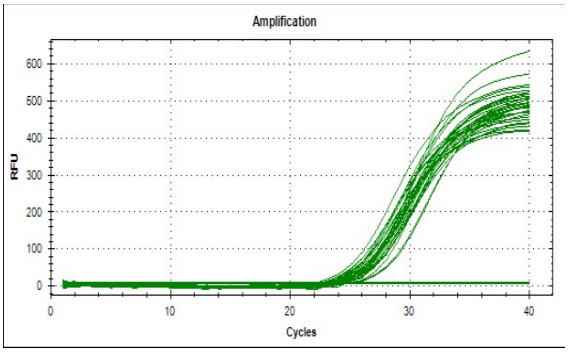
(C) **(D)**



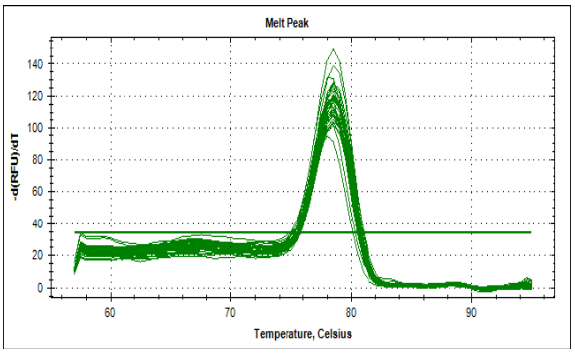


(E) **(F)**

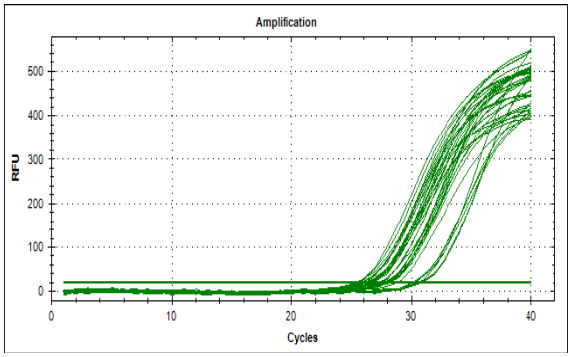
Fig. 4.4.2: Amplification (A, C, E) and dissociation (B, D, F) curves of iNOS gene mRNA during qRT-PCR in bursa, spleen and thymus tissues, respectively in selected pure strain of RIR chicken



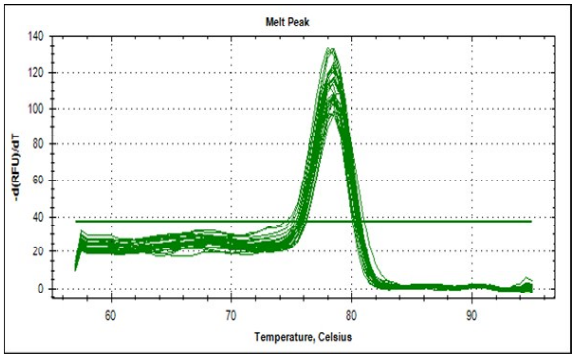
(A)



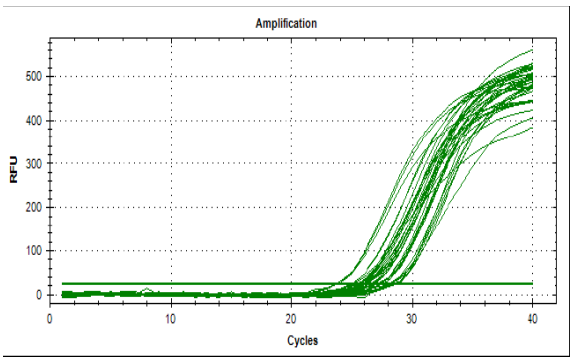
(B)



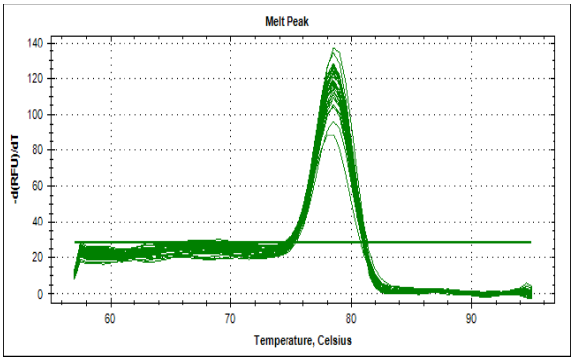
(C)



(D)



(E)



(F)

Fig. 4.4.3: Amplification (A, C, E) and dissociation (B, D, F) curves of TLR15 gene mRNA during qRT-PCR in bursa, spleen and thymus tissues, respectively in selected pure strain of RIR chicken

Table 4.4.2: Least squares mean ± standard error of 40-ΔC_t values of mRNA expression levels of immune response genes in bursa

Factors	N	IL1-β	iNOS	TLR15
Overall	12	36.110±0.472	34.268±0.425	30.301±0.522
Male	6	35.427±0.668	34.000±0.601	30.370±0.739
Female	6	36.793±0.668	34.537±0.601	30.232±0.739

N: Number of observations

The LS ANOVA revealed that the sex had non-significant (P>0.05) effect on the expression of all three immune response genes studied; wherein the mean 40-ΔC_t values for IL1-β, iNOS and TLR15 were 35.427±0.668, 34.000±0.601 and 30.370±0.739 in males and 36.793±0.668, 34.537±0.601 and 30.232±0.739 in females, respectively.

4.4.4.2 Gene expression in Spleen

The LS ANOVA for assessing the influence of sex on the mRNA expression of three immune response genes viz., IL1-β, iNOS and TLR15 in the spleen tissues are presented in **table 4.4.3** and the factor-wise least squares means ± standard error of 40-ΔC_t values are presented in **table 4.4.4**. Graphical representation of relative least squares means ± error of 40-ΔC_t values has also been presented in **figure 4.4.5.**

Table 4.4.3: Least squares analysis of variance of 40-ΔC_t values of mRNA expression levels of immune response genes in spleen

SV	df	IL1-β		iNOS		TLR15	
		MSS	p value	MSS	p value	MSS	p value
Sex	1	15.098	0.1018	9.293	0.3578	1.015	0.5472
Remainder		10	46.517		99.991		26.152

MSS: mean sum of squares; df **:** *degree of freedom*

Table 4.4.4: Least squares mean ± standard error of 40-ΔC_t values of mRNA expression levels of immune response genes in spleen

Factors	N	IL1-β	iNOS	TLR15
Overall	12	34.707±0.623	31.377±0.913	29.252±0.467
Male	6	33.585±0.881	30.497±1.291	29.543±0.660
Female	6	35.828±0.881	32.257±1.291	28.962±0.660

N: Number of observations.

The sexes did not differ significantly for expression of three immune response genes studied in spleen also. The means 40-ΔC_t values for IL1-β, iNOS and TLR15 were 33.585±0.881, 30.497±1.291 and 29.543±0.660 in males and 35.828±0.881, 32.257±1.291 and 28.962±0.660 in females, respectively; the differences being non-significant (P>0.05).

4.4.1.3 Gene expression in thymus

The LS ANOVA for assessing the influence of sex on the mRNA expression of three immune response genes viz., IL1-β, iNOS and TLR15 in the thymus tissues are presented in **table 4.4.5** and the factor-wise least squares means ± standard error of 40-ΔC_t values are presented in **table 4.4.6**. Graphical representation of relative least squares means ± error of 40-ΔC_t values has also been presented in **figure 4.4.6.**

Table 4.4.5: Least squares analysis of variance of 40-ΔC_t values of mRNA expression levels of immune response genes in thymus

SV	df	IL1-β		iNOS		TLR15	
		MSS	p value	MSS	p value	MSS	p value
Sex	1	1.449	0.7410	0.357	0.8247	0.193	0.7578
Remainder		10	125.458		69.080		19.166

MSS: mean sum of squares; df **:** *degree of freedom*

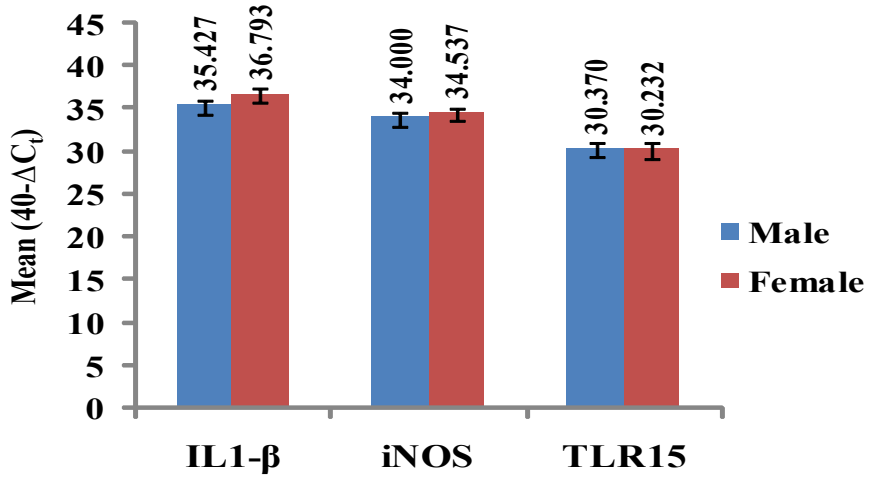


Fig. 4.4.4: Relative expression of IL1-β, iNOS and TLR15 genes in bursa of male and female pure selected strain of RIR chicken

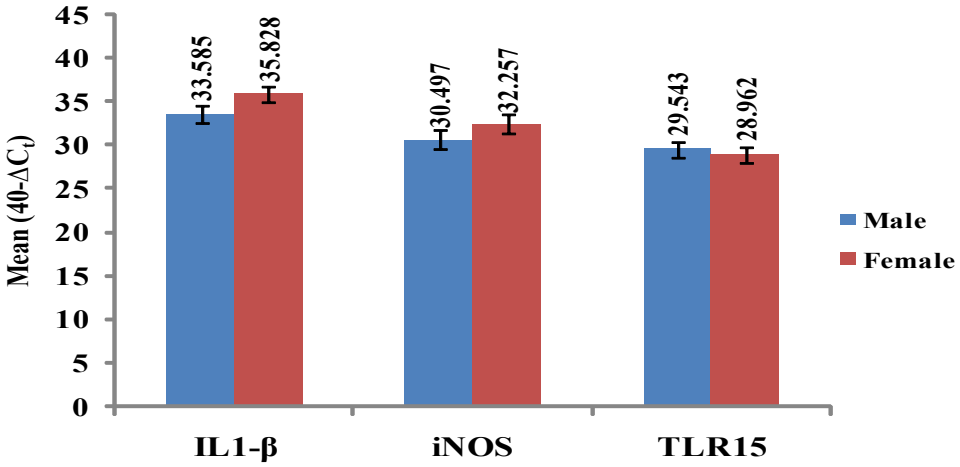


Fig. 4.4.5: Relative expression of IL1-β, iNOS and TLR15 genes in spleen of male and female pure selected strain of RIR chicken

Table 4.4.6: Least squares mean ± standard error of 40-ΔC_t values of mRNA expression levels of immune response genes in thymus

Factors	N	IL1-β	iNOS	TLR15
Overall	12	34.928±1.022	30.172±0.756	30.203±0.400
Male	6	35.275±1.446	30.345±1.073	30.077±0.565
Female	6	34.580±1.446	30.000±1.073	30.330±0.565

N: Number of observations.

The LS ANOVA reveals that, sex effect was non-significant (P>0.05) on expression of all three immune response genes studied. The mean 40-ΔC_t values for IL1-β, iNOS and TLR15 were 35.275±1.446, 30.345±1.073 and 30.077±0.565 in males and 34.580±1.446, 30.000±1.073 and 30.330±0.565 in females, respectively; again the difference were non-significant.

4.4.2: Gene wise differential mRNA expression of immune related genes in different tissues

Analysis of data on relative mRNA expression of three immune response genes, estimated as 40-ΔC_t, were carried out in RIR chicken by incorporating tissue as fixed effect in the statistical model.

The LS ANOVA showing effects of tissue on expression of mRNA of IL1-β, iNOS and TLR15 genes in RIR chicken are presented in **table 4.4.7** and the factor-wise least squares means ± standard error of 40-ΔC_t values are presented in **table 4.4.8**. Graphical representation of relative least squares means ± standard error of 40-ΔC_t values has also been presented in **figure 4.4.7**.

Table 4.4.7: Least squares analysis of variance of 40-ΔC_t values of mRNA expression levels of immune response genes in three lymphoid tissues of RIR chicken

SV	df	IL1-β		iNOS		TLR15	
		MSS	p value	MSS	p value	MSS	p value
Tissue	2	13.666	0.3714	106.350	0.0009*	8.050	0.2029
Remainder		33	220.881		201.269		79.324

MSS: mean sum of squares.

Table 4.4.8: Least squares mean ± standard error of 40-ΔC_t values of mRNA expression levels of immune response genes in lymphoid tissue of RIR chicken

Factors	N	IL1-β	iNOS	TLR15
Bursa	12	36.110±0.747	34.268±0.713[a]	30.301±0.448
Spleen	12	34.707±0.747	31.377±0.713[b]	29.253±0.448
Thymus	12	34.928±0.747	30.173±0.713[b]	30.203±0.448

N: Number of observations; Means with different superscripts in a column differ significantly ($p \leq 0.05$).

The expression of iNOS gene differed significantly ($P \leq 0.001$) among tissues in RIR chicken. Bursa tissue demonstrated highest expression of iNOS gene with mean 40-ΔC_t value as 34.268±0.713, which was significantly more than those in spleen and thymus, where the estimates were 31.377±0.713 and 30.173±0.713, respectively.

The three tissues did not differ significantly ($P \leq 0.05$) for the expression of IL1-β and TLR15 genes; the overall mean 40-ΔC_t values in bursa, spleen and thymus were 36.110±0.747, 34.707±0.747 and 34.928±0.747 for IL1-β and 30.301±0.448, 29.253±0.448 and 30.203±0.448 for TLR15 gene, respectively.

4.4.3 Influence of levels of immunocompetence traits on relative gene expression (40-ΔC_t) in various lymphoid tissue of RIR chickens

In order to assess the influence of IC traits on relative gene expression (40-ΔCt), HA titre, serum lysozyme and serum IgG concentrations in the sampled birds were classified in to

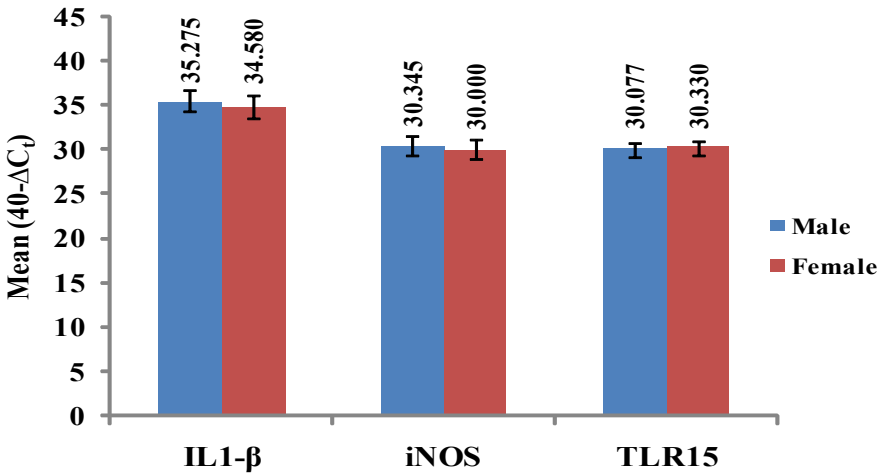


Fig. 4.4.6: Relative expression of IL1-β, iNOS and TLR15 genes in thymus of male and female pure selected strain of RIR chicken

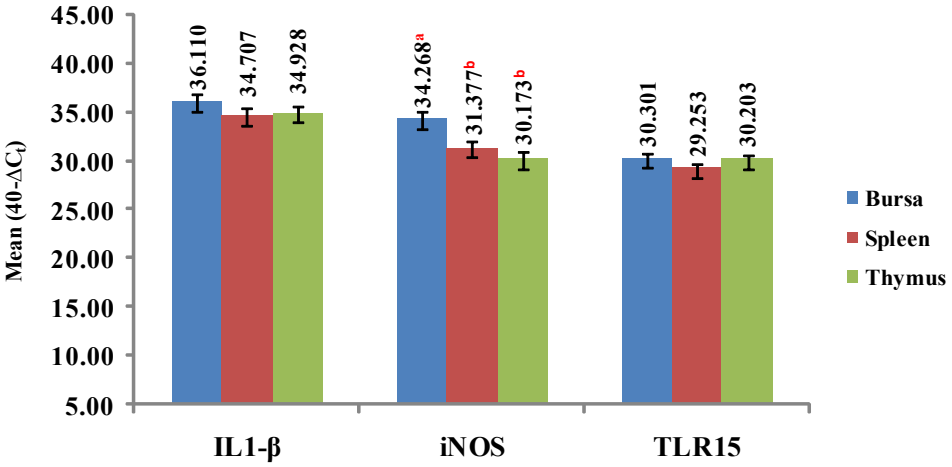


Fig. 4.4.7: Relative expression of IL1-β, iNOS and TLR15 genes in bursa, spleen and thymus of pure selected strain of RIR chicken

Table 4.4.9: P values showing effect of level of immunocompetence traits on gene expression (40-ΔC_t) of various lymphoid tissues

Source of variation	P values								
	B-IL1-β	**B-iNOS**	**B-TLR15**	**S-IL1-β**	**S-iNOS**	**S-TLR15**	**T-IL1-β**	**T-iNOS**	**T-TLR15**
Influence of level of HA on gene expression									
HA label	0.8576	0.9780	0.2482	0.0818$^{\#}$	0.6899	0.5924	0.4044	0.1913	0.3337
Influence of level of lysozyme on gene expression									
Lysozyme label	0.2243	0.4495	0.6101	0.4459	0.6749	0.0119*	0.0542**	0.3708	0.7629
Influence of level of IgG on gene expression									
IgG label	0.6590	0.9585	0.7195	0.8541	0.7220	0.6021	0.3770	0.5734	0.6517

$^{*}P \leq 0.01$; $^{**}P \leq 0.06$; $^{\#}P \leq 0.09$; B= Bursa; S= Spleen; T=Thymus

Table 4.4.10: Level-wise mean 40-”Ct of three genes expression of various lymphoid tissues

Factor	Least squares means								
	B-IL1-β	B-iNOS	B-TLR15	S-IL1-β	S-iNOS	S-TLR15	T-IL1-β	T-iNOS	T-TLR15
Influence of level of HA on gene expression									
High	36.14	34.38	30.81	32.99	30.77	29.81	35.78	30.79	30.53
	±0.83	±0.71	±0.73	±0.89^{b}	±1.50	±0.73	±1.52	±1.03	±0.58
Medium	35.75	34.18	30.77	36.26	32.58	29.03	32.97	28.34	30.56
	±0.93	±0.79	±0.82	±0.99^{a}	±1.67	±0.82	±1.70	±1.16	±0.65
Low	36.54	34.20	28.82	35.50	30.78	28.62	36.11	31.59	29.18
	±1.08	±0.91	±0.94	±1.14^{a}	±1.93	±0.95	±1.96	±1.33	±0.75
Influence of level of lysozyme on gene expression									
High	35.14	34.68	30.80	33.84	32.31	27.22	31.27	28.32	30.13
	±0.93	±0.84	±1.04	±1.38	±1.93	±0.61^{b}	±1.57^{b}	±1.44	±0.82
Medium	36.98	33.72	29.77	34.37	30.51	29.77	36.77	30.81	29.98
	±0.66	±0.59	±0.74	±0.98	±1.36	±0.43^{a}	±1.11^{a}	±1.02	±0.58
Low	35.34	34.95	30.87	36.25	32.18	30.25	34.89	30.74	30.72
	±0.93	±0.84	±1.04	±1.38	±1.93	±0.61^{a}	±1.57^{ab}	±1.44	±0.82
Influence of level of IgG on gene expression									
High	36.36	34.48	30.64	34.36	32.58	29.07	36.22	31.41	29.92
	±1.05	±0.91	±1.06	±1.48	±1.94	±0.95	±1.95	±1.51	±0.81
Medium	35.74	34.17	29.95	34.60	30.73	29.02	33.74	29.51	30.08
	±0.68	±0.60	±0.70	±0.97	±1.27	±0.62	±1.27	±0.99	±0.53
Low	37.04	34.31	31.04	35.62	31.83	30.35	37.15	30.63	31.05
	±1.28	±1.11	±1.30	±1.82	±2.38	±1.16	±2.38	±1.85	±0.99

Means within a factor having different superscripts in a column differ significantly ($P \leq 0.05$)

three groups based on descriptive analysis into low (birds eliciting lower estimates than mean minus one standard deviation), medium (values between mean + standard deviation and mean minus standard deviation) and high (values higher than mean plus one standard deviation) groups. The IC traits groups were taken as fixed effect in the statistical model used for least squares analysis of variance along with other relevant factors.

Least square analysis of variance of influence of immunocompetence traits on relative gene expression and factor-wise least square means are presented in **table 4.4.9** and **4.4.10** respectively and graphical representation of relative least squares means ± standard error of 40-"C_t values has also been presented in **figure 4.4.8**.

Least squares analysis of variance reveals that the influence of HA titre levels had significant effect on relative gene expression of IL1-β ($P \leq 0.09$) in spleen and serum lysozyme level also significant on relative gene expression of TLR15 ($P \leq 0.01$) in spleen and IL1- β ($P \leq 0.06$) in thymus tissue. Influence of serum IgG levels was statistically non-significant ($P > 0.05$) on all genes studied in all lymphoid tissues. It was observed that birds with medium or low HA tire revealed significantly higher IL1-β gene expression in spleen than birds having high HA titre. Similarly, birds with medium or low serum lysozyme level revealed significantly higher mRNA expression of IL1-β in thymus and TLR15 in spleen tissue than birds having high lysozyme level.

4.4.4 Fold expression in IL1-β, iNOS and TLR15 genes

IL1-β gene expression was 2.65 and 1.27 folds more in bursa and thymus, respectively in comparison to spleen. iNOS gene expressed 17.12 and 2.30 folds more in bursa and spleen, respectively, in comparison to thymus. TLR15 gene expression was 2.67 and 1.93 folds more in bursa and thymus, respectively, in comparison to spleen (**Fig. 4.4.11**). The basal level of all three immune response gene was more in bursa than thymus and spleen in all the lymphoid tissue studied, which might be due to fact that bursa is the principal site for production of B-lymphocytes in chicken .

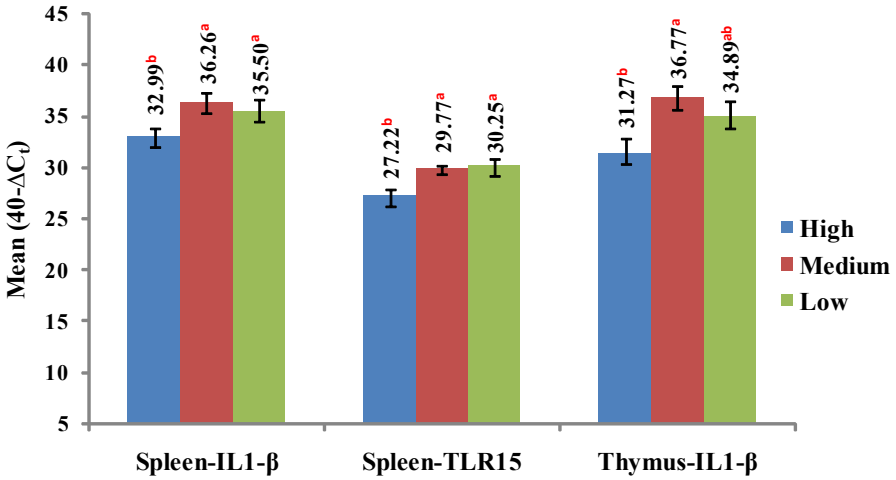


Fig. 4.4.8: Influence of IC traits' levels on gene expression in lymphoid tissues of selected pure strain of RIR chicken

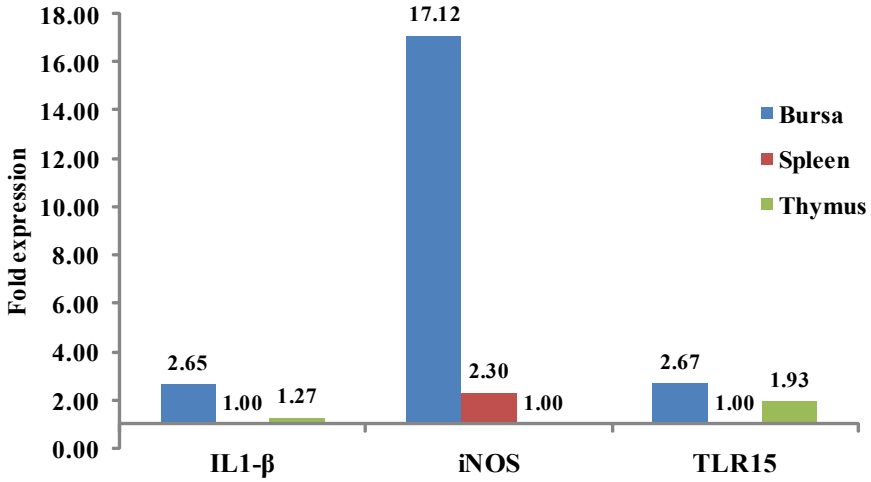


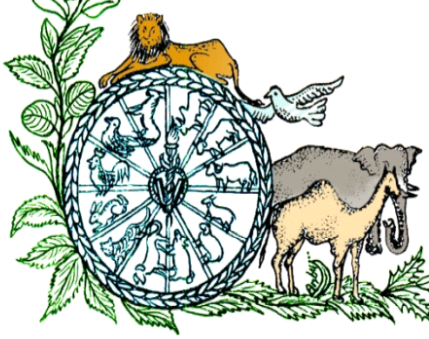
Fig. 4.4.9: Fold expression of IL1-β, iNOS and TLR15 genes in bursa, spleen and thymus of pure selected strain of RIR chicken

Table 4.4.11: Fold expression of mRNA of IL1-β, iNOS, and TLR15 genes in different lymphoid tissues of RIR chicken

Tissue	N	Mean $\Delta C_t \pm SE$			$-\Delta C_t$			$2^{(-\Delta\delta Ct)}$		
		IL1-β	iNOS	TLR15	IL1-β	iNOS	TLR15	IL1-β	iNOS	TLR15
Bursa	12	3.89±1.64	5.37±1.37	9.70±1.65	-1.40	-4.10	-1.05	2.65	17.12	2.67
Spleen	12	5.30±2.27	8.62±3.02	10.75±1.50	0.00	-1.20	0.00	1.00	2.30	1.00
Thymus	12	4.95±3.43	9.83±2.41	9.80±1.27	0.34	0.00	-0.95	1.27	1.00	1.93

ΔC_t = (C_t target–C_t control); $-\Delta C_t$ = (ΔC_t target - ΔC_t calibrator); Tissue showing the highest ΔCt among all tissues was used as Calibrator.

Discussion



5 DISCUSSION

Present investigation revealed several interesting findings which are discussed below in light of the recent available literature.

5.1 Objective 1: To analyze effects of various genetic and non-genetic factors on layer economic traits in Rhode Island Red chicken.

The percent fertility, estimated as 73.24%, in this investigation was close to pervious reports of Das *et al.* (2014c); Debnath *et al.* (2015) as 75.86% and 76.98% in selected strain of RIR chicken and Bhardwaj *et al.* (2006) as 75.21% in RIR chicken. However Zelleke *et al.* (2005) and Khan *et al.* (2014) reported higher percent fertility in RIR chicken which was 82.97±0.67 and 78.26%, respectively and Ahmed *et al.* (2012) reported it as 87.00% in WLH X RIR crossbred chicken. Malago and Baitilwake (2009) also reported percent fertility as 77.90% in RIR chicken. In the present finding, the percent fertility was lower than those reported in CARI Annual Report, 2010-11 wherein it was 87.13, 87.04, 85.64 and 73.78% in RIR selected, RIR control, CARI-Sonali, CARI-Debendra, respectively. Percent fertility observed in the present study was somewhat higher than those reported earlier by Kamar *et al.* (1984) in RIR (71.6%).

The percent hatchability was estimated as fertile eggs set and total eggs set basis was 78.13% and 57.22%, respectively. These estimates were close to those reported by Das *et al*. (2014c) where the corresponding estimates in RIR^S chicken were 75.65 and 57.46%. Zelleke *et al.* (2005) reported higher percent hatchability on total egg set (59.49±2.30) and fertile egg set (76.67±1.66) basis in pure RIR chicken. In the previous year (CARI Annual

Report, 2010-11), the hatchability in RIR^S chicken was higher than those estimated in the present study. Debnath *et al.* (2015) also reported higher percent hatchability on total egg set (61.44%) and fertile egg set (79.85%) basis in – RIR^S chicken, however Bhardwaj *et al.* (2006) and Khan *et al*. (2014) reported lower hatchability percentage on total egg set as 38.01% and 51.41% and fertile egg set basis as 50.39% and65.69% in comparison to the present estimates in RIR^S chicken. The fertility and hatchability are interrelated heritable traits that vary among breeds, varieties (Kingori, 2011). Variation in different reproductive traits are influenced by several genetic and non-genetic factors such as semen storage temperature, care, age of males, quality of eggs, age of pullets, season, nutrition, pre-incubation warming, and humidity etc. Therefore, the variations in different reports might be due to differences in some or all of these factors in various populations or genetic stocks. Thus the selected strain of RIR chicken revealed comparable fertility and hatchability indicating their reproductive fitness under long-term selection based on part-period egg production.

5.1.1 Body weights at different weeks of age

Least squares ANOVA revealed significant effect of sire and sex ($P \leq 0.001$) on body weights at all ages (Table 4.1.1). Males were significantly heavier than females at all ages (Table 4.1.2).

Shivaraman *et al*. (2003) also reported significant ($P \leq 0.01$) effect of sire and sex on body weights at BW4, BW5 and BW6 in synthetic broiler dam line. Das (2013) reported significant effect of sire on BW40 in RIR^S (selected) and RIR^C (control) chicken strains.

The overall mean of body weight at 16 (BW16) weeks of age in combined sexes was 1362.6±21.4g, which was lower, than reported by Das *et al.* (2014c) in combined sexes (1446.63±12.72g). The mean body weight at 16 weeks in males in present study was 1492.9±24.0g, and in females, it was 1232.4±25.5g. These estimates were lower than those reported by Das *et al.* (2014c) in selected strain RIR, as the average BW16 was 1639.10±18.08g in male and in females it was 1254.16 ± 15.22g. Das *et al*. (2014d) estimated body weight at 16 weeks as 1206.07±25.47g with mean body weight in males being 1333.11±34.39g, and in females being 1079.03 ±37.57g in RIR^S strain, which was lower than the present study.

The overall means of BW20 and BW40 on combined sex basis were 1791.6± 24.6g and 2184.6±26.2g, respectively. The corresponding mean body weights in males were 2040.3±28.1g and 2624.4±31.3g and in females 1542.9±30.2g and 1744.8±33.7g. The LS means of BW20 and BW40 of pullets of RIR selected strain in the present study were comparable to those reported earlier in RIR pullets as 1517.69±0.40g and 1783.43±0.73g (Kumar *et al.,* 2002), 1395.68±5.05 and 1673.24±5.64g (Jilani *et al.,* 2005), 1395.68±5.05 and 1673.24±5.64g (Jilani *et al.,* 2007), 1369±20.7 and 1836±34.2g (Saini *et al.,* 2011) and 1589.74 ± 10.25 and 1744.78 ± 8.86g (Das *et al.*, 2014c), respectively.

The overall least-squares mean of BW64 on combined sex basis was 2433.8±34.2g and corresponding males and female body weights were 2784.5±41.3g and 2083.1±42.9g. In the absence of reports on BW64 on combined sex basis in RIR, the estimates cannot be compared or contrasted. However, females weighed slightly more than the previous generation where BW64 was 1933.1±23.4g (CARI Annual Report, 2014-15).

5.1.2 Layer economic traits

The least-squares mean of AFE was 134.5 ± 0.9 days, which was lower than those reported in earlier reports such as 149.18 ± 0.14 days in RIR chicken (Kumar *et al.,* 2002), 146.16±0.33 days (Jilani *et al.,* 2005, 2007), 152.00 ± 3.8 days (Saini *et al.,* 2011), 147.00 ± 1.15 days (Khawaja *et al.,* 2013) and 148.86 ± 0.78 days (Das *et al.,* 2014c) and close to that reported in the contemporary generation involving three hatches (CARI Annual Report, 2014-15) in selected strain of RIR chicken. The strain in the present study was under continuous selection for part-period egg production and there was no selection for ASM, hence, the variation in different studies in ASM may be attributed to the correlated effect of selection or due to genetic differences.

LS means of egg weight at EW28, EW40 and EW64 weeks of age were 44.8±0.3g, 47.7±0.4g and 51.5±0.7g, respectively (Table 4.1.4). The estimated egg weight at 28 weeks of age was quite comparable to 44.98±0.20g which was reported by Das *et al.* (2014c) in selected RIR strain. However, the EW40 was lower than 51.6±0.62g reported by Saini *et al.* (2011) in RIR chicken and 51.75 ±0.09g reported by Das *et al.* (2014c) in selected RIR

strain which might be due to comparatively shorter ASM observed in the present study. Comparable estimates of EW28, EW40 and EW64 was reported in selected and rendom-bred control of RIR chicken (CARI, Annual Report, 2014) however, Khawaja *et al.* (2013) reported higher egg weight at 72 weeks of age as 56.82 ±0.54 in RIR Chicken.

LS means egg production up to 40 weeks (EP40) and 64 weeks (EP64) were 118.3±1.2 and 214.5±4.8 eggs, respectively. Present estimate of egg production upto 40 weeks corroborated well with the earlier reports of Kumar *et al.* (2002), Jilani *et al.* (2005) and Jilani *et al.* (2007) who reported eggs production up to 40 weeks as 112.49±0.54, 107.19±0.10 and 107.16±0.10 in RIR strains. Das *et al.* (2014c) and Saini *et al*. (2011) reported lower egg production as 96.45±0.99 and 74±3.2 eggs up to 40 weeks in RIR strain. However, Rao *et al.* (2012) reported egg production up to 72 weeks of age as 156±1.927 eggs, considering it as annual egg production in synthetic variety of RIR. The estimated EP40 and EP64 which were relatively higher than reported previously might be due to differences in the genetic architecture of the strains and also probable difference in the feeding regime in different studies.

5.1.3 Genetic parameters

The heritability estimates for body weights at various ages were high in magnitude and associated with low standard errors suggested them to be more reliable and precise (Table 4.1.5). The estimates indicated that there was significant scope for improvement of body weight traits. The heritability estimates for body weights at different weeks were quite comparable to those reported by Qadri *et al.* (2013) in IWP strain of White Leghorn chicken for BW16, BW20 and BW40. High heritability estimates for BW40 were also reported by Kumar *et al.* (2002) in RIR strain. Similarly, Jilani *et al.* (2005) and Jilani *et al*. (2007) reported high heritability for BW20 and BW40 in RIR chicken.

The heritability estimate of AFE was very low 0.01±0.30 which indicated non-additive genetic variance for this trait. It was in close agreement with the estimates reported by Reddy *et al.* (2001) who reported heritability of ASM in IWH as 0.01± 0.09 and in IWI as 0.02± 0.04 lines of white leghorn chicken. Jayalaxmi *et al.* (2010) also reported low heritability (0.020±0.067) of AFE in IWP stain of White leghorn chicken. Lower heritability estimates indicated that it was difficult to improve this trait through genetic selection

Heritability estimates were low for EW28 (0.17±0.30) and EW40 (0.09±0.32) and high for EW64 (0.52±0.45). Lower heritability of EW28 and EW40 were also reported by Qadri *et el*. (2013) in IWP strain of White Leghorn chicken, and for EW28 by Jayalaxmi *et al.* (2010) and for EW40 by Vasu *et al*. (2004), Barot *et al.* (2008) and Anees *et al.* (2010). High heritability at 64 weeks egg weight of age is in agreement with that reported by Jayalaxmi *et al.* (2010) IWP strain of White Leghorn chicken. However, a few reports (Jilani *et al.,* 2007) and Jayalaxmi *et al.* (2010) for EW40 also recorded somewhat higher estimates which might be due to differences in the genetic groups.

The heritability estimates for egg production upto 40 and 64 weeks of age were 0.02±0.28 and 0.86±0.43, respectively. These estimates were in corroboration with the reports of Rajkumar *et al.* (2011) (0.02±0.17) in sex linked dwarf chicken; Jayalaxmi *et al* (2010) in IWK strain (0.059±0.072) of White Leghorn for egg production upto 40 weeks of age. The heritability of EP64 was much higher than that reported by Rajkumar *et al.* (2011). Higher h^2 estimate of EP64 in this study suggested that selection based on a longer production period would yield better results than that based on part- period egg production.

5.1.4 Genetic and phenotypic correlations:

The AFE showed negative genetic correlation with BW16, BW20, EW28 and EP40, which indicated that lower body weight at housing increased the age of sexual maturity (Table 4.1.5). Qadri *et al*. (2013) reported a negative correlation of AFE with BW16 in IWN and IWP strains of White Leghorn and Choadhary *et al*. (2009) also reported a negative correlation of AFE with BW16 in IWN strain while, Jilani *et al*. (2005) reported the negative and significant association of AFE with BW20, EW35 and EP40 in RIR strain at genetic and phenotypic level, which was similar to present finding. Similarly, Jayalaxmi *et al*. (2010) reported negative genetic correlations of ASM with BW16, BW20 and BW40 weeks of age and egg production. The genetic and phenotypic correlations of ASM with EP40 were found to be highly negative as reported by Qadri *et el*. (2013) and Jayalaxmi *et al.* (2010).

The genetic and phenotypic correlations among body weights were high and positive and were in close agreement with the estimates reported by Jilani *et al.* (2005), Jilani *et al.*

(2007) in RIR strain. Barot *et al*. (2008) and Rajkumar *et al.* (2011) also reported strong and positive correlation among growth traits.

Genetic correlations of BW16 were positive with EW64 and EP40 and negative with EP64. Jilani *et al.* (2007) also reported positive correlation of BW16 with egg weights and negative genetic correlations of BW16 with EP40. The genetic correlations between body weight at various ages and egg weights or egg numbers were either not estimable or were less precise. However, the phenotypic correlations of body weights with egg weights were moderately positive and with egg numbers they were low and inconsistent.

Genetic correlations among egg weights were inconsistent however the phenotypic correlations among egg weights were positive and higher in magnitude. Both genetic as well as phenotypic correlations between egg weights and egg numbers did not show any definite trend as also reported by Nwagu *et al.* (2007). The EP40 had high and positive genetic (0.89±0.15) as well as phenotypic (0.63) correlations with EP64 suggesting the usefulness of 40-weeks part-period egg production as selection criterion for genetic improvement of annual egg production (EP64). The genetic correlations among layer traits either could not be estimated or had estimates beyond range in the present study which might be due to the reason that the number of progenies per sire (k-values) were very low for layer traits (k=4.38) in comparison to growth traits (k=12.05), where the estimates could be estimated with precision.

5.1.5 Percent mortality

Percent mortality recorded in combined sexes of selected strain of RIR was 1.54%, 0.31%, 2.60%, 6.35% and 7.86% during different period from 0 to 4, 5 to 8, 9 to 16, 17 to 40 and 41 to 64 weeks of age. The overall mortality from day zero up to 64 weeks of age was 17.57%. The mortality were close to those reported earlier in different studies. Sivaraman *et al*. (2005) recorded mortality on 303 chicks of both sexes over the period from 0 to 4, 4 to 5 and 5 to 6 weeks of age, which was 2.77 %, 0.63 % and 0.0 %, respectively. Khawaja *et al*. (2013) estimated the percent mortality as 10.00±0.23% during 18-72 weeks period in RIR chicken. Das *et al.,* (2014c) calculated the percent mortality in selected strain of Rhode Island Red chicken for different periods and reported it to be approx. 5.15% during first week, 4.80% during brooding, 5.60% during growing and 7.73% during laying stages.

5.2. Objective 2: To determine allelic polymorphism at egg production – associated microsatellite loci in Rhode Island Red chicken and their association with layer economic traits.

Allelic polymorphism was studied at 10 microsatellite loci reported to be associated with layer economic traits. Nine out of ten microsatellite loci were found to be polymorphic (Table 4.2.1). Arya (2012) studied 47 microsatellites loci in high and low egg production sub-population of White leghorn chicken using 3.4% MetaPhor Agarose and reported nine out of 47 microsatellite loci were polymorphic. Deshmukh *et al.* (2015) also genotyped 25 microsatellite markers in Hill fowl, Rhode Island Red, Kadaknath, White Leghorn and White Cornish using 3.4% MetaPhor Agarose and reported 17 (70%) loci were polymorphic among the breeds. Total number of alleles in the present study ranged from two to six in different loci and average number of alleles per locus was 3.50 ± 0.29 (Table 4.2.2). Deshmukh *et al.* (2015) reported two to three alleles at polymorphic locus and average number of alleles per locus across the breeds was 2.41.

Microsatellite locus **ADL0020** generated four alleles with sizes of 126, 118, 110 and 102 bp in RIR chicken. Panday *et al.* (2002) also reported four alleles in Aseel and Nicobari and five alleles in Miri chicken at this locus. Vijh and Tantia (2004) observed five alleles in Aseel, Nicobari, Miri and Kashmir Favorella native chicken breeds, Pandey *et al*. (2005) reported four alleles ranging from 95-105 bp at this locus in 40 samples of Ankleshwar indigenous chicken breed. Chatterjee *et al*. (2008 a, b) studied five polymorphic microsatellites using 8% non-denaturing PAGE and reported three different alleles at ADL0020 locus with sizes of 97, 107 and 112 bp in six crossbred chicken populations of White leghorn. Kim *et al*. (2008b) reported two alleles for ADL0020 in Korean native chicken breed with sizes of 101 and 105 bp. The frequencies of two alleles were 0.262 and 0.257, respectively. Chatterjee *et al*. (2010b) also studied the same locus along with fourteen other loci and reported six number of alleles with sizes of 98, 100, 102, 108,112 and 116 bp in two indigenous native breed (Aseel and Kadaknath) and three different chicken lines. The differences in numbers and sizes of alleles could be attributed to the differences in the genetic makeup of the genome analyzed or may be probably due to loss and fixation of alleles after long term selection.

Microsatellite locus **ADL0023** resolved three alleles of 202, 188 and 176bp with frequencies of 0.105, 0.158, and 0.737, respectively in the selected RIR population. However, Panday *et al.* (2002) used the same primer pair for this locus and revealed five alleles in Aseel and Miri and three alleles in Nicobari chicken breed. Vijh and Tantia (2004) observed eight alleles for ADL0023 locus in four native chicken breeds. Pandey *et al*. (2005) reported eight alleles ranging from 164-182 bp in Ankleshwar chicken breed. Almost similar number of alleles and size range was reported by Chatterjee *et al*. (2008 a,b), where three alleles were observed with sizes of 176,184 and 204 bp through 8% non-denaturing PAGE in six crossbred chicken populations of White Leghorn. Present observation were in close agreement to the finding of Khan *et al*. (2010), who resolved the same locus through high resolution MetaPhor Agarose and reported allele size ranging from 175-200 bp in Kadaknath, 178-189bp in Ankleshwar and only one i,e 178 bp in White Leghorn chicken breed. Present finding were also quite similar to the report of Chatterjee *et al*. (2010b) where five alleles at ADL0023 locus with sizes of 166, 170, 178, 182 and 194 bp were observed in three different chicken lines and two native Aseel and Kadaknath breeds. Difference in the allele size and number may be attributed to genetics differences.

Microsatellite locus **ADL0102** resolved three alleles of 110, 114, and 122 bp in RIR chicken. The frequency of A, B and C alleles were 0.289, 0.408 and 0.303, respectively. However, Panday *et al.* (2002) used the same primer pair for this locus and reported three, seven and five alleles in Aseel, Miri and Nicobari native chicken breed, respectively. Vijh and Tantia (2004) reported eight alleles at ADL0102 locus in four native chicken breeds. Pandey *et al*. (2005) reported eight alleles in Ankleshwar chicken breed ranging in size from 97-127 bp for ADL0102 locus, which was almost similar to the size range observed in present investigation. Chatterjee *et al*. (2008a, b) explored same locus and reported three alleles with sizes of 92,108 and 118 bp in different six crossbred of White Leghorn chicken, which were in accordance with present finding. Chatterjee *et al*. (2010b) reported five alleles at ADL0102 locus with sizes of 96, 104, 108, 114 and 120 bp among three different chicken lines and two native Aseel and Kadaknath breed. However Das (2013) revealed four alleles with sizes of 136, 146, 166 and 174 bp with corresponding frequencies of 0.20, 0.20, 0.40 and 0.20 in

selected strain of RIR chicken. Differences in various reports might be due to either presence or absence of other alleles at this locus or due to smaller variations in the method employed and their limitation.

Microsatellite locus **ADL0176** resolved four alleles of 220, 214, 206, and 202bp with frequency of 0.053, 0.243, 0.474 and 0.230, respectively for A, B, C and D alleles. Panday *et al.* (2002) used the same primer pair and reported eight, seven and four alleles in Aseel, Nicobari and Miri chicken breed, respectively. Vijh and Tantia (2004) reported five alleles in four native chicken breeds. Pandey *et al*. (2005) reported five alleles ranging from 188-210 bp in Ankleshwar chicken breed. Haunshi and Sharma (2006) genotyped same locus in 76 backcross progenies of Nacked neck population and resolved three alleles of 198, 190, 181 bp sizes. .The 190 bp allele was most prevalent in the population. Chatterjee *et al*. (2008a, b) reported six alleles with sizes of 138,150,160, 168,176 and 188 bp in six crossbred chicken populations. Chatterjee *et al*. (2010b) also reported six alleles at this locus with sizes of 190, 196, 200, 202, 206 and 218 bp among three different chicken (Vanaraja male line, Vanaraja female line and Gramapriya female line) lines and two native Aseel and Kadaknath chicken breeds. Das (2013) reported three different alleles at this locus in 6% urea PAGE with sizes of 194, 196, 236 bp and 200, 202 and 236 bp in selected and control line of RIR, respectively. However, Arya (2012) revealed a monomorphic pattern at this locus with the same pair of markers in 3.4% MetaPhor Agarose and reported only one allele of 188 bp in both high and low egg production subpopulations of WLH chicken. Suh *et al,* (2014) who employed same locus using capillary electrophoresis in 450 chickens belonging to six different Korean native chicken breeds (Korean Reddish Brown, Korean Yellowish Brown, Korean Grayish Brown, Korean Black, Korean White, Korean Ogye,) and three imported breeds (White Leghorn, Rhode Island Red, Cornish) and reported eight numbers of allele across breeds ranging in size from 186 to 208 bp. Deshmukh *et al.* (2015) revealed three alleles each with 194, 204 and 216 bp size in randomly selected Hill fowl, Rhode Island Red, Kadaknath, and White Leghorn and two alleles of 194 and 204 bp in White Cornish chicken.

Microsatellite locus **ADL0210** resolved two alleles of 124 and 132bp with their frequency of 0.053, and 0.947 in the selected RIR population studied. However, Panday *et*

al. (2002) used the same primer pair for this locus and revealed five alleles in Aseel, eight alleles in Miri and six alleles in Nicobari chicken breed. Vijh and Tantia (2004) observed nine alleles at this locus in four native chicken breeds. Pandey *et al*. (2005) reported 5 alleles ranging from 115-131 bp for ADL0102 locus in Ankleshwar chicken breed. Haunshi and Sharma (2006) observed 2 different alleles of sizes as 124 and 131bp in backcross progenies of Nacked neck population. Present finding was quite similar to Chatterjee *et al*. (2010a) who reported four alleles with sizes of 124, 128, 130 and 134 bp in six crossbred chicken populations of WLH. Chatterjee *et al*. (2010b) reported three alleles at ADL0210 locus with sizes of 128, 134 and 142 bp among three lines and two native chicken breeds. The alleles observed in the present study have also been reported by others workers but the number of alleles as well as frequency varied, due to different selection programmes being followed in the population studied thereby causing fixation or loss of some of the alleles.

Microsatellite locus **MCW0007** resolved three alleles of 302, 275 and 262 bp. The frequency of A, B and C allele were 0.105, 0.586 and 0.309, respectively. However, Panday *et al.* (2002) used the same primer pair for this locus and reported three alleles in Aseel and Miri and two alleles in Nicobari chicken breed. Vijh and Tantia (2004) observed 7 alleles at this locus in four native chicken breeds. Pandey *et al*. (2005) reported four alleles ranging from 295-317 bp in Ankleshwar chicken breed, which were close to present observation. Chatterjee *et al*. (2008a, b) who analyzed same locus and reported three alleles with sizes of 292, 298 and 320 bp, in six different crossbred white leghorn chicken, which were quite similar to the present findings. Chatterjee *et al*. (2010b) reported two alleles with sizes of 290 and 318 bp among three different chicken lines and two native Aseel and Kadaknath breeds. Arya (2012) studied polymorphism at MCW0007 microsatellite loci by 3.4% MetaPhor Agarose gel electrophoresis and reported only two alleles of 227 and 303 bp in both high and low egg production subpopulations of White leghorn chicken. Deshmukh *et al.* (2015) studied same locus in RIR chicken and reported alleles of 316 and 340 bp with frequencies of 0.27 and 0.73, respectively. The differences in number and sizes of alleles could be attributed to the differences in the genetic makeup of the genetic groups analyzed.

Microsatellite locus **MCW0014** resolved two alleles of 192bp and 176 bp with frequencies of 0.138 and 0.862 in the selected RIR population studied. However, Panday *et al.* (2002) used the same primer pair for this locus and revealed five alleles in Aseel and Miri and two alleles in Nicobari chicken breeds. Vijh and Tantia (2004) observed eight alleles at MCW0014 locus in four native chicken breeds. Pandey *et al*. (2005) reported five alleles ranging from 173-188bp in Ankleshwar chicken breed. Almost similar size range as observed in this study were also reported by Bao *et al,* (2007 and 2008) who employed same marker and reported 11 alleles ranging from 160-186bp in 14 Chinese indigenous chicken breed and red jungle fowl using 8% polyacrylamide gel. Present observation were in close aggrement to the finding of Chatterjee *et al*. (2010a) who analyzed the same locus and reported two alleles with sizes of 180 and 192 bp in six crossbred chicken populations of White Leghorn. Present finding was also quite similar to the report of Chatterjee *et al*. (2010b) who reported four alleles at MCW0014 locus with sizes of 180, 182, 190 and 206 bp among three different chicken lines and two native Aseel and Kadaknath breeds. Arya (2012) also analyzed MCW0014 microsatellite loci in 3.4% MetaPhor Agarose and revealed only one allele of 246 bp in both high and low egg production sub-populations of WLH chicken. Deshmukh *et al.* (2015) studied same locus in 3.4% MetaPhor Agarose and reported two alleles of 200 and 220 bp with frequencies of 0.29 and 0.71 in RIR chicken. Variation in various reports might be due to variations in the genetic groups analyzed.

Microsatellite locus **MCW0041** revealed monomorphic pattern and shown only one allele of 164 bp in the selected RIR population studied. Panday *et al.* (2002) revealed four alleles in Aseel and Miri and two alleles in Nicobari chicken breed at this locus. Vijh and Tantia (2004) observed eight alleles at MCW0041 locus in four native chicken breeds. Pandey *et al*. (2005) resolved five allele at this locus in Ankleshwar with a size range of 152 to 172 bp. Allele found in the present finding had size with range as per earlier finding of Chatterjee *et al*. (2010a) who reported three alleles with sizes of 150, 160, and 170 bp in six crossbred chicken populations of White Leghorn. Das (2013) studied same locus and reported alleles of 148, 156 and 162 bp with the frequencies of 0.083, 0.583 and 0.333 in RIR^S chicken. The difference in allele number might be due to absence or loss of other alleles during long term

genetic selection in the population used in present investigation or due to limitation of the methodology used.

Microsatellite locus **MCW0069** resolved six alleles of 232, 221, 210, 194, 184 and 172bp with frequencies of 0.026, 0.059, 0.198, 0.138, 0.408 and 0.171, respectively. However, Bao *et al.* (2007) and Bao *et al.* (2008) who studied the same locus in 14 Chinese indigenous chicken breeds and red jungle fowl using 8% PAGE and reported nine alleles ranging from 158-176 bp. Arya (2012) studied MCW0069 microsatellite locus using 3.4% MetaPhor Agarose and reported 2 alleles of 126 and 160 bp only in high egg production and only one allele of 110 bp in low egg production subpopulation of WLH chicken. Deshmukh *et al.* (2015) reported two alleles at this locus with sizes of 174 and 198 bp and frequencies of 0.62 and 0.38 in RIR chicken breed. The wide variation in number of alleles and sizes at this locus might be due to differences in genetic architecture and the different populations under various studies.

Microsatellite locus **MCW0103** revealed two alleles of 298 and 276 bp with frequency of 0.263 and 0.737, respectively. The Present finding were in agreement with the report of Bao *et al.* (2007) and Bao *et al.* (2008) who also revealed two alleles but with the sizes of 266 and 270 bp in 14 Chinese indigenous chicken breeds and one red jungle fowl using 8% PAGE. Arya (2012) analyzed MCW0103 microsatellite loci on 3.4% MetaPhor Agarose and reported four alleles of 359, 401, 439 and 479 bp in high egg subpopulations and only one allele of 359bp in low egg production subpopulation of WLH. Suh *et al.* (2014) who analyzed the same locus in ABI 3130xl Genetic Analyzer using capillary electrophoresis and reported two alleles with sizes of 269 and 273 bp in six Korean native and three imported chicken breeds. Deshmukh *et al.* (2015) reported only one allele of 324 bp in five chicken breeds. Differences in allele numbers and sizes may be attributed to the sensitivity of techniques employed in these studies, beside the genetic differences.

5.2.1 Average heterozygosity and polymorphic information content (PIC)

Nei's heterozygosity with microsatellite analysis in present study ranged from 0.0997 (ADL0210) to 0.7421 (MCW0069). Four microsatellite loci demonstrated heterozygosity

more than 0.50 and five microsatellite loci demonstrated heterozygosity less than 0.50. The average heterozygosity pooled over all polymorphic loci was 0.4119± 0.2475 in selected RIR chicken (Table 4.2.3). Similar to the present finding, Vijh and Tantia (2004) studied in four native chicken breed (Nicobari, Miri, Aseel and Kashmir Favorolla) and Pandey *et al*. (2005) in Ankaleshwar chicken and reported higher Nei's heterozygosity at ADL0020, ADL0023, ADL0102, ADL0176, ADL0210, MCW0007, MCW0014 and MCW0041 MS loci and mean Nei's heterozygosity than the present investigation. Das (2013) also reported high Nei's heterozygosity for ADL0102 (0.6720) ADL0210 (0.6921), MCW0014 (0.4992), MCW0041 (0.4599) and low for ADL0176 (0.4918) in selected strain of RIR chicken. The low level of heterozygosity estimated in present study might be due to small population of RIR chicken and that too under long term selection programme.

Polymorphic information content (PIC) is a parameter indicative of the degree of informativeness of a marker (Parmar *et al.,* 2007). Nine out of ten microsatellite loci under the present study were found to be polymorphic in RIR^S chicken. The estimated mean PIC value was 0.313±0.064 and it ranged from 0.0947 (ADL0210) to 0.6020 (ADL0176). Pandey *et al*. (2002) in Aseel, Nicobari and Miri chicken, Vijh and Tantia (2004) in four native chicken breed (Nicobari, Miri, Aseel and Kashmir Favorolla) and Pandey *et al*. (2005) in Ankaleshwar chicken who analyzed the same eight loci viz., ADL0020, ADL0023, ADL0102, ADL0176, ADL0210, MCW0007, MCW0014 and MCW0041, and reported higher PIC values than the present investigation. Chatterjee *et al.* (2008a) analyzed the same five MS loci viz., ADL0020, ADL0023, ADL0102, ADL0176, and MCW0007 used in present study in six cross bred of IWH, IWI and IWK strain of WLH chicken selected for egg number, egg weight and feed efficiency over 10 generations of selection and reported higher PIC value at ADL0020 (0.54) and ADL0023 (0.58) and somewhat close to present study at ADL0102 (0.58), ADL0176 (0.72), and MCW0007 (0.55). Bao *et al*, (2007) and Bao *et al*, (2008) reported high PIC value for MCW0014 and low for MCW0069 than the present study in Chinese chicken breeds and red jungle fowl.

The PIC value at ADL0020 locus was reported as 0.7582 in Korean Native chicken (Kim *et al*., 2008b) and as 0.59 in WLH, Kadaknath, Ankleshwar and CARI-Uttam quail

(Khan *et al*., 2010). Chatterjee *et al*. (2010a) reported PIC value as 0.61 (ADL0210), 0.38 (MCW0014) and 0.52 (MCW0041) in six crossbred chicken of WLH. Chatterjee *et al*. (2010b) a evaluated seven loci viz., ADL0020, ADL0023, ADL0102, ADL0176, ADL0210, MCW0014 and MCW0041 as also used in present investigation along with other microsatellite in two indigenous native breed (Kadaknath and Aseel) and three different chicken lines (Vanaraja male line, Vanaraja female line and Gramapriya female line) and reported higher PIC value at ADL0020 (0.656), ADL0023 (0.712), ADL0176 (0.694), ADL0210 (0.456), MCW0014 (0.364) and MCW0041 (0.512) and somewhat close to present study at ADL0102 (0.573). Suh *et al.* (2014) reported PIC value as 0.703 for ADL016 and 0.258 for MCW0103 in six Korean native chicken and three imported (White Leghorn, Rhode Island Red and Cornish) breeds. Deshmukh *et al*. (2015) reported low PIC values at ADL0176 (0.656) and MCW007 (0.712), and high at MCW0069 (0.250) and MCW0103 (0.154) in RIR chicken than the present estimates. The PIC values indicated that all the loci, except ADL0102 and ADL0176 under study were moderately polymorphic. The differences in polymorphic information content of various microsatellite loci may be due to the differences in genetic architecture of population analysed or may be probably due to loss and or fixation of some of the alleles after long term selection. It may be also due to different techniques employed by different workers.

5.2.2 Population genetic analysis of microsatellite data

The mean ± SE of observed and effective number of alleles and Shannon's index were 3.0000±1.4142, 2.0324± 0.9416 and 0.7342± 0.4649 (Table 4.2.4). Effective number of allele ranged from 1.1108 (ADL0210) to 3.8778 (MCW0069).

Present findings of population genetic analysis were compared to previous reports on some of these loci in different native chicken breeds or strains. High number of effective alleles reported by Pandey *et al.* (2002) in Aseel, Nicobari, and Miri native chicken breed and high number of effective alleles as well as Shannon's index was reported by Pandey *et al*. (2005) in Ankaleshwar chicken at ADL0020, ADL0023, ADL0102, ADL0176, ADL0210, MCW0007, MCW0014 and MCW0041 locus than the present investigation. Chatterjee *et al.* (2010b) reported high observed and effective number of allele as well as Shannon's index

for ADL0020, ADL0023, ADL0102, ADL0176, ADL0210, MCW0014 and low for MCW0007 in Kadaknath and Aseel breed and three different chicken lines than the present study. Das (2013) reported the observed and effective number of allele as well as Shannon's index as 4, 3.5714 and 1.3322 at ADL0102; 3, 2.3810 and 0.9433 at ADL0176; 5, 3.7895 and 1.4452 at ADL0210; 3, 2.2727 and 0.9503 at MCW0014 and at MCW0041 as 3, 2.1818 and 0.8877 respectively, in selected strain of RIR chicken.

5.2.3 Hardy-Weinberg equilibrium

The mean ± SE of observed and expected heterozygosity were 0.2776±0.211 and 0.4147±0.2491, respectively (Table 4.2.5). If $H_o \simeq H_e$, then the population is considered to be in H-W equilibrium. In the present study, the mean H_e was more than the mean H_o which indicated that population was not in Hardy-Weinberg equilibrium but under the influences of some forces like selection for some economic traits that might be associated with microsatellite loci. The results of Chi-square test and G-square tests also revealed significant differences between H_o and H_e frequencies demonstrating that the population was under H-W disequilibrium for all loci, which might be due to influence of external forces. Since, the studied population was continuously being selected for part-period egg production and also small in size, this might have been the reason for it being in Hardy-Weinberg disequilibrium. The observed and expected heterozygosity was also reported by Pandey *et al*. (2005) in Ankaleshwar at ADL0020 as 0.850 and 0.640, at ADL0023 as 0.333 and 0.804, at ADL0102 as 0.410 and 0.709, at ADL0176 as 0.816 and 0.740, at ADL0210 as 0.342 and 0.527, at MCW0007 as 0.692 and 0.675, at MCW0014 as 0.539 and 0.624 and at MCW0041as 0.300 and 0.369, respectively. Chatterjee *et al.* (2010b) reported high observed and expected heterozygosity for ADL0020 (0.74 and 0.76), ADL0023 (0.91 and 0.79), ADL0102 (0.59 and 0.76), ADL0176 (0.90 and 0.80), ADL0210 (0.99 and 0.62), MCW0007 (0.83 and 0.49) and MCW0014 (0.72 and 0.67) in Kadaknath and Aseel breed and three different chicken lines than the present study Das (2013) reported the observed and expected heterozygosity as 0.8000 and 0.7579 for ADL0102, 0.2000 and 0.6105 at ADL0176, 0.1667 and 0.7681 at ADL0210, 0.0000 and 0.5895 at MCW0014 and 0.3333 and 0.5652 at MCW0041 respectively, in selected strain of RIR chicken. Deshmukh *et al.* (2015) estimated high observed

and expected heterozygosity in RIR chicken for ADL0176 (0.571 and 0.561), MCW0007 (0.546 and 0.416), MCW0014 (0.250 and 0.431) and MCW0069 (0.154 and 0.492) respectively**.**

5.2.4 Association of microsatellite genotypes with growth traits in selected pure strain of RIR chicken

Least squares analysis of variance elucidated significant ($P \leq 0.05$) MS-genotype effect for ADL0020 (Table 4.2.6) and ADL0210 (Table 4.2.14) on body weights at 16, 20, 40 and 64 wks of age, MCW0007 (Table 4.2.16) on BW40, and MCW0014 (Table 4.2.18) on BW16 and BW40 in selected strain of RIR chicken. Critical difference test showed that some MS genotypes had significantly different body weights (Tables 4.2.7, 4.2.15, 4.2.17, 4.2.19).

Present findings were quite similar to earlier report by Chatterjee *et al*. (2008b), who studied five microsatellites and their association with growth and immunocompetence traits in six crossbred population of white leghorn chicken selected for egg number, egg weight and feed efficiency over 10 generations of selection and reported significant ($P \leq 0.05$) association between genotypes at MCW007 with body weight at zero, BW8, BW12, BW20, BW28 and BW40, genotypes at ADL0020 with BW8, BW12 and BW40 and genotypes at ADL0176with BW40. Kim *et al*. (2008b) Studied 17 polymorphic microsatellite markers on chromosome one used for allelic association tests with phenotypic traits in Korean native chicken and reported that UMA1.117, ADL0020, UMA1.019, LMA1 and ADL0238 loci showed significant differences in allelic distribution for the trait of body weights (BW). Chatterjee *et al*. (2010a) reported that MCW0041 genotypes were significantly ($P \leq 0.05$) associated with body weights at 28 and 40 weeks of age. Das (2013) reported that MCW0014, MCW0051 and ADL0176 genotypes were significantly ($P \leq 0.05$) associated with body weight at 40 weeks of age in selected strain of RIR chicken.

Different workers have also reported significant association between genotypes at various MS loci and body weight at different ages. Sewalem *et al.* (2002) genotyped 101 microsatellite markers on chromosome 1 in F_2 chicken population which was established by crossing of a broiler sire-line and an egg laying (White Leghorn) line and found that the microsatellite LEI0068, LEI0146, and MCW0018 were associated with body weights at 3, 6

and 9 weeks of age and microsatellite ROS0025 affected the body weight at 6 weeks of age only. Pandya *et al.* (2005) studied LEI-146 and MCW-43 microsatellites association with growth traits in Bantam, White Leghorn and Bantamised White Leghorn chicken. The birds having 1,1 and 1,3 genotypes for LEI0146 microsatellites marker, had lowest body weight at all the ages, while birds with genotypes 2,2 and 3,3 were having highest body weight at all the ages. Birds with1,3 and 4,4 genotypes for MCW043 microsatellites markers were having highest body weight while birds with 1,2 and 2,3 genotypes at this locus were having lowest body weight at all the ages. Atzmon *et al*. (2006) genotyped 76 microsatellite markers and established association between microsatellite markers and growth related traits and reported that MCW0102 was significantly associated with BW at 7 weeks in a commercial broiler line. Nones *et al*. (2006) found that LEI0068 and LEI0079 markers were associated with BW at 35 and 42 days and MCW0058 with BW at 42 days in F_2 experimental population, developed by two generations of crossbreeding between a broiler sire line and a layer line. Nassar *et al*. (2012) analysed seven microsatellite markers in the 6th selected generations of Cairo B-2 line and compared with the control line (C line). The allelic frequencies of the simple sequence repeats (SSR) loci, ADL0328, were higher (six alleles) in the Cairo B-2 line, males and females, while the C line showed only five alleles and reported that heavier birds had more alleles for the ROS0025, MCW0010, MCW0018, c3-46151949, c5-4999025, and MCW0097, than the lighter birds.

5.2.5 Association of microsatellite genotypes with layer economic traits in selected pure strain of RIR chicken.

Least squares analysis of variance elucidated significant ($P \leq 0.05$) effect of MS-ADL0020 genotypes on EP40 and EP64 and also on EW64 ($P \leq 0.08$) (Table 4.2.6), ADL0023 genotypes on AFE and EP40 of age (Table 4.2.8), ADL0210 genotypes on AFE, EW28, EW40 and EW64 (Table 4.2.14) and MCW0014 genotypes on EW28 ($P \leq 0.11$) weeks of age (Table 4.2.18) in selected pure strain of RIR chicken. Critical difference test showed that some MS genotypes differed significantly for variety of layer economic traits (Tables 4.2.7, 4.2.9, 4.2.15, 4.2.19).

Present finding was in accordance with the earlier report of Chatterjee *et al*. (2008a) who studied genetic variability at five microsatellites and their association with egg production

traits in six crossbred population of White Leghorn chicken exploring same loci as used in the present investigation and reported that only genotypes at ADL023 microsatellite were significantly ($P \leq 0.05$) associated with egg production upto 64 and 72 weeks and egg weight at 28 weeks of age. Genotypes 11, 12, 13 and 23 produced more number of eggs than genotype 22. Genotype 22 produced poor performance by about 25% lower than genotype 13 which was the highest producing genotype. Egg weight was better in case of genotype 12, 13 and 23 and the lower egg weight was observed in birds with genotype 11 and 22 for microsatellite ADL023. Chatterjee *et al*. (2008b) also studied five microsatellites used in present study for their association and reported that MCW0007, ADL0020, ADL0023 and ADL0176 microsatellite were significantly associated to age of sexual maturity in crossbred population of white leghorn chicken, selected over 10 generations of selection. Kim *et al*. (2008b) studied allelic association tests with phenotypic traits in Korean native chicken and reported that allelic frequency of MCW0160 showed a significant difference between the high and the low groups for egg weight. ADL0101 and ADL0238 show significant differences in allelic pattern for egg production trait and ADL0234, UMA1.125 and ADL0101 for age at first egg lay. Chatterjee *et al*. (2010a) explored nine microsatellite markers to study genetic variability of microsatellites and possible relationship with growth, egg production, and immunocompetence traits in six genetic groups of White Leghorn and reported that MCW0041, ADL0210, and MCW0110 were significantly ($P \leq 0.05$) associated with egg production traits. Genotype 33 at MCW0041 had the highest egg production, up to 64 and 72 weeks of age. Genotypes 11 and 13 of this marker produced the lowest number of eggs. Genotypes 11 and 13 at ADL0210 marker produced the lowest number of eggs. The heterozygous genotype 34 at ADL0210 had the highest egg production, up to 52, 64, and 72 weeks of age. Homozygote 11 at MCW0110 produced the highest number of eggs, up to 28 weeks of age. No microsatellite polymorphism was significantly associated with egg weight at any age, with age at sexual maturity, or with immune response to sheep RBC. Das (2013) reported that genotypes at MCW0044, ADL0102 and ADL0158 locus had significant ($P \leq 0.05$) effect on EP40, MCW0075 locus on AFE, MCW0005 and MCW0014 loci on EW28 in selected pure strain of RIR chicken.

5.3 Objective 3: To estimate immunocompetence traits and their association with layer economic traits in Rhode Island Red chicken.

The immunocompetence traits estimated in the selected strain of RIR chicken that has undergone long term selection for pert-period egg production are discussed below in light of the recent available literature.

5.3.1 Antibody response to SRBC

Results revealed wide variability in HA titres which ranged between 2-17 with its overall least-squares mean in selected strain of RIR as 9.35 ± 0.29 (Tables 4.3.1 and 4.3.2).

Wide variation in the antibody response to sheep RBC, measured at 5th dpi in selected strain of RIR as in accordance with the earlier reports. Various factors have been reported to alter and influence the antibody response to SRBC in chickens viz. genetic factors (Gyles *et al.,* 1986; Sivaraman *et al.,* 2003; Das *et al.,* 2014a), higher dose elicited higher responses (Ubosi *et al*., 1985; Boa-Amponsem *et al.,* 2000), intravenous route inoculation initiated higher antibody responses than other routes (Van de Zijpp and Nieuland, 1986; Boa-Amponsem, 2001; Kumar, 2006) and age of the bird affected the response (Ubosi *et al.,* 1985). One or more of these factors might have been responsible for wide variation in antibody titres observed in the present investigation.

The estimate was quite comparable to previous reports in different chicken breeds. The mean HA titre was within range as reported by Sivaraman *et al*. (2005) in the base population of SDL broiler chicken in response to SRBC with its least squares mean as 6.289±0.246. Chatterjee *et al.* (2007a) reported HA titre as 7.25 ± 0.18 in Aseel and 5.70 ± 0.25 in Kadaknath native chicken. Chatterjee *et al.* (2007b) estimated the HA titre as 8.79 ± 1.44 and 7.60 ± 1.78, respectively in a non-inbred (NB) and full-sib mated (FS) populations of Dahlem Red chicken, which corroborated well with the present findings. Saini *et al.* (2008) estimated low HA titre then the present study at 4th dpi in RIR-C (CARI strain) and RIR-B (Bhubaneswar strain) chicken as 5.20±0.47 and 4.70±0.41, respectively. Singh *et al.* (2009) recorded HA titre as 7.49 ± 0.25 in Kadaknath and Singh *et al*. (2010) as 9.22 ±0.20 in

Aseel native chicken breed. Gupta *et al.* (2010) also reported HA titre in HSRBC and LSRBC lines of white Leghorn chicken to be 8.06±0.22 and 7.87±0.26, respectively. Rajkumar *et al.,* (2010) evaluated the effect of naked neck (Na) gene on immunocompetence traits in three genotypes (NaNa, Nana and nana) of the naked neck chicken under a tropical climate of Southern India and reported that antibody titre was significantly higher in NaNa (7.00±0.29) followed by Nana (6.88±0.65) and nana (4.62±0.38). Tomar *et al.* (2012) estimated in five indigenous developed broiler parent lines through long-term selection for 5 week body weight and obtained highest titre in Naked neck (7.923±0.383) and least in SML (6.280±0.296) broiler. However, Revagade *et al.* (2013) determined 5th dpi HA titre as 8.810±0279 and 8.429±0.279 in tropical oriented chicken with Naked neck (Na) and Frizzle (F) major genes Kokate *et al.* (2013) recorded higher HA titre in Aseel, Kadaknath and White leghorn chicken as 10.84±0.18, 11.62±0.21 and 11.94± 0.15, respectively. Das *et al*. (2014a) estimated 5dpi HA titre as 5.739±0.436 in CARI-Dabendra; Jaiswal *et al*. (2014) reported 5dpi HA titre as 7.93 ± 0.24 in Kadaknath native chicken. Das *et al.* (2014b) reported the same in CARI-Sonali as 6.001±0.441. The 5dpi HA titre in present study was in approximation to the findings of Das *et al.* (2014c) who recorded 5 dpi HA titre in control, selected and white strain of RIR chicken as 8.837±0.473, 10.393±0.473 and 6.511±0.504, respectively. The differences in various reports might be due to the reason that these genotypes were not selected for any IC trait or may be due to different genetic backgrounds of breeds studied.

5.3.2 Serum lysozyme concentration

Least-squares mean of serum lysozyme level was 4.77±0.15 µg/ml and 4.51±0.16 µg/ml in male and females of selected strain of RIR chicken, respectively. The overall average was 4.64±0.11 µg/ml (Tables 4.3.1 and 4.3.2).

The mean observed in the present finding were comparable with earlier reports in various breeds of chicken. Sivaraman *et al*. (2005) estimated serum lysozyme level as 1.860±0.047 mg/ml in synthetic dam line of broiler chicken. Gupta *et al.* (2010) reported the lysozyme concentration in low and high SRBC response lines of white Leghorn chicken to be 2.77±0.09 µg/ml and 2.85±0.08 µg/ml, respectively. Kumar and Kumar (2011) estimated it

as 3.42 ± 0.19 µg/ml in Aseel and Jaiswal *et al*. (2014) as 5.07 ± 0.29 µg/ml in Kadaknath native chickens. Tomar *et al.* (2012) reported that SDL broiler parent line had highest serum lysozyme (4.180±0.049 mg/ml) concentration among the five germplasm (coloured plumaged CSML and CSFL, white plumaged SML and SDL and naked neck lines) analyzed. However, Kokate *et al.* (2013) recorded higher serum lysozyme concentration as 21.28±0.78 µg/ml in Aseel, 16.91±0.93 µg/ml in Kadaknath and 9.42±0.68 µg/ml in White leghorn chicken, respectively. Das *et al.* (2014c) reported relatively higher estimates of serum lysozyme level in RIR^W (6.996±0.435) followed by RIR^S (6.336±0.437), RIR^C (5.174±0.428), CARI-Debendra (6.000±0.47) and CARI-Sonali (5.692±0.404) chicken.

5.3.3 Serum IgG concentration

Overall least squares mean of serum IgG level was 8.61±0.34 mg/ml. It was slightly higher in females (8.67 ±0.45 mg/ml) than males (8.56 ±0.42 mg/ml) in pure selected strain of RIR chicken (Tables 4.3.1 and 4.3.2).

Present findings corroborated well with the earlier reports in different genotypes or breeds. Sivaraman *et al*. (2005) estimated serum IgG concentration as 6.287±0.194 mg/ml in synthetic dam line of broiler chicken. Saini *et al.* (2008) reported serum IgG level in two selected strains (PL1 and PL2) and one random bred control line (PL3) of single comb White Leghorn and two Rhode Island Red (RIR-C and RIR-B) strains and found that IgG level was higher at 4dpi in RIR-C (1.83±0.28) among all strains studied. Singh *et al.* (2009) estimated higher serum IgG concentration (10.07±0.20 mg/ml) in Kadaknath chicken. Singh *et al.* (2010) obtained relatively higher IgG level (10.61±0.25 mg/ml) and Kumar and Kumar (2011) reported IgG level of 20.73±0.83 mg/ml in Aseel native chicken breed. Gupta *et al.* (2010) also reported higher serum IgG level in both HSRBC and LSRBC lines of white Leghorn chicken to be 33.91±1.68 mg/ml and 31.65±1.28 mg/ml, respectively. Tomar *et al.* (2012) found highest serum IgG (5.27±0.26 mg/ml) concentration in SDL broiler parent lines then the others germpalsm studied. Das *et al*. (2014c) estimated serum IgG concentration as 6.597±0.361, 7.780±0.361, 7.749±0.390, 5.151±0.398 and 6.002±0.398 µg/µl in RIR^S, RIR^C, RIR^W, CARI-Sonali and CARI-Debendra chicken, respectively. Jaiswal *et al*. (2014) reported overall serum IgG concentration as 12.15± 0.48 mg/ml in Kadaknath breed; it was higher in males

(12.47 ± 0.61) then females (11.82 ± 0.64). The HA titre in present study was close to the findings of Das *et al.* (2014b) who recorded 5 dpi HA titre in control and selected line of RIR chicken as 8.837±0.473 and 10.393±0.473, respectively. Das *et al*. (2015) obtained overall serum IgG concentrations as 6.98 ± 2.95 mg/ml in selected and control line of RIR chicken. The variation in the serum IgG concentration might be due to the differences in genetic makeup of different stocks and other factors like differences in feeding, management, environment, and age at the time of estimation of traits.

5.3.4 Influence of sex on IC traits

The influence of sex on HA titre, serum lysozyme and serum IgG concentration was found to be statistically non-significant (P>0.05), although males had higher antibody titre and serum lysozyme level than the females. But, IgG concentration was somewhat higher in females than males. It was suggestive of non-significant effect of sex on immunocompetence traits.

The non-significant effect of sex on IC traits was consistent with the reports of Sivaraman *et al.* (2005) in SDL broiler chicken; Singh *et al*. (2010) in Aseel chicken; Gupta *et al.* (2010) in WLH chicken and Jaiswal *et al.* (2014) in Kadkanath native chicken breed. Kokate (2013) Kumar and Kumar (2011) reported non-significant influence of sex on HA titre, although males revealed higher mean antibody titre and serum lysozyme level than females. Kokate (2013) in Aseel, Kadaknath and White leghorn chicken and Das *et al.* (2014a) in CARI-Debandra chicken also reported non-significant effect of sex on HA titre, serum lysozyme level and serum IgG level although males demonstrated higher estimates of HA titre, serum lysozyme and serum IgG concentration than female birds. Das *et al.* (2014b) also reported non-significant effect of sex on all IC traits in CARI-Sonali chicken although males demonstrated higher estimates of HA titre, serum lysozyme than female birds and serum IgG concentration was somewhat equal in both sexes. Present results indicated that genetic mechanism responsible for mounting of antibody response to SRBC and regulation of serum lysozyme and serum IgG levels in RIR might be sex-independent.

5.3.5 Genetic and phenotypic parameters of immunocompetence and growth traits in RIR chicken

Least Square ANOVA revealed that sire had significant effect on serum IgG ($P \leq 0.01$) level and body weight at BW16, BW20, BW40 and BW64 weeks of age (Table 4.3.5).

5.3.5.1 Heritability

The **heritability** estimates of serum IgG level was high (0.302±0.188) and it could not be estimated for HA titre and serum lysozyme level. Jaiswal *et al.* (2014) reported high heritability estimates for serum IgG (0.257 ± 0.228) in Kadaknath native chicken. Gupta *et al.* (2010) reported very low (0.009±0.059 to 0.049±0.083) estimates of heritability for IC traits in the HSRBC line and could not be estimate in LSRBC line of White Leghorn. Present finding are in accordance with the reports of Kokate (2013) in White leghorn chicken, who also could not estimate h^2 of HA titre and serum lysozyme and obtained very low estimates of serum IgG (0.01±0.31) level. The low heritability value for immune response traits indicated that effective improvement in these traits can be achieved through some form of family selection or combined selection based on individual's own and family values for IC traits.

The heritability estimates for BW16, BW20, BW40 and BW64 in RIR chicken were 00.516± 0.22, 0.468 ± 0.219, 0.302±0.188 and 0.344±0.214, respectively. High h^2 for BW16, BW20 and BW40 in IWP strain of White Leghorn by Qadri *et al.* (2013); high h^2 for BW40 by Kumar *et al.* (2002); BW20 and BW40 by Jilani *et al.* (2005) and Jilani *et al*. (2007) in RIR chicken were quite similar to the present finding.

5.3.5.2 Genetic and phenotypic correlations

The genetic correlation (r_G) among IC traits could not be estimated as the heritability of HA titre and serum lysozyme level could not be estimated. Gupta *et al.* (2010) in IWJ-high SRBC line; Kokate (2013) in Aseel, Kadaknath and White leghorn chicken also reported that the r_G among IC traits was not estimable. However, Jaiswal *et al.* (2014) reported high and negative r_G between HA titre and serum lysozyme, very low and negative r_G between serum lysozyme and serum IgG level which however were associated with high standard errors. Serum IgG concentration had positive and low genetic correlation with BW16 (0.162 ± 0.395)

moderate with BW20 (0.302±0.392) although associated with high standard error, but positive and high genetic correlation with BW40 (0.928±0.459) and BW64 (0.796± 0.507), that was associated with low standard error. Sivaraman *et al.* (2005) had also reported low to high negative genetic correlations of serum IgG concentration with body weights at four, five and six weeks of age in synthetic dam line of broiler chickens associated with high standard error. Saini *et al.* (2007) reported positive genetic correlations between HA titre and body weights at 12, 24, 28 and 40 weeks of age in selected strain of White Leghorn.

The Phenotypic correlations (r_p) among IC traits were lower in magnitude. The HA titre had negative r_p with serum lysozyme and IgG level. But serum lysozyme levels had low and positive r_p with serum IgG level. Sivaraman *et al.* (2005) in synthetic dam line (SDL) of broiler chicken, Singh *et al.* (2009) and Jaiswal *et al.* (2014) in Kadaknath native chicken, Singh *et al*. (2010) in Aseel chicken, Singh *et al*. (2010) in third generation of specialized white Leghorn chicken, Das *et al*. (2014a) in CARI-Dabendra and Das *et al*. (2014b) in CARI-Sonali also reported lower phenotypic correlations among IC traits. HA titre had low and negative phenotypic correlation with BW40 and BW64 and positive with BW16 and BW20. Serum lysozyme concentration also had positive and low phenotypic correlation with BW16, BW20, BW40 and BW64. HA titre and Serum IgG concentration had positive and low phenotypic correlation with early body weights such as BW16 and BW20 and very low and negative correlation with BW40 and BW64. Martin *et al.* (1990) also observed very low phenotypic correlation of SRBC response with growth traits of chicken lines selected for high or low antibody response to sheep RBCs. Kundu (1997) observed no consistent trend either in magnitude or in direction of phenotypic correlations for different immunocompetence traits with growth traits. Sivaraman *et al.* (2005) had also reported very low phenotypic correlations of SRBC response with body weights at four, five and six weeks of age in synthetic dam line of broiler chicken. Saini *et al.* (2007) reported positive r_G and negative r_p between HA titre and body weights at 12, 24, 28 and 40 weeks of age in selected strain of White Leghorn. Eid *et al.* (2010) reported negative phenotypic correlations between antibody titres at 7 (-0.049), 14 (-0.008) and 21 (-0.041) dpi and body weight at seven weeks of age in both broiler (ISA Hubbard and Ross 308) strains, respectively.

5.3.6 Immunocompetence and layer economic traits and their inter-relationship in RIR chicken

5.3.6.1 Heritabilities

Heritability estimates were moderate to high for serum lysozyme level, BW16, EW28 EW40, EW64, EP40 and EP64 and lower for serum IgG level (Table 4.3.8). IC traits h^2 estimates were associated with high standard errors making them less precise. Singh *et al,* (2009) in Aseel and Jaiswal *et al,* (2014) in Kadaknath native chicken also obtained higher heritability estimates for serum lysozyme level. It was in consonance with the reports of Singh *et al*. (2009) in Aseel and Gupta *et al.* (2010) in HSRBC line of IWG chicken. The heritability estimates for egg weights at 28 and 40 weeks of age were moderate (0.21-0.24) but were high heritable for BW16, EW64, EN40 and EP64. The heritability estimates indicated that sufficient genetic variance existed in the flock for further genetic improvement of these traits. For IgG, being lowly heritable trait, effective improvement can be achieved through some form of family selection or combined selection based on individual's own and family values.

5.3.6.2 Phenotypic and genetic correlations

Hemagglutination titre had very low phenotypic correlations with all the traits except EW64 where it was low and positive. Saini *et al.* (2007) had shown negative phenotypic and positive genetic correlation of HA titre with body weights at 12, 24, 28 and 40 weeks of age and also with egg production traits in WLH chicken. The genetic correlation of HA titre with other traits could not be estimated, which might be attributed to sampling variation due to small sample size. Similar trend were reported by Singh *et al*. (2009), Singh *et al*. (2010), Kokate (2013) in Aseel, Kadaknath and White Leghorn chicken, respectively. Serum lysozyme level had high negative r_G with serum IgG level, EP40 and EP64; low to moderate r_G with EW28 and EW64, positive and high r_G with BW16 and EW40 a. Das *et al*, (2014a) reported negative genetic correlation of serum lysozyme with serum IgG level in CARI-Debendra chicken. Serum IgG concentration had high and positive r_G with BW16 and egg weights at different ages but negative correlation with EP40 and EP64. Sivaraman *et al,* (2005) reported positive and high genetic correlation amongst body weights at 4th, 5th and 6th weeks of age in synthetic broiler dam line, although higher standard error was associated with these estimates, which

made them less precise. Present investigation demonstrated low inheritance pattern of immunocompetence traits and hence suggested their incorporation in selection programme along with layer economic traits. Selection based on an index that incorporates different immunocompetence and economic traits, may help in genetic improvement of overall general immunocompetence of chicken.

5.3.7 Influence of levels of immunocompetence traits on growth traits in RIR chicken

LS ANOVA revealed that serum IgG level significantly affected the BW40 and BW64 (Table 4.3.9). It was observed that birds with low serum IgG concentration showed highest BW40 than those having non-significantly different medium and high levels of serum IgG. Similarly, birds with low IgG level demonstrated highest BW64 (Table 4.3.10).

Varied reports are available in the literature on this and related aspects. Parmentier *et al.* (1998) studied the antibody response and body weights of chicken lines selected for high and low humoral responsiveness to sheep red blood cells and reported that the high immune response line chickens were significantly lower in body weight at 38 weeks of age than the control and low line selected for antibody response to SRBCs. Eid *et al*. (2010) evaluated the effect of both strain and sex against SRBCs antigen on live body weight, at different ages in two different commercial strains (ISA Hubbard and Ross 308) of broiler chicken. Results showed that chicks which had high antibody titers were low in body weight as compared to birds with low antibody levels. The difference between both low line (LL) and control (CL) and high lines (HL) was significant ($P \leq 0.05$) at different age. Das *et al.* (2014a) reported that serum lysozyme level had significant effect ($P \leq 0.05$) on body weight at 40 weeks of age in CARI-Debendra chicken. Birds having low or medium serum lysozyme level revealed comparatively heavier body weight at 40 weeks of age than the birds having high lysozyme level ($P \leq 0.05$). Das *et al*. (2015) reported that birds with high serum IgG levels had significantly ($P \leq 0.05$) more body weights at 20th week of age than those having medium and low IgG level in control line of RIR chicken.

5.3.8 Influence of levels of immunocompetence traits on layer economic traits in RIR chicken

Least square analysis of variance revealed significant influence of serum lysozyme level on EP64 (Table 4.3.11). It was observed that birds with highest serum lysozyme concentration produced highest number of eggs up to 64 weeks of age than those having medium or low levels. Females with medium serum lysozyme level produced the next lower number of eggs and those with low level which produced the lowest number of eggs up to 64 weeks of age (Table 4.3.12).

Significant influence of the level of various immunocompetence traits on some layer economic traits was observed in the present findings. Reports in this regard are very limited in the literature reviewed. Van der Zijpp and Nieuwland (1986) reported higher egg number and egg weight for those birds containing high HA titre than low HA titre in ISA Warren chicken line. Das *et al*. (2015) reported that HA titre and serum IgG levels have significant ($P \leq 0.05$) association with egg weights in selected strain of RIR chicken. Pullets containing high HA titre and serum IgG levels laid heavier ($P \leq 0.05$) eggs at 40th week of age than those with medium or low levels of HA titre and serum IgG. Again pullets having high and medium serum IgG levels also laid heavier ($P \leq 0.05$) eggs at 28th week of age than those with low IgG level.

Influence of level of HA titre was statistically non-significant on any of the layer economic traits in selected strain of RIR chicken, which was similar to the finding of Das *et al.* (2014a) in CARI-Debendra pullets, who reported that serum IgG levels had significant effect ($P \leq 0.05$) on egg weight 28 weeks of age (EW28). Egg weight at 28 weeks of age was significantly ($P \leq 0.05$) heavier for those hens containing high serum IgG level than low or medium IgG level. Das *et al.* (2014b) revealed significant ($P \leq 0.05$) effect of HA titre and serum lysozyme levels of on EW40 and AFE in CARI-Sonali chicken and reported that birds with high or medium HA titre levels had significantly ($P \leq 0.05$) more EW40 than those with low level of HA titre. Birds with medium serum lysozyme level had significantly ($P \leq 0.05$) lower AFE than those with high level of serum lysozyme. Van der Zijpp and Nieuwland, (1986) reported that, egg number and egg weight were higher in high SRBC response line than low SRBC line in chicken

5.3.9. Association of microsatellite genotypes with immunocompetence traits

Least squares analysis of variance revealed that none of the MS-genotypes had non significant (P>0.05) effect on any of the IC traits.

Present finding were similar to the reports of Chatterjee *et al*. (2008b) in six crossbred populations of White leghorn chicken selected over ten generations of selection, employed same five microsatellites (ADL0020, ADL0023, ADL0102 ADL0176, and MCW0007) used in present study, reported that none of the microsatellites were significantly associated with immune response against SRBC at 4-5week of age. Chatterjee *et al*. (2010a) also explored nine microsatellite markers to study the genetic variability and its possible relationship with growth, egg production, and immunocompetence traits in six crossbred populations of White leghorn chicken selected over ten generations of selection and reported that none of the microsatellites were significantly associated with immune response against SRBC.

5.4 Objective 4: To analyze relative expression of important immunity related genes in various tissues of Rhode Island Red chicken by quantitative reverse transcription PCR (qRT-PCR).

The primary data on mRNA expression were recorded and statistically analyzed, then the results were compiled and are being discussed below comprehensively with relevant and recent literatures: -

5.4.1 Differential mRNA expression of immune related genes in different sexes

Relative mRNA expression of three genes viz., IL1-β, iNOS and TLR15 was analyzed in three lymphoid tissues viz., bursa, spleen and thymus for assessing sex differences using GLM of SAS. Analysis revealed that the difference between two sexes was non-significant (P>0.05) for all the three genes in all the three tissues in selected pure strain of RIR chicken (Table 4.4.1, Table 4.4.3, Table 4.4.5).

Similar to the present finding Kokate (2013) also reported non-significant (P>0.05) effect of sex on mRNA expression of IL1-β, IFN-γ, iNOS gene in bursa and spleen tissue of Aseel, Kadaknath, and White leghorn chicken. Kumar *et al*. (2011) studied the effect of immunomodulators and genetic lines on expression of innate immunity genes IL1-β, IL-2,

iNOS, TLR4 and TLR15 and reported that there were no significant differences in relative expression of genes among different lines viz. broiler, layer and inbred Fayoumi and sexes except for IL1-β gene, which was significantly ($P \leq 0.05$) affected by sex and males exhibited higher ($P \leq 0.05$) IL1-β expression than females. Differences in reports might be due to the differences in the germplasm analyzed.

5.4.2 Differential mRNA expression of immune related genes in different tissues

Out of the three genes studied, the mRNA expression of iNOS gene differed significantly ($P \leq 0.001$) among lymphoid tissues in RIR chicken (Table 4.4.7). Highest expression (mean $40\text{-}\Delta C_t = 34.27 \pm 0.71$) was observed in Bursa tissue, followed by in spleen (31.38 ± 0.71) and thymus tissues (30.17 ± 0.71) (Table 4.4.8).

Similar to the present finding, Higgs *et al.* (2006) observed higher expression of TLR15 mRNA in bursa followed by spleen in chicken. However, Kokate (2013) while studying the basal expression of four immune response genes, iNOS, IFN-γ, IL1-β and TLR15 in three tissues viz., bursa, spleen and thymus tissue of Aseel, Kadaknath, and White leghorn chicken reported that the mRNA expression of all the four genes varied significantly ($P \leq 0.01$) among tissues of all breeds. The mRNA expression of IL1-β and TLR15 were highest in spleen whereas IFN- γ and iNOS were highest in thymus tissue of all the breeds studied. Very limited literature was available on this aspect and so is the comparison presented.

5.4.3 Influence of levels of immunocompetence traits on relative gene expression ($40\text{-}\Delta C_t$) in various lymphoid tissue of RIR chickens

Least squares analysis of variance revealed that the influence of HA titre levels had significant effect on relative gene expression of IL1-β ($P \leq 0.09$) in spleen. Serum lysozyme levels had significant effect on relative gene expression of TLR15 ($P \leq 0.01$) in spleen and IL1-β ($P \leq 0.06$) in thymus tissue (Table 4.4.9). Influence of serum IgG levels was statistically non-significant on all genes studied in all lymphoid tissues. It was observed that birds with medium or low HA tire revealed significantly higher IL1-β gene expression in spleen than birds having high HA titre. Similarly, birds with medium or low serum lysozyme level revealed significantly higher mRNA expression of IL1-β in thymus and TLR15 in spleen tissue than birds having

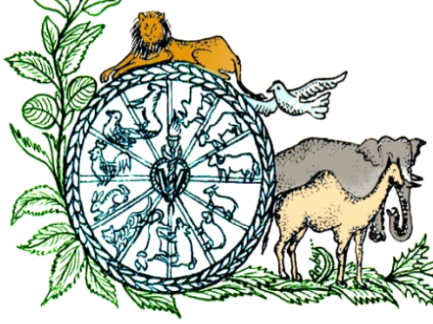
high lysozyme level (Table 4.4.10). There was no report in the literature on this aspect and hence the results could not be compared or contrasted. The findings paved way in chalking out programmes for genetic improvement for disease resistance and production and utilization of IC traits information therein.

5.4.4 Fold expression of IL1-β, INOS and TLR15 genes in different tissues

In RIR selected strain, the IL1-β gene expression was 2.65 and 1.27 folds more in bursa and thymus, respectively in comparison to spleen, iNOS gene expressed 17.12 and 2.30 folds more in bursa and spleen, respectively, in comparison to thymus and the TLR15 gene expression was 2.67 and 1.93 folds more in bursa and thymus, respectively, in comparison to spleen (Table 4.4.11).

The basal mRNA expression levels of all three immune response genes were more in bursa than thymus and spleen in all the three lymphoid tissues, which might be due to fact that bursa is the principal site for production of B-lymphocytes in chicken. There are a few reports available on fold expression studies in chicken but they are on breed/ genetic differences (Patel *et al.,* 2013, Annamalai *et al.,* 2014). However, reports on comparison of basal mRNA expression levels across tissues in RIR chicken could not be found. Thus, it can only be concluded that bursa tissue revealed maximum fold expression of the genes studied.

Summary and Conclusions



6 SUMMARY AND CONCLUSIONS

Present investigation was carried out in Rhode Island Red (RIR) chicken to analyze the effects of various genetic and non-genetic factors on layer economic traits, to determine allelic polymorphism at egg production-associated microsatellite loci and their association with layer economic traits, to estimate immunocompetence traits and their association with layer economic traits and to analyze relative expression of important immunity related genes in various tissues by quantitative reverse transcription PCR (qRT-PCR). Three hundred twenty five chicks were obtained in a single hatch after mating five males with 125 females in 1:5 ratio using A.I. All the experimental birds were maintained in similar conditions at Experimental Layer Farm, Central Avian Research Institute, Izatnagar, (India).

Data were recorded on growth, production and reproduction performance traits. Data on growth traits of combined sex at 16, 20, 40 and 64 weeks of age were analyzed by least squares ANOVA taking sire as random and sex as fixed effects in the model. Data on layer economic traits, viz., age of first egg, egg weight at 28, 40 and 64 weeks of age and egg production up to 40 and 64 weeks of age were also analyzed by least square ANOVA taking sire as random effect in the model. The overall percent fertility was 73.24% and hatchability on fertile eggs set (FES) and on total eggs set (TES) basis were 78.13% and 57.22%, respectively. Least squares analysis of variance revealed significant ($P \leq 0.01$) effect of sire and highly significant effect of sex ($P \leq 0.001$) on body weights at all ages. The overall least-squares means of body weight at 16, 20, 40 and 64 weeks of age on combined sex basis were 1362.6±21.4g, 1791.6± 24.6g, 2184.6±26.2 and 2433.8±34.2g, respectively. The corresponding mean body weights in males were 1492.9±24.0g, 2040.3±28.1g, 2624.4±31.3g and 2784.5±41.3g and

in females 1232.4±25.5g, 1542.9±30.2g, 1744.8±33.7g and 2083.1±42.9g; the differences between sexes were significant at all ages. Least squares analysis of variance revealed significant ($P \leq 0.01$) effect of sire on egg production up to 64 weeks of age and non- significant ($P > 0.05$) on all other layer traits. Least squares means of ASM, EW28, EW40, EW64, EN40 and EN64 were 134.5±0.9days, 44.8±0.3g, 47.7±0.4g, 51.5±0.7g, 118.3±1.2 eggs and 214.5±4.8 eggs, respectively.

The heritability estimates were high for growth traits and low to high for layer economic traits. Heritability estimates for growth traits were 0.55±0.22 (BW16), 0.42±0.19 (BW20), 0.29±0.18 (BW40) and 0.36±0.21 (BW64), respectively and variable for economic traits 0.01±0.30 (AFE), 0.17±0.30 (EW28), 0.09±0.32 (EW40), 0.52±0.45 (EW64), 0.02±0.28 (EP40) and 0.86±0.43 (EP64). Genetic correlations of AFE with BW16, BW20, EW28 and EP40 were negative and less than unity, but positive and more than unity with EW40. The r_G of BW16 with BW20 and EW64 were positive but highly positive and more than unity with BW40 and BW64 and low with EP40 and could not be estimated with EW28 and EW40. The r_G of BW20 was highly positive with BW40 and BW64 but could not be estimated with any other trait. Similarly, BW40 had highly positive correlation with BW64. The r_G of BW40 and BW64 with other traits could not be estimated. The r_G of EW28 was negative and less than unity with EW40 but positive and more than unity with EP40, other r_G were not estimable. EW40 had highly positive genetic correlation with EW64 and more than unity with EP40, but could not be estimated with EP64. The r_G between EW64 and EP64 was moderately negative, however, EP40 had highly positive r_G with EP64 (0.89±0.15). Phenotypic correlations of AFE with all the traits were very low and close to zero except with BW20 and EW64 where they were low and positive. The r_P among all body weights and egg weights were high and positive. However, r_P between body weights at various ages was close to zero except between BW16 and EP40, where it was lowly positive. Similarly, egg weights had highly positive r_P amongst them but had very low r_P with EP40 and EP64, although r_P was lowly positive between EW64 and EP64. The EP40 had highly positive phenotypic correlation with EP64 (0.63). Percent mortality recorded in combined sexes of selected strain of RIR was 1.54%, 0.31%, 2.60%, 6.35% and 7.86% during different period from 0 to 4, 5 to 8, 9 to 16, 17 to 40 and 41 to 64 weeks of age.

Seventy six RIR^S pullets were screened for genetic polymorphism at 10 egg production associated microsatellite (MS) loci and alleles were separated through 3.4% MetaPhor Agarose gel electrophoresis and their sizes were estimated with the help of GelDoc using Quantity One software. Allelic data were analyzed by POPGENE version 1.32 and various population genetic parameters were estimated.

Microsatellite analysis revealed 2-6 alleles with varied frequencies. Their molecular sizes ranged from 102bp at ADL0102 to 302bp at MCW0007. A total 30 alleles with average no. of alleles per locus 3.50 ±0.29 were revealed. Out of nine polymorphic loci, MCW0069 revealed high degree of polymorphism with six number of alleles and their varied frequencies. Alleles' frequency ranged from 0.013 to 0.947 in the population at 10 loci and most frequent allele was 124 bp allele (94.7%) at ADL0210 locus.The mean ± SE of Nei's heterozygosity of ten microsatellite loci was 0.4119± 0.2475, which ranged from 0.0997 (ADL0210) to 0.7421 (MCW0069). The mean ± SE PIC value was 0.313±0.064, which ranged from 0.0947 (ADL0210) to 0.6020 (ADL0176), respectively, indicated that all the nine loci were moderately polymorphic and informative. The mean ± SE of observed and effective number of alleles and Shannon's index were 3.0000±1.4142, 2.0324± 0.9416 and 0.7342± 0.4649. Effective number of allele ranged from 1.1108 (ADL0210) to 3.8778 (MCW0069). Effective number of alleles at each locus was less than the observed number of alleles indicating the prevalence of heterozygosity at each locus. Mean H_e > mean H_o which indicated that population was not in Hardy-Weinberg equilibrium but under the influences of some forces like selection for some economic traits that might be associated with microsatellite loci. The results of Chi-square test and G-square tests also revealed significant differences between H_o and H_e frequencies demonstrating that the population was under H-W disequilibrium for all loci. Out of nine polymorphic microsatellites, five microsatellites were found to have significant effect on growth and or layer economic traits.

Least squares analysis of variance revealed significant effect of MS-genotypes at ADL0020 on BW16, BW20, BW40, BW64, EW64, EP40 and EP64; ADL0023 MS-genotypes on AFE and EP40, ADL0210 MS-genotype on AFE, BW16, BW20, BW40, BW64, EW28, EW40 and EW64; MCW0007 MS-genotype on BW40 only and MS-

genotype at MCW0014 on BW16 (P≤0.06), BW40 (P≤0.05) and EW28 (P≤0.11), respectively.

The immunocompetence traits in pure strain of RIR^S were evaluated by assessing important parameters related to various facets of immunity such as antibody response to SRBC, serum lysozyme activity and serum IgG level. The data generated on immunocompetence traits was analyzed by Least-squares analysis of variance. The Least squares ANOVA revealed significant (P≤0.01) effect of sire on serum IgG level only. Overall least-squares means of HA titre, serum lysozyme and serum IgG were 9.35 ± 0.29, 4.64±0.11 µg/ml and 8.61 ±0.34 mg/ml. The influence of sex on IC traits was statistically non-significant (P>0.05), although males had higher antibody titre and serum lysozyme level than females and IgG concentration was somewhat higher in females than males. The heritability estimate of serum IgG conc. was high (0.302±0.188) and could not be estimated for serum lysozyme and HA titre. The genetic correlation (r_G) among IC traits could not be estimated and phenotypic correlations (rp) among IC traits were lower in magnitude. Serum IgG concentration had positive and low genetic correlation with BW16 (0.162 ± 0.395) moderate with BW20 (0.302±0.392), although associated with high standard error, and positive and high genetic correlation with BW40 (0.928±0.459) and BW64 (0.796± 0.507). Genetic correlation of HA titre and serum lysozyme with body weights could not be estimated. Genetic correlations among body weights were highly positive. HA titre had negative phenotypic correlation with serum lysozyme, serum IgG level, BW40 and BW64 and positive with BW16 and BW20. Serum lysozyme concentration had positive and low phenotypic correlation with serum IgG conc., BW16, BW20, BW40 and BW64. HA titre and Serum IgG concentration had positive and low phenotypic correlation with early body weights such as BW16 and BW20 and very low and negative correlations with BW40 and BW64.

Data on IC traits individually recorded on females and layer economic traits and their inter-relationship were analyzed using mixed model least-squares (LS) analysis of variance. Least-squares means of HA titre, serum lysozyme and serum IgG in RIR pullets were 9.33±0.34, 4.42±0.18 µl/ml and 8.72±0.41 mg/ml, respectively. Heritability estimates were low for serum IgG level (0.109±0.48) and high for serum lysozyme level (0.414±0.38) and could not be

estimated for HA titre. Serum lysozyme level had highly negative genetic correlation with serum IgG level, EP40 and EP64; low to moderate and negative with EW28 and EW64, positive and high genetic correlation with BW16 and EW28 and could not be estimated with AFE. Serum IgG concentration had high and positive correlation with body and egg weights at different ages, but highly negative genetic correlation with EP40 and EP64. The genetic correlation of HA titre could not be estimated, which may be attributed to sampling variation due to small sample size. Hemagglutination titre had very low and negative phenotypic correlations with serum lysozyme level, serum IgG conc. and EP40 and moderately positive correlation with EW64. The correlations with all other traits were very low and positive. Serum lysozyme level had low positive phenotypic correlation with serum IgG level, BW16, EW40 and EP40; highly positive r_p with EW64 and EP64, negative and low phenotypic correlation with AFE and EW28. Serum IgG concentration also had very low phenotypic correlation with all the traits. Least squares analysis of variance revealed significant effect of serum IgG levels on BW40 ($P \leq 0.06$) and BW64 ($P \leq 0.05$). The birds with low serum IgG concentration had highest BW40 ($P \leq 0.06$) in comparison to those having non-significantly different medium and high levels of serum IgG. Similarly, birds with low IgG level demonstrated high BW64 ($P \leq 0.05$) than those having non-significantly different medium and high levels of serum IgG. Least squares analysis of variance revealed that serum lysozyme levels had significant ($P \leq 0.05$) effect on egg production up to 64 weeks of age (EP64) in RIR chicken. The birds with high serum lysozyme concentration produced highest number of eggs up to 64 weeks of age ($P \leq 0.05$) followed by birds with medium and then low levels. Least squares analysis of variance was carried out to assess the effect of MS genotypes at polymorphic loci on immunocompetence traits. None of the microsatellites was found to have significant effect on any of the immunocompetence traits.

Relative mRNA expressions of three immune response genes viz., IL1-β, iNOS and TLR15 were studied by quantitative reverse transcriptase PCR (qRT-PCR) method in bursa, spleen and thymus tissue, collected from selected strain of RIR chicken. Sex differences were assessed using GLM of SAS. Analysis revealed that the difference between two sexes was non-significant ($P>0.05$) for all the three genes in all the three tissues. Out of the three genes

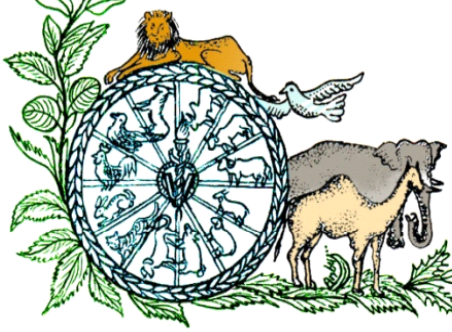
studied, the mRNA expression of iNOS gene differed significantly (P<0.001) among lymphoid tissues. Highest expression (mean 40-ΔC_t = 34.27±0.71) was observed in Bursa tissue, followed by in spleen (31.38±0.71) and thymus (30.17±0.71) tissues. Least squares analysis of variance revealed that the influence of HA titre levels had significant effect on relative gene expression of IL1-β (P≤0.09) in spleen and serum lysozyme levels on relative gene expression of TLR15 (P≤0.01) in spleen and IL1-β (P≤0.06) in thymus tissues. Influence of serum IgG levels was statistically not significant (P>0.05) on all genes studied in three lymphoid tissues. It was observed that birds with medium or low HA tire revealed significantly higher IL1-β gene expression in spleen than birds having high HA titre. Similarly, birds with medium or low serum lysozyme level revealed significantly higher mRNA expression of IL1-β in thymus and TLR15 in spleen tissue than birds having high lysozyme level.

Conclusions

1. Improved AFE (134.5±0.9 days) and EP40 (118.3±1.2 eggs) over previous generation and highly positive genetic (0.89±0.15) as well as phenotypic (0.63) correlations between egg production up to 40 (EP40) and 64 (EP64) weeks demonstrated usefulness of selection based on part-period egg production (up to 40 weeks) for genetic improvement of annual egg production in RIR chicken.
2. Males of RIR-selected strain exhibited higher body weights than females from 16 weeks to 64 weeks of age.
3. Out of the 10 egg production-associated microsatellite loci analyzed, nine were polymorphic revealing 2-6 no. of alleles. The PIC ranged from 0.095 to 0.60 at these polymorphic loci.
4. All the polymorphic loci exhibited prevalence of heterozygosity as the effective numbers of alleles were lesser than the observed number of alleles.
5. RIR-selected strain demonstrated Hardy-Weinberg disequilibrium as mean exp. heterozygosity was more than mean obs. Heterozygosity and significant Chi square and G-square estimates and suggested that selection for part-period egg production might have association with microsatellite loci studied.

6. Five MS loci exhibited significant differences among genotypes at these loci for growth and layer economic traits suggesting their utility in marker-assisted selection for the associated trait. However, this might be validated on large numbers of samples.
7. Immunocompetence traits in RIR-selected strain did not differ significantly between sexes.
8. Serum IgG concentration had highly positive genetic correlation with housing body weight (BW16) and all egg weights and highly negative genetic correlation with EP40 and EP64.
9. Levels of serum IgG concentration had significant influence on body weight. Birds with low serum IgG conc. demonstrated highest BW40 and BW64 than those having medium or high levels of serum IgG.
10. Levels of serum lysozyme concentration had significant influence on egg production. Birds with high serum lysozyme concentration produced highest number of eggs up to 64 weeks of age followed by those having medium and then low serum lysozyme levels.
11. Out of the three studied genes, the basal mRNA expression of iNOS gene differed significantly ($P \leq 0.001$) among three lymphoid tissues, viz., bursa, spleen and thymus. The highest expression was observed in bursa, followed by spleen and thymus tissues.
12. Levels of different IC traits influenced the basal mRNA expression in different tissues. Birds with medium or low HA tire revealed significantly higher IL1-β gene expression in spleen than birds having high HA titre. Similarly, birds with medium or low serum lysozyme level revealed higher mRNA expression of IL1-β in thymus and TLR15 in spleen tissue than birds having high lysozyme level.
13. The basal gene expression of the three genes was several folds higher in bursa as compared to spleen and thymus.

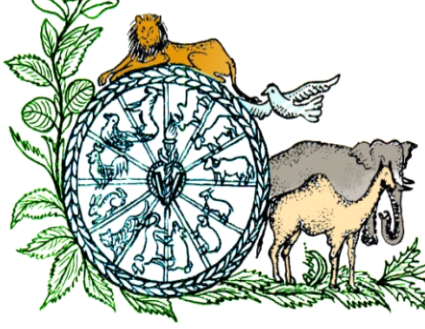
Mini Abstract



7 MINI ABSTRACT

Present investigation was carried out in Rhode Island Red (RIR) chicken to analyze the effects of various genetic and non-genetic factors on layer economic traits, to determine allelic polymorphism at egg production – associated microsatellite loci and their association with layer economic traits, to estimate immunocompetence traits and their association with layer economic traits and to analyze relative expression of important immunity related genes in various tissues by quantitative reverse transcription PCR. Three hundred twenty five single-hatched chicks produced and maintained at this institute were used for this purpose. It was revealed that average. AFE and egg production up to 40 weeks of age were 134.5±0.9 days and 118.3±1.2 eggs, respectively. The genetic and phenotypic correlations between egg production up to 40 and 64 weeks were highly positive. Males of RIR-selected strain exhibited higher body weights than females at all ages. Out of the 10 egg production-associated microsatellite loci analyzed, nine revealed polymorphism. All the polymorphic loci exhibited prevalence of heterozygosity. RIR-selected strain demonstrated Hardy-Weinberg disequilibrium. Genotypes at five MS loci demonstrated significant association with growth and layer economic traits suggesting their utility in marker-assisted selection. There was no difference between sexes for immunocompetence traits in. Levels of serum IgG concentration had significant influence on body weight. Birds with low serum IgG conc. demonstrated highest body weights at 40 and 64 weeks of age than those having medium or high levels of serum IgG. Levels of serum lysozyme concentration had significant influence on egg production. Birds with high serum lysozyme concentration produced highest number of eggs up to 64 weeks of age followed by those having medium and then low serum lysozyme levels. Out of the three genes studied, the basal mRNA expression of iNOS gene differed significantly among three lymphoid tissues, viz., bursa, spleen and thymus. The highest expression was observed in bursa, followed by spleen and thymus. Levels of different IC traits influenced basal mRNA expression in different tissues. Birds with medium or low HA tire revealed significantly higher IL1-β gene expression in spleen. Similarly, birds with medium or low serum lysozyme level revealed higher mRNA expression of IL1-β in thymus and TLR15 in spleen. The basal gene expression of the three genes was several folds higher in bursa as compared to spleen and thymus. This study generated significant findings which could be used for improvement of egg production, body weights and diseases resistance in chicken.

लघु सारांश



8 लघु सारांश

यह शोध रोड आई लैण्ड रेड (आर.आई.आर) मुर्गियों में अण्डा उत्पादन वाले गुणों पर विभिन्न कारकों के प्रभाव का अध्ययन करना, अण्डा उत्पादन से संबन्धित माइक्रोसेटेलाईट लोसाई में बहुरूपता/अध्ययन और इस बहुरूपता का अण्डा उत्पादन वाले गुणों से सम्बन्ध विभिन्न इम्युनोकाम्पीटेस गुणों का आंकलन एवं उनका अण्डा उत्पादन वाले गुणों से संबन्ध तथा तीन प्रतिरक्षा संबधित जीनों के तुलनात्मक, मात्रात्मक, अनुवांशिक अभिव्यक्ति की जानकारी क्यु.आर.टी.पी.सी.आर. विधि द्वारा जानने हेतु किया गया। इस कार्य हेतुसंस्थान में ही उत्पादित आर. आई आर प्रजाति के 325 चूँजों का उपयोग किया गया। इस अध्ययन में प्रथम अण्डा देने की औसत उम्र 134.5±10.9 दिन व 40 हफ्ते की उम्र तक का औसत अण्डा उत्पादन 118.3±1.2 अण्डा पाया गया। साथ ही 40 एवं 64 सप्ताह की उम्र तक का अण्डा उत्पादन में उच्च जेनेटिक एवं फीनोटिपीक कोरेलेशन पाया गया। नर पक्षियों का वजन मादा पक्षियों की अपेक्षा हर उम्र पर अधिक पाया गया। दस मे से नौ माइक्रोसैटेलाइट लोसाई में बहुरूपता देखी गई। इन सभी बहुरूपी माइक्रो सैटेलाइट लासाई में हैट्रोजाइगासिटी की बहुलता देखी गयी एवं आर. आई.आर. मुर्गियेां में हार्डी वीनवर्ग डीस इक्यूली ब्रीयम भी पाया गया। पाँच मार्डक्रोसेटेलाईट लोसाई पर विभिन्न जीनोंटाईट का संबन्ध ग्रोथ एवं अण्डा उत्पादन वाले गुणों के साथ भी पाया गया। जो इनकी मार्कर असीस्टेड सेलेक्रान में उपयोगिता दर्शता है आर. आई. आर. मुर्गियों में विभिन्न इम्यूनोकम्पीटेन्स वाले गुणों के लिए नर एवं मादा लिगों में कोई भेद नहीं देखा गया। यह भी पाया गया कि जिन मुर्गियों में सीरम आई.जी.जी. की मात्रा कम थी उनका 40 एवं 64 सप्ताह पर वजन अधिक था। साथ ही साथ यह भी देखा गया कि जिन मुर्गियों में सीरम लाईसोजाईम की मात्रा अधिक थी उन्होंन64 सप्ताह की आयुतक अधिक अण्डा उत्पादित किया। प्रतिरक्षा सम्बन्धित जीनों के तुलनात्मक अनुवांशिक अभिव्यक्ति के अध्ययन से पता चला कि तीन लीम्फॉयड उतको वर्सा, तिल्ली एवं थाईमस में तीन जीनों में से आई. नॉस जीन की अभिव्यक्ति भिन्न थी। सर्वाधिक अभिव्यक्ति वर्सा में उससे कम तिल्ली में तथा न्यूतम स्तर थाईमस में देखा गया। यह भी पाया गया कि इम्यूनोकाम्पीटेन्स गुणों के विभिन्न स्तरो (उच्च, मध्ययम एवं निम्न) वाली मुर्गियों में उपरोक्त जीनों की अभिव्यक्ति में विभिन्नता भी थी। मध्ययम अथवा निम्न एच.ए. टाइटर वाली मुर्गियों में तिल्ली में आई0एल. वन वीटा का बीच जीन की सर्वाधिक अभिव्यक्ति पायी गयी। इसी प्रकार मध्ययम अथवा निम्न सीरम लाइसोजाईम स्तर वाली मुर्गियों मे थाईमस उतक में आई.एल. वन वीटा का बीच तथा तिल्ली में टी. एल.आर. 15 जीनों की अभिवयक्ति अन्य स्तर वाली मुर्गियेां की तुलना में अधिक पायी। तीनों जीनों में सर्वाधिक आनुवंशिक अभिव्यक्ति वर्सा में प्रदर्शित की। इस शोध के परिणामों का उपयोग मुर्गियों में अण्डा उत्पादन, शारीरिक भार तथा प्रतिरक्षा सम्बन्धित जीनों की आनुवांशिक अभिव्यक्ति के आनुवंशिक सुधार हेतुकरने में महत्वपूर्ण निष्कर्ष पाये गये।

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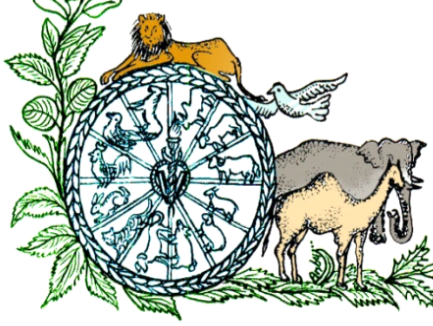

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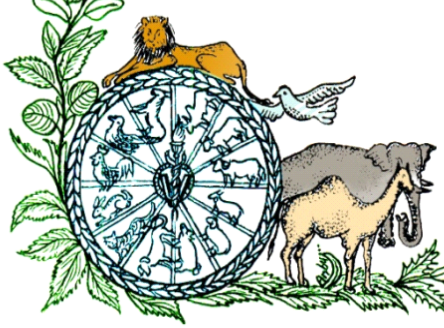
Annexure



ANNEXURE-I

Chemicals/Equipments/Lab wares/Miscellaneous items
Molecular genetics studies
(A). Reagents for DNA Isolation

1. 2.7% EDTA as anticoagulant for blood collection

Dissolve 2.7 g of EDTA (molecular weight 292.25g) in 100 ml of autoclaved ddH_2O or Heparin @ 20 IU per ml of blood.

2. Phosphate buffer saline (PBS) (pH 7.2-7.4)

Sodium Chloride (**NaCl**)	8.00 g
Potassium Chloride (**KCl**)	0.20 g
Sodium biphosphate (**Na_2HPO_4**)	1.44 g
Potassium biphosphate (**KH_2PO_4**)	0.24 g

Dissolve in 800 ml of autoclaved distilled water and then adjust the pH to 7.2 to 7.4 with HCl. Make the final volume up to 1000 ml with autoclaved distilled water, sterilize by autoclaving and store at 4ºC.

3. 5M NaCl

NaCl	292.2g
Autoclaved distilled water	1000ml

Sterilize by autoclaving and store at 4ºC.

4. 2M Tris (pH 8.0)

Tris HCl	242.2 g

Dissolve in 800 ml of autoclave double distilled H_2O and then adjust the pH to 8.0 by adding concentrated HCl. Make the final volume up to 1000ml with autoclave distilled H_2O. Sterilize by autoclaving and store at 4ºC.

5. 0.5 M EDTA (pH 8.0)

EDTA disodium salt	186.12g

Dissolve in 500 ml of autoclaved double distilled water with the help of magnetic stirrer for 1-2 hours. And adjust the pH to 8.0 with NaOH pellets (approximately 20g NaOH) at which EDTA gets dissolved thoroughly. Make the final volume upto 1000 ml with autoclaved distilled water. Dispense into aliquots, sterilize by autoclaving and store at 4ºC.

Functions of EDTA

1. Chelates Mg^{++} ions
2. Protects from nucleases
3. Makes plasma membrane more fragile.

6. Lysis buffer

2M NaCl	5.0 ml
2M Tris HCL	2.5 ml

0.5M EDTA	5.0 ml
2M NaCl	5.0 ml

Add autoclave distilled water upto 100ml, sterilize by autoclaving and store at 4ºC.

7. **10% Sodium Dodecyl Sulfate (SDS) /Sodium Lauryl Sulfate (SLS)**

SDS/SLS	10gm
Autoclaved distilled water	100ml

No need to sterilize SDS/SLS and store at room temperature

Function of SDS/SLS

1. Helps in cell membrane lysis,
2. Acts as a catalyst.

8. **Proteinase K**:

Weigh empty eppendorf, add 5 mg of Proteinase K and add 1000 µl of autoclaved tdH_2O. Store in freezer after proper mixing.

Proteinase K

(1) Stock solution – 5 mg/ml

(2) Storage temp. - –20°C

(3) Concentration in reaction - 50 µg/ml

(4) Reaction buffer - 0.01 M Tris (pH - 7.8)
0.05 M EDTA
0.5% SDS/SLS

(5) Temperature - 37-56°C.

Proteinase 'K' is highly active protease of the subtilisin type that is purified from the mold Tritirachium album Limber. The enzyme has two binding sites for $Ca^{++,}$ which lie some distance from the active site and are not directly involved in the catalytic mechanism. However, when Ca^{++} is removed from the enzyme, approximately 80% of the catalytic activity is lost because of long-range structural changes Because the residual activity is usually sufficient to degrade proteins that commonly contaminate preparations of nucleic acids, digestion with Proteinase 'K' is usually carried out in the presence of EDTA (to inhibit the action of Mg^{++} - dependent nucleases). However, to digest highly resistant proteins such as keratin, it may be necessary to use a buffer containing 1 mM Ca^{++} and no EDTA. At the end of the digestion, the Ca^{++} should be chelated by addition of EDTA (pH 8.0) to a final concentration of 2 mM before the nucleic acids are purified.

Function of Proteinase K- Digestion of proteins

9. **Tris- saturated phenol**

1. Melt phenol at 68°C by keeping in water bath.
2. Measure the require volume.
3. Add 8- hydroxyl quinolene to a final concentration of 0.1% (It is an anti-oxidant, partial inhibitor of RNAse and a weak chelator of metal ions. In addition, its gives yellow color to phenol provides convenient way to identify the organic phase).

4. Equal volume of 0.5 M Tris HCl pH 8.0 (at room temperature) was added, stirred for ½ hr and kept overnight.
5. Next day supernatant was removed, 0.1 M Tris pH 8.0 was added, stirred for ½ hr and placed back in the refrigerator.
6. In the evening, the supernatant was removed, 0.1 M Tris pH 8.0 was added, stirred for ½ hr and placed back in the refrigerator overnight.
7. Extract phenol several times with equal volume of 1M Tris (pH 8.0)
8. Then, With 0.I M Tris,until the pH of the aqueous phase is more than 7.6.
9. Add 0.2% β- mercaptoetanol.
10. Mix thoroughly and store in amber coloured bottle at 4°C.

Crystalline phenol as such is not recommended because it must be redistilled at 160°C to remove oxidation products such as quinones that cause the breakdown of phosphodiester bonds or cause cross linking of RNA and DNA.

Functions of phenol-

1. **RNA with poly A tail is dissolved in alkaline phenol.**
2. **Inhibits RNAse**
3. **Weakly chelates metal ions**

Functions of 8- Hydrohxyquinolone -

Prevents oxidation of phenol

Yellow colour provides convenient way to identify the organic phase

Caution:

Phenol is highly corrosive and can cause severe burns .Wear gloves, protective clothing and safety glasses when handling phenol. All manipulations should be carried out in a chemical hood. Any areas that come in contact with phenol should be rinsed with large volume of water and washed with soap and water.

10. Phenol: Chloroform: Isoamyl alcohol (25: 24: 1)

Tris- saturated phenol	**25ml**
Chloroform	**24ml**
Isoamyl alcohol	**1ml**

Store at 4°C in amber coloured bottle under 0.1M Tris HCl (pH-8.0) for a period of 1 month.

Function of chloroform

1. **Denatures proteins.**
2. **Facilitates the separation of aqueous and organic phases.**
3. **Removes phenol as phenol causes breaks in phosphodiester bonds**

Function of isoamyl alcohol- **Reduces foaming during extraction**

11. Chloroform: Isoamyl alcohol (24:1).

Chloroform	**96 ml**
Isoamyl alcohol	**4 ml**

Store at 4°C in amber coloured bottle

12. 70% ethanol

Absolute ethanol	35ml
Autoclaved distilled water	15ml

Chill it at -20°C.

13. 3 M Sodium Acetate (pH=5.2)

Sodium Acetate	408.1 g
Autoclaved distilled water	15ml

Adjust the pH to 5.2 with glacial acetic acid and then make the volume upto 1000 ml with H_2O. Dispense into aliquots and sterilize by autoclaving and store at 4°C.

Function of 3M Sodium Acetate- Precipitates DNA

14. 2-Mercaptoethanol (2ME)

Usually obtained as a 14.4 M solution. Store in dark bottle.

B. Reagents for PCR

1. Reconstitution of dNTP's (pH=7.0)

Working solution of dNTP mix with 10mM of each dNTP from 100 mM of each dNTP stock.

10 µl of each dNTP is taken and the volume is made upto 100 µl *i.e.,* 10 µl of dATP + 10µl of dGTP + 10µl dTTP +10µl dCTP + 60µl of tdH_2O (nuclease free). The effective concentration of each dNTP becomes 10 mM in the mix.

$$N_1V_1 = N_2V_2$$

$$10\ mM \times V_1 = 200\mu M \times 50\mu l$$

(Final concentration should be 200µM of each dNTP in 50µl)

$$x = 1\mu l$$

Therefore in a 50 µl reaction to have 200 µM of each dNTP 1µl of working solution is added. For getting a concentration of 300 µM in 50µl reaction 1.5µl of working solution is added.

Working solution of 2.5 mM of each dNTP from 10 mM of each dNTP stock

10 µl of dATP + 10 µl dGTP + 10 µl of dTTP + 10 µl of dCTP are added in an eppendorf making the effective concentration of each dNTP to 2.5 mM.

$$N_1V_1 = N_2V_2$$

$$2.5mM \times V1 = 200\mu M \times 50\mu l$$

(Final concentration should be 200µM of each dNTP in 50µl)

$$V_1 = 4\mu l$$

Therefore in a 50 µl reaction to have 200 µM of each dNTP 4µl of working solution is added.

2. Primer Reconstitution

From the stock of 1000 pM/µl, working solution of 20 pM/µl is prepared by taking 1µl of stock in an eppendorf tube and making up the volume to 50µl. In a PCR reaction 1µl of working solution is added for a 50µl reaction to get an effective concentration of 0.4 µM.

$$N_1V_1 = N_2V_2$$

$$20\ pM \times V1 = 0.4\ \mu M \times 50\mu l$$

$$V_1 = (0.4\ \mu M \times 50\mu l)/20\ pM$$

Similarly if 1.5µl is added the effective concentration becomes 0.6µM in 50µl reaction.

$$N_1V_1 = N_2V_2$$

$$20\ pM \times V1 = 0.6\ \mu M \times 50\mu l$$

$$V_{1} = (0.6\ \mu M \times 50\mu l)/ 20\ pM$$

$$= 1.5\ \mu l$$

$MgCl_2$

1µl of 25 mM is added for a 50 µl reaction to get an effective concentration of 0.5 mM.

$$N_1V_1 = N_2V_2$$

$$25\ mM \times V1 = 0.5\ mM \times 50\mu l$$

$$V_1 = 1\mu l$$

4. 10 X buffer with $MgCl_2$ or without $MgCl_2$

The buffer is diluted to make 1X in the PCR reaction. The buffer may contain $MgCl_2$. The requirement of the $MgCl_2$ can be taken care by the buffer. If the PCR reaction needed more concentration than in the buffer it should be provided additionally.

10X Taq buffer

Tris HCl(pH=8.8)	100 mM
KCl	500 mM
$MgCl_2$	15 mM
Triton X-100	1%

Store at -20ºC. Use at a final concentration of 2.5 mM.

5. Taq DNA Polymerase enzyme (3 units/µl)

Store at -20ºC and use at a final concentration of 0.75 U.

C. Reagents for Agarose Gel Electrophoresis

Agarose of electrophoresis grade

1. TBE (Tris, boric acid, EDTA) buffer (5X)

Tris base	54.0 g
Boric acid	27.5 g
0.5 M EDTA	20.0 ml

Adjust pH to 8.0

2. 6 X loading dye for Agarose gel

Type-1	Type-2
Bromophenol blue 0.25%	0.25%
Xylene Cyanol 0.25%	0.25%
Sucrose in DW 40%	40%

Mix and store at 4°C

-

These gel loading buffers serve three purposes: They increase the density of the sample ensuring that the DNA drops evenly into the well; they add colour to the sample, thereby simplifying the loading process; and they contain dyes that, in an electric field, move toward the anode at predictable rates. Bromophenol Blue migrates through agarose gels approximately 2.2-fold faster than Xylene Cyanol FF, independent of the agarose concentration. Bromophenol Blue migrates through agarose gels run in 0.5 X TBE at approximately the same rate as linear double-stranded DNA 300 bp in length, whereas Xylene Cyanol FF migrates at approximately the same rate as linear double stranded DNA 4 kb in length. These relationships are not significantly affected by the concentration of agarose in the gel over the range of 0.5% to 1.4%.

4. 20,000 X Ethidium Bromide

Ethidium Bromide 10 mg

Autoclaved dH_2O 1000 ml

Wrap in an aluminium foil and store at room temperature in a dark place (as Et.br. is photosensitive).

Function of Et.br.- acts as intercalating agent to make complex with DNA/RNA molecules which illuminates when exposed over UV light.

5. 20bpDNA ladder (0.1µg/µl) or low range DNA Ruler

20 bp ladder contains 11 fragment ranging from 20 to 300 bp i.e. 20, 40, 60, 80, 100, 120, 140, 160, 180, 200 and 300 bp. Low range DNA Rular contains 10 fragment ranging from 25 to 700 bp i.e. 25, 50, 75, 100, 150, 200, 300, 400, 500, 600 and 700 bp

Store at 4ºC for within-use or at -20ºC for ever.

D. Reagents for immunological studies

1. 1M Na_2HPO_4

Na_2HPO_4	14.196 g
Autoclaved distilled water	100ml

Sterilize by autoclaving and store at 4ºC.

2. 1M Na_2HPO_4

NaH_2PO_4	15.601 g
Autoclaved distilled water	100ml

Sterilize by autoclaving and store at 4ºC.

3. Dibasic Buffer Solution (0.066M, pH 6.3)

1M Na_2HPO_4	21.65ml
1M NaH_2PO_4	78.35ml

Mix thoroughly and then take 66 ml of this mixture and makeup the final volume upto 1000 ml by adding autoclaved dH_2O to get 0.066 M dibasic buffer.

OR

Take 66 ml of 1M Na_2HPO_4 and add autoclaved dH_2O to make approx. 700 ml, then adjust pH to 6.3 by adding 1M NaH_2PO_4 and finally make up the volume to 1000 ml.

4. ***Micrococcus lysodieketicus*** **bacterial stock solution**

Micrococcus lysodieketicus	2.0 mg
Dibasic Buffer (0.066M)	1 ml

Mix gently and then take 66 ml of this mixture and makeup the final volume upto 1000 ml by adding autoclaved dH_2O to get 0.066 M dibasic buffer

5. **Standard Lysozyme (2µg/µl) stock solution**

Lysozyme	2.0 mg
Dibasic buffer	1ml

6. **Rabbit Anti-chicken IgY**

Use as such @ 35 µl/ml of 0.1M Tris HCl required for gel preparation in lysoplate.

7. **Standard Chicken IgG (4 mg/ml) stock solution**

Dilute 4 mg of standard chicken IgG in to 1000µl of 0.1M Tris-HCl.

8. **0.2% Coomassie Brilliant Blue (CBB)**

Coomassie Brilliant Blue	200 mg
Methanol	2 ml
Acetic acid	2 ml

Make the final volume up to 100 ml with autoclaved distilled water, sterilize by autoclaving and store at 4ºC

9. **Destaning solution (500 ml)**

Methanol	150ml
Acetic Acid	50ml
Distilled water	300ml

,

VITAE

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PUBLICATIONS FROM THIS WORK

1. **Rahim A**., Kumar S., Jagadeesan K. Ullah S., Debnath J., Yadav R and Bhanja S.K. (2015).Genetic analysis of growth, production and reproduction traits after long-term selection in Rhode Island Red chicken. *Indian Journal of Animal Research* (Accepted).
2. **Rahim A**., Kumar S., Debnath J., Yadav R and Jagadeesan K. (2015) Immunocompetence traits and their association with layer economic traits in Rhode Island Red chicken. *Indian Journal of Poultry Science* (Accepted).