GENETIC ASPECTS OF EGG PRODUCTION TRAITS, IMMUNE RESPONSIVENESS AND MICROSATELLITE-BASED DIVERSITY IN LAYER CHICKENS

Thesis

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By

SATYENDRA PAL SINGH
[L-2005-V-03-M]

DEPARTMENT OF ANIMAL BREEDING AND GENETICS
COLLEGE OF VETERINARY SCIENCE

GURU ANGAD DEV VETERINARY AND ANIMAL SCIENCES UNIVERSITY
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CERTIFICATE – I

This is to certify that the thesis entitled "**GENETIC ASPECTS OF EGG PRODUCTION TRAITS, IMMUNE RESPONSIVENESS AND MICROSATELLITE-BASED DIVERSITY IN LAYER CHICKENS**" submitted for the degree of **Master of Veterinary Science** in the subject of Animal Breeding and Genetics **(Minor subject:** Veterinary Biochemistry**) of the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana**, is a bonafide research work carried out by **Satyendra Pal Singh** [L-2005-V-03-M] under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

[Dr. Gurvinder Singh Brah]
MAJOR ADVISOR
Professor of Animal Breeding & Genetics
Deptt. of Animal Breeding and Genetics
GADVASU, Ludhiana -141004

CERTIFICATE – II

This is to certify that the thesis entitled **“GENETIC ASPECTS OF EGG PRODUCTION TRAITS, IMMUNE RESPONSIVENESS AND MICROSATELLITE-BASED DIVERSITY IN LAYER CHICKENS”** submitted by **Satyendra Pal Singh** [L-2005-V-03-M] to the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, in the partial fulfillment of the requirements for the degree of **Master of Veterinary Science** in the subject of **Animal Breeding and Genetics** (**Minor subject:** Veterinary Biochemistry) has been approved by the Student’s Advisory Committee along with Head of department after an oral examination on the same.

______________________________	______________________________
[Dr. M. L. Chaudhary]	**[Dr. Gurvinder Singh Brah]**
Head of the Department	**Major Advisor**

____

(Dr. S. K. Jand)
Dean Post-Graduate Studies

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ABSTRACT

The present study was conducted on two selected strains, a control line, a naked-neck strain of WLh, two strains of RIR and their crosses with the objectives to evaluate growth, egg production and quality traits, immunocompetence and genetic diversity among the strains by using microsatellite markers. Of all the strains, RIR-C had highest body weight. Selected WLh, PL1 and PL2 did not differ for body weight, while both had higher body weight than the control at 12, 16 and 20 weeks of age but thereafter control tended to maintain higher body weight. The egg production was recorded from the onset of production upto 44 weeks of age. The control strain matured significantly later than the other strains and breed cross PL2♂ X RIR-B♀ attained earlier sexual maturity. PL2 had higher egg number than the control during the first phase of laying period. PL2 had higher egg weight, egg mass, egg production efficiency index than the control during all the three age periods. RIR-B had better performance for the egg production traits as compared to RIR-C. Egg quality and composition traits showed improvement with age but specific gravity, albumen height, HUS, % albumen declined with age. Control PL3 had higher specific gravity among all the groups. Specific gravity and yolk traits showed significant age x genetic group interaction. Immune response to Sheep Red Blood Cells was measured as HA titre at 0, 5 and 10 days post primary inoculation. The presence of natural antibody was evident in all the genetic groups. All groups showed an increase in HA titre upto 10 days post immunization. The control line (PL3) showed highest HA titre on day 5 and 10 post primary inoculation and highest response after PHA-P inoculation. Genetic diversity was evaluated among the selected strain PL2, RIR-B and their cross by using 14 microsatellite markers. All the loci exhibited polymorphism in all the populations except MCW-111, ADL-268 in PL2 strain. Marker MCW-104 showed highest polymorphism. The dendrogram based on Nei (1978) grouped PL2 and cross PL2♂ X RIR-B♀ under cluster 1 and RIR B under cluster 2.

Key words: Egg production, Immunocompetence, Sheep Red Blood Cells, Microsatellite, Genetic Diversity, White Leghorn, Rhode Island Red.

Signature of Major Advisor　　　　　　　　Signature of the student

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ABBREVIATIONS

°C	:	Degree centigrade
μl	:	Microlitre
μM	:	Micromolar
Ab	:	Antibody
A temp.	:	Annealing temperature
$AgNo_3$	:	Silver nitrate
ASM	:	Age at Sexual maturity
B	:	Chicks given booster inoculation
bp	:	Base pairs
BPR	:	Barred Plymoth Rock
Con-A	:	Concavalin A
cm	:	Centimeter
cM	:	Centi Morgan
CMI	:	Cell mediated immunity
Conc.	:	Concentration
Chr	:	Chromosome
d	:	Days
dATP	:	Deoxy adenosine triphosphate
ddH_2o	:	Double distilled water
df	:	Degree of freedom
dGTP	:	Deoxy Guanosine Triphosphate
DNA	:	Deoxy Ribonucleic Acid
dNTP	:	Deoxy nucleotide triphosphate
DPPI	:	Days post primary inoculation
dTTP	:	Deoxy thymine triphosphate
E. coli	:	Escherichia coli
EDTA	:	Ethylene Di Ammine Tetra acetic acid
eg	:	for example
EMFD	:	Egg Mass per functional day
EMS	:	Estimated Mean Sum of Squares
EPE	:	Egg Production efficiency
EI	:	Efficiency index
F	:	Fayoumi
FAO	:	Food and Agriculture Organization
Fig	:	Figure
FP	:	Forward Primer
g	:	Grams
G	:	Gauze
h^2	:	Heritability
HA test	:	Haemagglutination test
HA	:	High antibody selected line
HC	:	Hemolytic Complement Level
HIG	:	High IgG selected line
HIM	:	High IgM Selected line
HLB	:	Hyline Brown
HLW	:	Hyline White
hr(s)	:	Hour(s)
HUS	:	Haugh Unit Score

HW	:	High body weight selected line
HW	:	Hardy Weinberg Equilibrium
IgG	:	Immunoglobulin G
IgM	:	Immunoglobulin M
LA	:	Low antibody selected line
log	:	Logrithim
LIM	:	Low IgM selected line
LIG	:	Low IgG selected line
LSR	:	Lyallpur Silver Black
LSZ	:	Serum lysozyme level
LW	:	Low body weight selected line
MD	:	Marek's Disease
MER	:	Mercaptoethanol Resistant Titre
MES	:	Mercaptoethanol Sensitive Titre
Mg Cl_2	:	Magnesium Chloride
mg	:	Milli grams
MHC	:	Major Histocompatibility Complex
min(s)	:	Minutes
ml	:	Milli litre
mM	:	Milli molar
MSS	:	Mean Sum of Squares
Na_2Co_3	:	Sodium carbonate
NaCl	:	Sodium chloride
NaOH	:	Sodium hydroxide
Ne	:	Effective number of Alleles
NID	:	Normally Independently Distributed
NK	:	Natural Killer Cells
NN	:	Naked Neck
No.	:	Number
PAGE	:	Poly acrylamide gel electrophoresis
PBS	:	Phosphate Buffer Saline
PCR	:	Polymerase Chain Reaction
pH	:	Concentration of hydrogen ion
PHA-P	:	Phytohaemagglutinin-P
PIC	:	Polymorphic information content
PL	:	Punjab Layer
PPI	:	Post primary inoculation
PSI	:	Post secondary inoculation
RAPD	:	Random Amplified Polymorphic DNA
RBC	:	Red blood corpuscles
RFLP	:	Restriction fragment length polymorphism
RNA	:	Ribonucleic acid
RIR	:	Rhode Island Red
RM	:	Robusta Maculate
RP	:	Reverse Primer
rpm	:	Rotation per minute
SDL	:	Synthetic Dam Line
SG	:	Specific Gravity
SDS	:	Sodium Do-decyl Sulphate
SE	:	Standard Error

Sec(s)	:	Second(s)
SRBC	:	Sheep Red Blood Cells
SS	:	Sum of Squares
Taq	:	DNA polymerase
TBE	:	Tris borate EDTA
tdH_2o	:	Triple distilled water
TE	:	Tris EDTA Buffer
TBE	:	Tris Boric acid EDTA Buffer
TEMED	:	NNN'N'tetramethyl ethylene diamine
Tris	:	Trihydroxymethyl aminomethane
UV	:	Ultra Violet
V	:	Volts
VNTR	:	Variable Number Tandem Repeats
w/v	:	Weight/volume
WLh	:	White Leghorn Breed
WPR	:	White Plymoth Rock

CHAPTER – I

INTRODUCTION

The economic returns from layer chickens are dependent on a number of economically important traits like egg production, egg weight, body weight, feed consumption etc. In addition to these attributes, egg quality characteristics are also important in layer chickens as those affect the acceptability by the consumers (Stadelman 1977). Egg quality is more important price-contributing factor in table and hatching eggs. Therefore, the economic success of a laying flock does not solely depend on the total number of the eggs produced but also on external and interior quality of eggs as well as on proportion of yolk and albumen also.

Disease resistance, is also an important trait of layer chicken that directly determines the economics. Besides the economic benefits accruing from improving disease resistance, the possibility of reducing medication would be an attractive feature from the stand point of public health, ethics, product quality and animal welfare (Pinard *et al*, 1992). The genetic variation for disease resistance has not frequently been exploited due to easy access of vaccines and the random nature of infection. Traditionally genetic improvement for disease resistance has been made through selection for general viability based on the family averages but the method has proved to be rather slow due to very low heritability of viability. Non-specific mechanisms of genetic resistance offer a good prospect of reducing the expenditure on prophylactics and vaccination programmes (Gavora and Spencer 1978). Immune responsiveness in poultry can be evaluated by challenging the birds with a non- pathogenic, non- specific antigens such as

Sheep Red Blood Cells (SRBC) (Siegel and Gross 1980), Bovine Serum Albumen (BSA) (Parmentier *et al*, 1998b), synthetic glutamic acid–alanine-tyrosine (Cheng and Lamont 1988), Mollusk haemocyanin, chicken egg white lysozyme and bacterial lipo-polysaccharide but SRBC is most widely used antigen to study the genetic aspects of immunocompetence in poultry (Vander Zipp 1983).

The vast poultry genetic resources comprise a number of breeds and strains distributed in various agro-climatic zones. Variability between breeds or strains has traditionally been studied using a wide range of morphological and/or economic traits. A number of workers have studied the variability at the level of proteins and enzymes. Current advances in molecular biology using DNA based markers have made the study of between breeds/strains more precise. A marker is an identified genomic site and the present marker alleles represent polymorphism at this site. Marker polymorphism is used to determine genetic variation within and between breeds i.e. genetic distance, heterozygosity and inbreeding (Nei 1978). Different types of locus-specific markers are available i.e. minisatellite, microsatellite, Random Amplified Polymorphic DNA (RAPD) and Restriction Fragment Length Polymorphism (RFLP). The microsatellite markers are numerous, offer good reproducibility, randomly distributed in the genome, highly polymorphic, easy to identify have low mutation rates, show multiple allelism and co-dominant inheritance (Crooijmans *et al*, 1996). All these properties make the microsatellite the preferred genetic marker for estimating genetic variation (Milligan *et al*, 1994).

The White Leghorn (WLh) and Rhode Island Red (RIR) are the two important breeds which contribute to most of the eggs produced from commercial poultry enterprises. Divergent strains of the White Leghorn (WLh) and Rhode Island Red (RIR) breeds are being maintained by several breeding organizations in India for the production of crosses for commercial poultry enterprise: the WLh for production of white eggs and RIR for production of tinted eggs. Simultaneous comparative evaluation of egg production, egg quality, immunological responses and genetic diversity of layer stocks in WLh and RIR genomes are scanty. Such a comparison would be useful in the development of stocks suited to cater to specialized markets and production systems. The present study has been conducted on four pure strains of White Leghorn (WLh); two pure strains of Rhode Island Red (RIR) and the breed/strain crosses being maintained at the Poultry Research Farm, GADVASU, Ludhiana with following objectives:

1. To evaluate the egg production and quality traits of pure strains of White Leghorn, Rhode Island Red, and their crosses at different stages of laying.
2. To compare the immune responsiveness of pure strains of White Leghorn, Rhode Island Red and their crosses.
3. To study the genetic diversity among pure strains of White Leghorn, Rhode Island Red; and their cross using microsatellite molecular markers.

CHAPTER – II

REVIEW OF LITERATURE

The review of literature for present study is presented under the following headings:

2.1 Egg productivity and quality traits

2.2 Immune responsiveness

2.3 Microsatellite-based genetic diversity

2.1 EGG PRODUCTIVITY AND QUALITY TRAITS

Differences between distinct breeds and strains within breeds with respect to differences for egg production, several workers have studied the growth and egg quality. The present review cover some of the latest references

Farooq *et al* (2002) studied the feed consumption and egg production from 109 flocks in Chakwal, Pakistan over 52.5 weeks including 6 weeks of brooding, 12 weeks of growing and 34.5 weeks of laying. Mean total feed consumption/bird during the period of 52.5 weeks was 37.0±0.55 Kg, including 1.16±0.02, 4.17±0.05 and 31.7±0.56 Kg during brooding, growing and laying periods, respectively. Layers kept on floor consumed more feed compared to those kept in cages. Higher feed consumption was recorded for babcock than hisex strains; for medium and small flocks compared with large flocks; for flocks under good compared with poor hygienic conditions; and in low-density compared with high-density housing. Feed conversion for egg production was better: in optimally utilized houses; in good compared with poor hygienic conditions; in larger than in small flocks; and in cage than in floor housing.

Ledur *et al* (2002) studied the genetic effects of aging on egg quality traits in White Leghorn strains and strain crosses. The mean heterosis was significant over time for egg weight. Heterosis increased in magnitude with age and mean heterosis for Haugh Unit Score (%) and albumen height was also influenced by age.

Miah *et al* (2002) compared the growth and egg production of Rhode Island Red (RIR), Single Comb White Leghorn (WLh), Fayoumi (F), and their crosses viz; F x RIR and F x WLh chickens. During the growing period, body weight of RIR was highest and was followed by F x RIR, F x WLh, WLh and Fayoumi in that order. RIR had significantly higher feed conversion efficiency followed by F x RIR; WLh; F x WLh and Fayoumi. Crossbreds started laying earlier than pure breeds. Fayoumi and F x RIR reached 50% egg production faster than F x WLh and other pure breeds while age at peak production was statistically similar between these

groups. Highest egg production was observed in F x RIR followed by F x WLh; WLh; RIR and Fayoumi while heaviest eggs were in RIR followed by WLh; F x WLh; F x RIR and Fayoumi in that order.

Hocking *et al* (2003) conducted an experiment to determine the extent of between-breed genetic variation for adult body weight, sexual maturity, rate of lay, egg weight and egg composition to 55 weeks of age in 25 commercial and traditional lines of laying fowl. The proportion of total variation associated with breed/line of origin was high (>0.8) for body weight, sexual maturity and shell colour; moderately high (0.4 to 0.7) for rate of lay, early and late egg weight, albumen; and low (<0.4) for egg weight at 42 to 45 weeks. There were no detectable differences between breeds within category (commercial and traditional line) for rate of lay. Commercial lines displayed earlier sexual maturity, greater rate and persistency of lay and higher egg weight at earlier (32 to 35 weeks) as well as at later (52 to 55 weeks) ages.

Monira *et al* (2003) studied the external and internal qualities of 80 fresh eggs from each Barred Plymouth Rock (BPR), White Leghorn (WLh), Rhode Island Red (RIR) and White Rock (WR) layers at 1; 7; 14 and 21 days holding periods. There were significant difference among the breeds and holding period for all the egg quality traits except egg width. Breed and holding period interactions were significant for egg length, shape index, albumen height and Haugh unit but not for other traits. The egg weight, egg length, egg width, albumen height and Haugh Unit Score of all breeds were higher in fresh eggs but breaking strength and shell thickness were higher in seven-day holding period eggs than the in eggs of other period eggs. The egg weights, egg length, egg width and breaking strength were superior in White Leghorn over other breeds. Shape index and albumen height were better in White Rock than other breeds.

Premavalli and Viswanathan (2004) studied the influence of age on egg quality characteristics in White Leghorn chicken. Egg weight, shape index and yolk colour increased significantly, however, egg specific gravity, egg shell thickness, albumen index, Haugh Unit Score (%) and yolk index decreased with advancing age.

Reddy *et al* (2004) evaluated the feed efficiency and related traits in egg-type chickens at 21-24; 29-32; 37-40 and 21-40 weeks of age. Actual feed consumption was lowest during 37-40 weeks and highest during 20-40 weeks of age. Feed consumption per day was highest at 29-32 and lowest at 37-40 weeks of age while overall feed consumption (21-40 weeks) was 97.73 g/d. Feed consumption per dozen of eggs was highest at 37-40 and 29-32 weeks of age. Total feed consumption between 21-40 weeks of age. Total feed consumption was 1.71± 0.005 Kg. Feed consumption per kg egg mass

was highest at 21-24 weeks and lowest at 29-32 weeks with value of 2.85±0.09.

Mohammed *et al* (2005) studied some characteristics in three local types of Sudanese indigenous fowls: large Baladi (LB), Bare-neck (BN) and Betwil (BT). The live weight of BN was heavier (1547±275 g) than either LB (1494±350g) or BT (1198±257g). The weekly hen-day egg production means were 3.7; 3.2 and 3.9 for BN, BT and LB respectively, while the corresponding hen-housed egg production means were 3.3, 2.7 and 3.4. The rate of egg production during the laying period (up to 36 weeks) was 47.1; 38.5 and 48.5 for BN, BT and LB respectively. There were significant differences ($p<0.05$) in average egg-shell thickness among local breed types. The means of egg-shell thickness for BT and BN were 36.2±4.2 and 36.2±4.0μ respectively and were significantly thicker than that of LB (34.3±3.6μ).

Rizzi and Cheiricato (2005) studied the effect of age on productivity and egg quality of hens of two hybrid lines (HyLine Brown – HLB and HyLine White – HLW) and two local breeds (Robusta maculate – RM and Ermellinata of Ravage – ER) at 30 and 42 weeks of age. Age significantly affected the hen-day egg production and the daily egg mass of HLB and RM hens but did not affect the daily egg production of ER hens although it had slight increase in daily egg mass. The egg weight, yolk colour, yolk percentage and yolk: albumen ratio increased with age in all the groups.

2.2 IMMUNE RESPONSIVENESS

The immune system of poultry is a complex network of different cell types that give rise to an effective response to foreign challenges. Proper and efficient function of immune system is directly associated with the health of birds. The phagocyte ability, cell-medicated and humoral immunities combine to provide birds with a complete spectrum of resistance.

Several workers have studied the genetic variation in the ability of immune system to respond to foreign challenges. Genetic resistance of poultry may be improved by selection for immune response to complex, non-infectious antigen because immune response to non-specific multi-determinant complex antigen provides an indication of the natural immunity status. Various antigens have been used to monitor immunocompetence in poultry. One of the most commonly used antigen for genetic analysis of humoral immune response in avians is

Sheep Red Blood Cells (SRBC). The SRBC is a complex, non-pathogenic, cell dependent antigen that requires the help of T-lymphocyte to produce antibody (Cheng *et al*, 1991). SRBC also produces more antibodies than variety of other antigens (Parmentier *et al,* 1998b)

Cell-mediated immunity is primarily mediated by T-cells, macrophages and natural killer (NK) cells, while phagocytosis is basic, non-specific body response to microbial infection. Mitogens are used to measure the proliferative capacity of certain cell types that are indicator of general cellular-immune responsiveness. Phytohaemagglutinin (PHA-P), Concanavaline (Con-A) and Poke weed mitogen (PWM) are the commonly used mitogens to induced cellular-immune responsiveness in avian species.

Existence of genetic control has been reported for both primary and secondary immune response to sheep erythrocyte (Siegel and Gross 1980; Miller *et al*, 1992). Significant effects of hatch, line, sex and B-group haplotype on response to sheep RBCS have also been reported (Gross *et al*, 1980; Vander Zijpp and Leenstra 1980; Dunnington *et al*, 1984). For the formulation of effective genetic improvement programme, the documentation of the breed and strain difference among various immunocompetence are of great value.

Vanderzipp and Leenstra (1980) determined the total agglutinin antibody titre, 2-Merceptoethanol sensitive (MES) and resistant (MER) titre in 598 White Leghorn chickens after SRBC inoculation. Antibody titres were determined at 0, 3, 7, 10 and 13 days post-injection. Total antibody titre on day 0 and 5 represented completely 2-

Merceptoethanol sensitive antibodies and natural antibodies were present in some chicks at day 0. Mean total titre was highest (5.2) on day 7-post immunization.

Cheng and Lamont *et al* (1988) studied the immunocompetence in White Leghorn chicken which were divided into eight sub-lines based on erythrocyte antigen, antibody response to Glutamic Acid alanine-tyrosine (GAT) and response to Rous sarcoma virus-induced tumors, antibody to *Pasteurella multocida* (PM*), Mycoplasma gallisepticum* and infectious bursal disease (IBD) vaccines using ELISA. Phagocytic activity and T-cell mediated response were measured by carbon clearance and phytohaemagglutinin (PHA-P) injection assay, respectively. Significant haplotype (sub-lines) differences and sire-family differences were observed for all three measurements. Significant sex differences were observed for phagocytic activity and T-cell mediated response. Haplotypes with high antibody response to GAT had significantly higher antibody titre to PM and MG vaccines than haplotype with low antibody responses. Significant positive correlation was observed between antibody levels to two vaccines. A significant negative correlation was seen between phagocytic activity and T-cell mediated response of female.

Martin *et al (*1989) determined the SRBC antibody response in high (HA) and low (LA) antibody selected lines of chicken. Primary response pattern of total, MER and MES antibodies differed according to origin. Total antibodies increased rapidly, peaked and persisted at moderate level in high antibody selected line (HA). MES level peaked

then declined in line HA but it persisted at low level throughout in line LA. Titre of MER antibodies was considerably higher in line HA than in line LA. Secondary total titre was greater at five days after injection than at three day and greater for HA chicks than for LA chicks.

Benda *et al* (1990) evaluated the immune responsiveness of White Leghorn and Rhode Island Red cocks immunized with sheep red blood cells and *Brucella abortus* crude antigen. Serum agglutination was determined after 7 days. There were significant differences between the breeds for humoral and cellular immune responses. The wattle response was much higher in WLh than the RIR birds.

Saxena *et al* (1997) evaluated the primary antibody response to sheep erythrocytes in guinea fowl, using haemagglutination test. Immune response to sheep erythrocytes was normally distributed in guinea fowl with mean titre 1.53 ± 0.01. The effects of sire and variety (feather colour) were significant on titre value while the sex and sex X variety interaction for titre values was non significant.

Parmentier *et al* (1998b) studied the antibody (Ab) responses to i/m administered SRBC and BSA, and i/p administered *Escherichia coli* lipo-polysaccharide (LPS), in chicken lines divergently selected for high (H) and low (L) antibody responses to SRBC, and in a random bred control line (C). The antibody responses to SRBC and BSA, but not LPS, were significantly affected by line by treatment interactions. Levels of antibodies to SRBC and BSA were higher in the H line than in either the C or L line ($p < 0.05$).

Administration of LPS did not affect Ab responses to SRBC, but Ab responses to BSA were decreased in bird.

Boa Amponsem *et al* (2000) studied temporal pattern of SRBC antibody response after primary and secondary inoculation in White Leghorn lines selected for high (HA) and low (LA) 5-day antibody titres at 50 days of age. Antibody titre against SRBC was measured at 3, 5, 7, 9, 11, 13 and 20 days after inoculation. At 70 days of age half of birds were given a booster inoculation and antibody titre was measured 3, 6, 9 and 13 days after secondary injection. Pattern of antibody response to SRBC differed according to line, resulting in interaction of line by dosage by day. Antibody response to booster inoculation differed between lines with old age effect present for LA but not for HA chicks.

Sarkar *et al* (2000) studied the humoral, cell-mediated immunities and phagocytic ability in two pairs of chicken lines (at 10 wk of age) that were divergently selected for levels of high serum Ig M (HIM), low serum Ig M (LIM), high serum Ig G (HIG), and low serum Ig G (LIG). Cell mediated immunity was examined by splenomegaly assay at 12 weeks of age. At 20 and 23 weeks of age, 20 birds from the respective lines were injected *Brucella abortus* (BA), and blood samples were collected at 7 and 14 d post primary immunization (PPI) and post secondary immunization (PSI). Phagocytic ability was measured by carbon clearance assay at 25 and 30 wk of age. The results showed that the LIG line had higher degree of splenomegaly indices than the HIG line in both generations. The HIM and HIG lines had significantly ($p < 0.05$) higher total antibody titers to BA than their low counterparts.

Similarly, mercaptoethanol-resistant (MER) antibody titers to BA, as measured only in the fourth generation, were significantly ($p < 0.05$) higher in the HIM and HIG lines than their low counterparts. In both generations, the HIM and HIG lines had significantly ($p < 0.01$) faster carbon clearance ability than the LIM and LIG lines. The results suggest that both pairs of selected lines exhibited divergence in immuno-competence, although they had been selected for serum Ig isotypes.

Haunshi and Sharma (2002) studied general immuno-competence status in four pure chicken breeds viz. Dahlem Red, Aseel, Kadaknath and White Leghorn along with their selective crosses. Significant breed differences were observed for antibody response to SRBC. Dahlem Red had higher HA titre (9.21±0.46) while crossbreds showed significant differences among them for HA titre to SRBC. No consistent and sizeable heterotic effects of immune response traits were observed.

Cheema *et al* (2003) conducted a study to compare immuno-competence of four commercial broiler strains [Ross 308, Ross x Cobb, Ross 308 and Cobb x Cobb (cc)] that were fed either a marginal protein diet (D1) or high protein diet (D2). Strain cc showed comparatively higher and more persistent antibody tires against SRBC. The Ross 308 strain had significantly greater cell mediated. Immune response, as measured by T-lymphocyte proliferation response to PHA-P and Con-A than other strains. Birds on the high protein diets showed higher cell-mediated immune response than the birds on the low protein diet when measured by Con-A and PHA-P response. An interaction between strains and diets was seen for antibody response with the Ross 308 showing

higher titers on D1 while the CC had greater antibody response when raised on D2. These results suggest the existence of genetic differences between lines for various measures of immune responsiveness.

Singh *et al* (2004) studied immunocompetence status in two turkey varieties in India as response to Sheep Red Blood Cells by Haem-agglutination test 5 days post immunization with SRBC, Mercaptoethanol Resistant (MER) and Mercaptoethanol sensitive titre. The black variety had a higher MES antibody titre than the white variety .Sex had an effect on all traits except on MER, females generally had higher levels. The variety X sex interaction effect was significant for MES titre.

Van Den Brand *et al* (2004) studied the eggs of selected lines which differed in shell and albumen characteristics after 22 generations of divergent selection for antibody response against sheep red blood cells (SRBC) in three lines (high antibody response (H), control (C) and low antibody response (L). Results revealed that hatchability differed between the selected lines and albumen height decreased with layer age, but this decrease differed between lines. Eggs of the C line were heaviest, followed by the L line and finally the H line (59·44 *vs* 55·50 *vs* 54·15g, respectively). Egg shell thickness, egg shell percentage, albumen height and albumen pH were lowest in the L line, and highest in the H line, whereas the C line was intermediate. It was concluded that selection on antibody response to SRBC affected both external and internal egg characteristics

2.3 GENETIC DIVERSITY

Modern developments in DNA technologies have uncovered a large number of genetic polymorphism at the DNA sequence level; and to use them as markers for evaluation of the observed phenotypic variability. A marker is usually considered as a constituent that determines the function of a construction. A genetic marker is any suitable and inherited variation that can be measured/detected by a suitable method and can be used

subsequently to detect the presence of a specific genotype/phenotype other than itself, which otherwise is very difficult to detect.

Microsatellite markers are tandem repeat loci with a core motif of 1 to 6 base pair repeated several times in the genome sequence (Hillel *et al*, 2003). They are useful for measurement of genetic parameters such as number of expected alleles, Polymorphic information content (PIC), detection of rare alleles (Bartfai *et al,* 2003, Shen 2004) as they are numerous, show co-dominant inheritance. Microsatellite markers occur predominantly in the non-coding regions of DNA and are present in all plants, animals and microorganisms except bacteria.

Genetic distance is a measure of the evolutionary divergence between populations and it serves as an important tool for authentication of pedigree, characterization of breed/ strain within species and for genetic changes in species over time.

It is widely accepted that all populations of domesticated chickens have descended from a single ancestor, the Red Jungle Fowl (RJF) (*Gallus gallus*). At present, the improved Mediterranean type populations are the most closely related over to the Red Jungle Fowl (RJF). Later, with the massive use of selection and crossbreeding, local breeds and lines were developed, and Asian breeds of the Chinese and Malay types were introduced. All of these sources contributed to the modern biodiversity of chicken populations.

Since the start of commercial poultry breeding in the middle of the 20th century, chicken genetic diversity has become partitioned among relatively few highly specialized lines. As a consequence, many dual-purpose breeds, resulting from centuries of domestication and breeding, are now at the risk of

being lost. These breeds may, however, represent a resource of genes for future breeding and research purposes. Many workers have studied genetic relationship between and within chicken populations (Ponsuksili *et al,* 1999; Romanov *et al,* 2001 and Vanhala *et al*, 1998).

Crooijmans *et al* (1996) estimated allele frequencies for 17 microsatellite markers in nine highly selected commercial broiler and six highly selected commercial layer lines and reported the average number of marker alleles as 5.8 over all lines, 5.2 over broiler lines and 3.0 over layer lines. Average number of marker alleles within lines was 2.9, 3.6 and 2.0 for all, broiler and layer lines respectively. Average percentage of heterozygosity was 42% for all lines, 53% in broiler lines and only 27% in layer lines.

Takahashi *et al* (1998) studied the microsatellite DNA polymorphism based genetic relationship among ten Japanese native breeds of chicken and one imported breed (White Leghorn). The eight microsatellite primers used were polymorphic. Total 45 alleles were detected and the average number of alleles per locus was 5.6. Japanese native breeds were differentiated into three groups and they were clearly separated from the White Leghorns based on microsatellite polymorphism.

Vanhala *et al* (1998) evaluated genetic variability and genetic distances between eight chicken lines, which included three White Leghorn hybrids, three Finnish landrace, a Rhode Island Red, and a broiler hybrid line using microsatellite markers. These were polymorphic with number of alleles varying from 4 to 13 per locus and 1

to 10 per line. Estimate of expected heterozygosity were in range of 0.38 to 0.67 and all expected heterozygosities were greater than observed. The phylogenetic tree constructed using neighbor joining method grouped the lines into three clusters.

Kaiser *et al* (2000) assessed the genotype of 2 independent broiler populations with microsatellite marker to determine genetic polymorphism within and among broiler populations. The average number of alleles per line per microsatellite loci was 2.8 and 2.5 in population L and C respectively. Seventy two percent of the total alleles scored were unique to one or the other population.

Wimmer *et al* (2000) studied the genetic variability in various local chicken populations derived from Bolivia, India, Nigeria and Tanzania using 22 microsatellite markers. Between 2 and 11 alleles per locus were detected. All the populations showed high levels of heterozygosity with the lowest value of 45% for the Aseel population from India and the highest value of 67% for Arusha from Tanzania.

Romanov *et al* (2001) determined the genetic variation and genetic distances between strains of different origins in 224 individuals of 20 populations with 14 microsatellite markers covering 11 linkage groups. Of the 14-microsatellite loci, the number of alleles ranged between 2 and 21 per locus, the mean number of alleles being 11.2 per locus. By using Nei's standard distance and the Neighbor-Joining method, a phylogenetic tree was reconstructed; three major phylogenetic tree groupings were found. The Red Jungle Fowl (*Gallus gallus*) formed a separate branch and

demonstrated a specific allele distribution when compared with domestic fowl breeds analyzed. The second branch comprised commercial layer lines and chicken breeds that were subject to intense selection in the past or had common ancestral breeds with commercial strains. The third group encompassed the German native breed populations.

Zhang *et al* (2002) studied the diversity in Chinese native chicken breeds and imported broiler and layer breeds based on protein polymorphism, Randomly Amplified Polymorphic DNA and microsatellite polymorphism. Nine microsatellite loci used were polymorphic in 12 populations. Number of alleles per population at each locus ranged from 4 to 19 and the number of alleles per locus ranged from 12 to 26. Chinese native breeds were having higher gene diversity and average heterozygosity within the population. Layer breeds had much lower diversity and a close relationship between Chinese native and broiler breeds was observed.

Hillel *et al (2003)* measured the genetic biodiversity of 52 chicken populations with 22 di-nucleotide microsatellite markers. The polymorphism measures for the average, the least polymorphic population (inbred C line) and the most polymorphic population (*Gallus gallus spadiceus*) were, respectively, as follows: number of alleles per locus, per population: 3.5, 1.3 and 5.2; average gene diversity across markers: 0.47, 0.05 and 0.64; and proportion of polymorphic markers: 0.91, 0.25 and 1.0. Unselected populations were found to be more polymorphic than selected breeds such as layers. The distribution of population specific alleles and the amount of

genetic variation shared among populations supports the hypothesis that the red jungle fowl is the main progenitor of the domesticated chicken.

Wong *et al* (2004) compared the sequences of 3 domestic chickens (broiler, layer and Silkie) to their wild ancestor Red Jungle Fowl (RJF). It indicates that at least 90% are true single nucleotide polymorphism (SNPs), and at least 70% are common SNPs that segregate in many domestic breeds. Mean nucleotide diversity is about 5 SNP/kb for almost every possible comparison between RJF and domestic lines and indicated idea that domestic animals are highly inbred relative to their wild ancestor.

Kuo *et al* (2006) compared the Red Jungle Fowl and commercial layer sequences for newly developed 876 microsatellite biomarkers included di-, tri-, tetra- and penta- repeats, with a repeat frequency of 6 or greater. Small subsets of these markers from 6 chromosomal regions were selected for sequencing. Markers were grouped into categories based on the repeat number reported for the Red Jungle Fowl sequence. These categories were then further subdivided into two classes depending on whether the observed allele sizes were similar to the Red Jungle Fowl or different. Sequencing reactions were carried out in both directions and aligned. Preliminary analysis showed differences in allele sizes between Red Jungle Fowl and the commercial layers. Variation was found in the number of repeats, and there were some SNP polymorphisms seen in the flanking regions. All types of repeats, di-, tri-, tetra-, penta-, and complex repeats exhibited considerable polymorphisms between the Jungle Fowl and commercial layers.

Lujiang *et al* (2006) genotyped 2740 birds of 78 indigenous chicken breeds for 27 microsatellite markers on 13 chromosomes. The

number of alleles of the 27 markers ranged from 6 to 51 per locus with mean of 18.74. Heterozygosity (H) value of all the 78 chicken breeds was more than 0.5. The average H value (0.622) and polymorphic information content (PIC =0.573) of these breeds suggested that the Chinese indigenous chicken possessed more genetic diversity than that reported in many other countries.

CHAPTER – III

MATERIALS AND METHODS

3.1 DESCRIPTION OF POPULATIONS

The present study was conducted on two selected strains of White Leghorn, PL1 and PL2, a control line of White Leghorn, PL3 and two selected strains of Rhode Island Red (RIR) viz; RIR-B and RIR-C; and on two crosses viz; PL2 ♂ X RIR-B ♀ and RIR-B ♂ X RIR-C ♀ maintained at the Poultry Research Farm of the University. The investigations were conducted on the birds hatched during the year 2006-2007.

3.1.1. History

The WLh Strain PL1 was originally derived from a reputed commercial stock imported from U.S.A. while strain PL2 was derived from the well-known purebred "Mount Hope" stock of U.S.A., referred to as "Mychick" strain in India. By the year 2005-06 strains PL1 and PL2 have undergone 34 and 31 generations of intra-population selection, respectively. The control line, PL3, was derived from PL2 in it's ninth selected generation (1984-85); and differed from PL2 during the present study by 22 generations of reproduction. Two strains of Rhode Island Red strains, designated as RIR-C and RIR-B were procured in the form of hatching eggs from Central Avian Research Institute, Izatnagar and Central Poultry Breeding Farm, Bhubhneshwar, respectively. The strains were pedigreed generations and had since then been selected for egg production and egg weight.

3.1.2 Selection Procedure

In White Leghorn strains (PL1 and PL2), selection was conducted on the basis of combination selection (Osborne, 1957) for egg production to 40 weeks of age. The combination index values for each individual pullet was computed by assigning appropriate weightage to individual's own egg production, average egg production of it's 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 cullings were also practiced for egg weight (individual

basis) and viability (family basis) during the course of selection. During 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. The selected individuals were mated at random in individual laying cages using one sire with 5-6 dams through artificial insemination except that matings between full and half - sibs were avoided. The RIR-B and RIR-C strain were selected for egg production to 40 weeks of age along with some independent cullings for egg weight.

3.1.3 Management

The pedigreed chicks for the present study were hatched during Jan. 2006 in one hatch. The day -old chicks were wing banded. All the chicks were pedigreed both by sire and dam. Cockerels were dubbed at day old stage. The chicks were raised from day-old to 14 weeks in colony (brooder and grower) cages and subsequently all the pullets were raised in individual laying cages. The general procedure at the farm has been to distribute the pullets in colony cages and individual cages with no conscious grouping by families so that pullets of different sire and dam families were randomized. Feeding of birds was done with a ration containing 21-22 % protein upto 8 weeks of age and thereafter containing 17-18 % protein. The following vaccination schedule was followed:

Vaccine	**Age (days)**
Mareck's	0
Ranikhet (Mukteshwar F_1 strain)	5-7
Gumboro	18-21

Lasota	28-32
Ranikhet (R_2B)	42-45
Fowl pox	56-60
Ranikhet (R_2B)	110-115

3.2 DESCRIPTION OF EXPERIMENTS AND MEASUREMENTS

3.2.1 Experiment 1: Comparison of Strains for Growth, Egg Production and Egg- Quality

A random sample of 100 female chicks per genetic group was grown intermingled up to 14 weeks of age under similar environmental, nutritional and managemental conditions. The following traits were recorded on individual bird basis:

1. Body weights: Body weight of each individual pullet was recorded at 12; 16; 20; 24; 28; 32; 36 and 40 weeks of age. The body weights were measured in grams on a pan type balance.

2. Egg production traits: The following egg production traits were recorded on individual-pullet basis**:**

i. **Age at sexual maturity (ASM):** 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 number (EN40):** Egg production of each pullet was recorded daily up to 40 weeks of age in individual laying cages; and was partitioned into different periods corresponding the periods in which feed consumption and egg quality traits were recorded.

iii. **Egg weight:** Egg weight of each pullet was recorded as average of 6-8 consecutive eggs for each period on electrical digital balance up to a fraction of 0.1 g.

3. Egg quality and composition traits: The external and internal egg-quality and composition were recorded at three stages viz; 30, 36, 44 weeks and the various measurements recorded were as follow:

i. **Specific gravity:** Specific gravity of eggs was determined by floatation technique using salt solutions of varying concentrations. The salt solutions corresponding to specific gravity values from 1.052 to 1.120 with increments of 0.004 were prepared (Holder and Bradford, 1979). Before determination of specific gravity, individual eggs were weighed and then they were placed in different salt solutions starting from that of the lowest specific gravity. The specific gravity of the salt solution in which the egg did not either float or sink to the bottom but lay fully suspended was recorded as specific gravity of the egg. The eggs, which did not lie fully suspended, were transferred to next solution and were continued in next solutions till they remained fully suspended.

ii. **Shape Index:** Shape Index of individual egg was calculated as ratio of width to length of egg and expressed as percent.

iii. **Egg shell colour:** The egg shell colour was recorded on a scale of 1 to 13 as shown in Plate 1.

4. Internal egg-quality and-composition traits: Each egg was picked up in the left hand with large end of egg to the left. Striking it with a scalpel and placing both thumbs in cracked position of shell, the egg was broken perpendicular with a hinge like movement of the two halves of the shell. The plate used for breaking open the eggs was a smooth clear glass plate placed over a leveled surface. The contents of broken egg were spread

out on the glass plate, showing clearly the yolk in center surrounded by thick-albumen and thin-albumen layer lying at periphery. The following observations were recorded on the contents of broken egg:

i. **Albumen height and Haugh Unit Score (HUS %)**: The height of thick albumen was measured in millimeters using spherometer on contents of broken-open egg. Haugh Unit Score, which is a measure of albumen quality, was computed by the method of Haugh (1937) and later modified by Brant *et al* (1951) as below:

HUS = [{100 log (H + 7.57 – 1.7 $W^{0.37}$)} X100] /2.3026

Where: H is height of albumen (mm) and W is weight of egg (g)

ii. **Yolk diameter, height and weight**: Yolk was separated from albumen with the aid of a spatula. Height of the yolk was measured with a spherometer (mm) and the diameter was measured with Verneir calipers (in cm). The albumen clinging to the yolk was permitted to drain off and yolk rolled on paper towel to remove any adhering albumen and chalazae. Yolk was weighed up to fraction of 0.1 g. The weight of yolk was expressed as percentage of egg weight. Yolk index was calculated as:

Yolk Index (%) = (Yolk diameter X 100)/ Yolk height

iii. **Shell weight (g) and shell thickness (mm):** Shell was washed with tap water to remove sticking traces of albumen, taking care to collect even the broken bits, and dried in the thermostatically controlled hot air oven overnight (24 hrs) at 100±5°C. Shells were cooled to room temperature and weighed upto fraction of 0.1 g. Shell weight was also expressed as percentage of egg weight. Shell thickness was measured with the help of spherometer at three different places on

the equatorial region of the egg shell, and the values were averaged.

5. Derived productivity traits:

The following traits were derived from the basic egg production traits recorded on the individual pullets-basis:

i. **Rate of lay (%): ROL** = (No. of eggs laid up to specified period × 100)/ No. of functional days in that period

ii. **No. of functional days for the first period;** for subsequent periods, number of functional days corresponded the length of that period **=** No. of days up to end of the period – ASM

iii. **Egg mass (g): EM** = Egg no. in the specified period × average egg weight (g) of that period

iv. **Egg production efficiency: EPE (g/Kg)** = Egg mass (g)/ B W (Kg)

v. **Egg mass per functional day: EMFD (g/d)** = Egg mass (g)/ No. of functional days

vi. **Efficiency index: EI (g/d/Kg)** = Egg mass per functional day (g/d)/ B W (Kg)

6. Feed consumption and efficiency:

Feeding trials (ad libitum) were conducted at each of the three stage of laying .The birds were provided with weighed quantity of standard layer ration for 7-8 consecutive days with in each period. Feeding was done twice daily in morning and evening with all possible measures adopted to reduce wastage of feed. The feed residue was weighed after each recording period, and the amount of feed consumed by individual pullet per day was calculated. Feed efficiency was expressed on-per dozen of eggs and per-kg of egg mass basis as follow:

i. **Feed efficiency per dozen of eggs: FEDZ** = (FCT x 12)/ EN

ii. **Feed efficiency per kg egg mass: FEEM** = (FCT)/ EM

Where FCT is feed consumption per pullet during recording period

EN is the number of egg laid per pullet during recording period

EM is the egg mass per pullet during recording period

3.2.2 Experiment 2: Immune Response Estimation:

The immune response traits of cell-mediated immune response to sheep red blood cells (SRBC) and *in vivo* cell mediated immune response to mitogen Phytohaemagglutinin were recorded.

Immune response to SRBC: Sixty birds from each genetic group were used for the estimation of immune response to SRBC. Birds were given ad-lib feed and water. Cell mediated immune response to SRBC was estimated as follows:

a) Preparation of SRBC suspension: For the preparation of SRBC suspension blood from healthy sheep was obtained from the Sheep and Goat farm of University. About 30 ml blood was collected from the jugular vein in anticoagulant Alseiver solution with the help of 18G needle. The red blood cells were washed three times with equal volume of phosphate buffer saline (PBS). After final wash the packed cells were brought to 2.5% and 0.25% solution in PBS.

b) Immunization/ SRBC challenge: At 16 weeks of age chicks were immunized with 0.1 ml of 0.25% SRBC suspension. Prior to injection of SRBC suspension, a blood sample was drawn from each chick at day 0 of primary inoculation, to measure the base titre level. Then each chick was injected

intravenously with 0.1ml of 0.25% SRBC suspension. The blood samples were taken from the same birds at 5 and 10 days post-primary injection.

c) Preparation of serum samples: Blood samples were collected from the wing vein of the chicks with 24G needle without adding any anticoagulant. The clotted blood samples were centrifuged at 6000 r.p.m. for 10 min to harvest serum, which was then stored at 4°C.

d) Haemagglutination assay: Total antibody titre of antibodies against SRBC was determined as per method described by Martin *et al* (1989). In 96 well dilution plates, 50 μl plasma and 50 μl PBS (7.4) were mixed in first well and then serial dilutions of serum sample were done with 50 μl PBS (7.4). Then the plates were incubated at room temperature for 2-3 hr. with 2.5% SRBC suspension. The last well giving visible agglutination was recorded (Plate 2) and titre value was expressed as $\log_2$ of the reciprocal of the highest dilution giving agglutination.

In-vivo cell mediated immune response to Phytohaemagglutinin:

A total of 300 birds with 40 birds of each genetic group were evaluated for *in vivo* cell-mediated immune response to mitogen Phytohaemagglutinin (PHA-P). Immune response was evaluated as per method of Cheng and Lamont (1988) using footpad response to mitogen PHA-P at 46 weeks of age. The thickness of foot pad was measured with micrometer in mm. Left foot was used as control in which PBS was injected and right foot was used as test foot in which PHA-P in PBS (1 mg/ml) was injected. Thickness of both feet was

measured before injection and 24 hours after injection. Foot index was calculated as:

Foot Index = {(Post Inj.- Pre Inj.) - (Post PBS - Pre PBS)}

Where: Post Inj. is the thickness of test foot 24-hrs after injection of PHA-P;

Pre Inj. is the thickness of test foot before injection of PHA-P;

Post PBS is the thickness of control foot 24-hrs. post injection of PBS and

Pre PBS is the thickness of control foot before injection of PBS.

3.2.3 Experiment 3: Genetic Diversity Analysis Using Microsatellite Markers

Collection of blood samples: A random sample of 15 birds was taken from each of the three genetic groups viz; PL2, RIR-B and cross PL2♂ X RIR-B♀. Strain for the genetic diversity analysis. Venous blood samples (1.5 ml) were taken from wing vein of each bird with a 24 G needle. After adding 50 μl of 2.7 % EDTA, Blood samples were thoroughly mixed with anticoagulant and were stored overnight at -20° C.

Isolation of DNA: DNA was extracted from whole blood as per Sambrook *et al* (2001):

1. To 50μl blood sample added 500 μl of Lysis buffer
2. Incubated overnight at 57° C
3. Centrifuged at 5000 r.p.m. for 10 min
4. Added equal volume of Phenol: Chloroform (1:1) to supernatant and mixed well

5. Centrifuged at 5000 r.p.m. for 10 min
6. Repeated Phenol: Chloroform (1:1) extraction three times
7. Added equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) to supernatant and mixed well
8. Centrifuged at 5000 r.p.m. for 10 min
9. Added equal volume of Chloroform: Isoamyl alcohol (24:1) to supernatant and mixed well
10. Centrifuged at 5000 r.p.m. for 10 min
11. Added equal volume of 100% ethanol to supernatant and examined the presence of white threads of DNA
12. Incubated at 37º C for half an hour to precipitate DNA
13. Centrifuged and washed the pellet with 70% ethanol
14. Dried the pellet by inverting centrifuge tube on tissue paper
15. Added 50-80 μl of TE buffer and incubate at 56º C to dissolve the pellet
16. Stored at -20º C for long period

Quality checking of genomic DNA: For checking the quality of DNA, the DNA samples were evaluated on Agarose gel by electrophoresis. 1.0% agarose solution in 1.0X TBE buffer was prepared and put in gel cast to prepare gel. This mixture was poured into a gel-casting tray fitted with acrylic comb and allowed to solidify. Once the gel has solidified, a few ml of 1.0X TBE was added, comb was removed carefully and the gel was immersed in the electrophoresis tank containing 1.0X TBE buffer.

Samples were mixed with 6X loading dye and loaded into the wells. Electrophoresis was carried out @ 5-6 volts/cm for ½ - 1 hr. The bands of genomic DNA were visualized under UV-illumination and the sizes of the

bands separated were calculated from the standard DNA molecular weight markers.

Polymerase Chain Reaction: *In vitro* amplification of genomic DNA was done using PCR-based microsatellite markers recommended by Food and Agriculture Organization (FAO) for poultry. Primer name, its sequence, chromosomal location and standardized annealing temperature are presented in Table 1. Polymerase Chain Reaction was done using 50 μl reaction mixture as follows:

Sr.No.	Component	Final Concentration	Volume (μl)
1	10X PCR Assay buffer	1X	5
2	Forward primer	0.4μM	1
3	Reverse primer	0.4μM	1
4	dNTPs	200μM	1
5	Magnesium Chloride	2.5mM	0-2
6	Taq DNA Polymerase	1 Unit	0.3-05
7	DNA template	_	5
8	Deionised distilled water	_	Make volume to 50 μl

The PCR reaction contents were mixed thoroughly before adding and the reaction mixture was placed in thermocycler with following programming:

Step	Process	Temp. (ºC)	Time given (min)
1	Initial denaturation	94	4
2a	Denaturation	94	0.45-1
2b	Annealing	52-62	0.45-1
2c	Elongation	72	0.45-1
3	Repeat step 2 for 35-40 times		
4	Final Elongation	72	10

Table 1: Primer name, its sequence, chromosomal location, standardized annealing temperature of microsatellite markers

Primer		Sequence	Chro. No.	Ann. temp (°C)	Allele range (bp)
MCW-14	FP	**AAAATATTGGCTCTAGGAACTGTC**	6	60	140-215
	RP	ACCGGAAATGAAGGTAAGACTAGC			
MCW-67	FP	GAGATGTAGTGCCACATTCCGAC	10	60	168-232
	RP	GCACTACTGTGTGCTGCAGTTT			
MCW-183	FP	ATCCCAGTGTCGAGTATCCGA	7	60	281-333
	RP	TGAGATTTACTGGAGCCTGCC			
LEI-166	FP	AAGCAAGTGCTGGCTGTGCTC	3	62	248-329
	RP	TCCTGCCCTTAGCTACGCAC			
ADL-268	FP	CTCCACCCCTCTCAGAACTA	1	52	103-125
	RP	CAACTTCCCATCTACCTACT			
MCW-206	FP	CTTGACAGTGATGCATTAAAT	2	54	187-299
	RP	ACATCTAGAATTGACTGTTC			
MCW-104	FP	TAGCACAACTCAAGCTGTGAG	13	60	137-223
	RP	AGACTTGCACAGCTGTGACC			
MCW-123	FP	CCACTAGAAAAGAACATCCTC	14	60	76-100
	RP	GGCTGATGTAAGAAGGGATGA			
MCW-330	FP	TGGACCTCATCAGTCTGACAG	17	60	256-300
	RP	AATGTTCTCATAGAGTTCCTGC			
MCW-165	FP	CAGACATGCATGCCCAGATGA	23	60	114-118
	RP	GATCCAGTCCTGCAGGCTGC			
MCW-248	FP	GTTGTTCAAAAGAAGATGCATG	W29	60	205-225
	RP	TTGCATTAACTGGGCACTTTC			
MCW-111	FP	GCTCCATGTGAAGTGGTTTA	1	60	96-120
	RP	ATGTCCACTTGTCAATGATG			
MCW-034	FP	TGCACGCACTTACATACTTAGAGA	2	60	212-246
	RP	TGTCCTTCCAATTACATTCATGGG			
LEI-234	FP	ATGCATCAGATTGGTATTCAA	2	60	216-364
	RP	CGTGGCTGTGAACAAATATG			

Evaluation of PCR product: The PCR product so obtained was first analyzed using 2.0% agarose gel and then evaluated for genetic diversity using Denaturing- Polyacrylamide Gel Electrophoresis (PAGE) as follows:

i) Analytical agarose gel electrophoresis

1. 600 mg (2%) of agarose in 30 ml of 1.0X TBE was dissolved, melted, allowed cooling to 50ºC, 1µl of ethidium bromide (10 mg/ml stock at a final conc. of 0.5 µg/ml) was added and mixed thoroughly.
2. This mixture was poured into a gel-casting tray fitted with acrylic comb and allowed to solidify.
3. Once the gel has solidified, a few ml of TBE was added, comb was removed carefully and the gel was immersed in the electrophoresis tank containing 1.0X TBE buffer.
4. Samples were mixed with 6X loading dye and loaded into the wells.
5. Electrophoresis was carried out @ 5-6 volts/cm for ½ to 1 hr. The bands were visualized under UV-illumination and photographed (Plate 3). The sizes of the bands separated were calculated from the standard DNA molecular weight markers.

ii) Denaturing poly acrylamide gel electrophoresis

6% Urea-Polyacrylamide gel was used for resolution and documentation of PCR products. The setting of the gel was done as follows:

1. Glass plates (16 x 16 cm) were washed with detergent, rinsed initially under running tap water till no remains of detergent were left and finally in tdH_20 before drying. Before use the glass plates were cleaned with methanol and dried.
2. Glass plates were prepared by putting 1 mm spacer in its position after proper sealing, the gel was poured into the space and comb was inserted immediately.
3. The gel was allowed to polymerize at room temperature for 2-3 hrs.
4. After polymerization, gel was put in electrophoresis tank with notched plate facing towards the buffer reservoir.

5. The reservoir of the electrophoresis tank was filled with 1X TBE, the comb was removed carefully and the wells were washed with 1X TBE.

6. Samples were mixed with 6 X formamide based loading dye.

7. Sample were denatured at 98^0 C for 10 minutes afterward immediately snapped into ice for 10 minutes then loaded into the wells

8. Electrophoresis was carried out @100 volts for 5-6 hrs. The sizes of the bands separated were calculated from the standard DNA molecular weight markers.

iii) Silver staining

Silver staining was carried out according to Bassam *et al* (1991) as follows:

1. The gel was placed on a clean surface by keeping the notched plate facing upwards.

2. The upper plate was detached gently, by taking care to avoid breakage of the gel.

3. Cutting the upper portion of the gel from that side marked the first row loaded.

4. The gel along with the plate was placed in a suitable-sized tray.

5. **Fixing the gel:** About 300ml of 10% Glacial Acetic Acid was added in the tray, shaken gently and the plate was removed carefully after the gel got detached. The gel was agitated for 20 min or until the tracking dyes were no longer visible. The glacial acetate solution was saved and used as stop solution to terminate the developing reaction.

6. **Washing the gel:** The gel was rinsed 3 times, 2 mins each with tdH_2O.

7. **Staining the gel:** 300 ml 0.1% silver nitrate was added to the tray and agitated for 30 min.

8. **Washing the gel:** The gel was rinsed for 30 sec with tdH_2O.

9. **Developing the gel:** 300 ml of 0.028 M sodium carbonate (3% Na_2CO_3 solution) with 450 µl of 37% formaldehyde (added at the last minute) was poured in the tray and gel was gently shaken for few min. till the bands became brownish and distinct.

10. **Fixing the gel:** The reaction was stopped by adding 10% glacial acetic acid for 2 min.

The gel was stored in distilled H_2O until vacuum drying. Finally the DNA bands separated on the denatured polyacrylamide gel were visualized and analyzed after the silver staining.

3.3 STATISTICAL ANALYSIS

Comparison of Strains for Egg Production, Quality Traits and Immune response: The differences between the genetic groups were compared for immune response to sheep red blood cells, immune response to mitogen Phytohaemagglutinin, growth, egg quality and production traits by using Analysis of Variance (one way; two way with interaction as per the STATGRAF statistical package). The significance of differences between strains was evaluated by multiple range Test.

Genetic diversity analysis:

The size of the alleles observed was estimated using the INCHWORM software (www.molecularworkshop.com). The statistical analysis was carried out using POPGENE software (Yeh *et al*, 1999). The following observations were recorded:

i. Observed and expected number of alleles (based on Hardy Weinberg Equilibrium)
ii. Allelic frequency
iii. Testing for Hardy-Weinberg Equilibrium
iv. Observed and expected heterozygosity

CHAPTER – IV

RESULTS AND DISCUSSION

4.1 GROWTH

Body weight, is generally considered a good measure of growth, and gives an indication about the genetic potential for growth under specific environmental conditions. Future egg laying performance of birds also depends on proper growth during growing and laying periods. The means (±SE) for body weights of different genetic groups of White Leghorn and Rhode Island Red at different ages are presented in Tables 2 and 3.

Comparison of body weights of the two pure strains of WLh viz; PL1 and PL2 indicated that except for 28 week body weight, the two strains did not differ from each other significantly ($p \leq 0.05$). The control line, PL3 was derived from strain PL2 in its 9th cycle of selection. Therefore a comparison of body weights of these two strains indicates the effect of selection primarily for egg production and egg weight, and to some extent for body weight, viability and fertility. The selected White Leghorn strain PL2 had significantly higher body weights than its unselected counterpart, the control strain (PL3) at 12, 16 and 20 weeks of the ages but thereafter, the control strain tended to maintain higher weight than the selected strain though the differences could not attain statistical significance. Selection for egg production traits in PL2 has thus modified its growth pattern.

Table 2: Means (± SE) of body weights (g) of different genetic groups

Genetic group	BW12	BW16	BW20	BW24
WLh:				
PL1	$642 \pm 15.9^{ab}_{(9.3)}$	$851 \pm 22.7^{b}_{(10.8)}$	$1146 \pm 20.4^{b}_{(8.4)}$	$1217 \pm 22.6^{a}_{(8.8)}$
PL2	$660 \pm 12.2^{b}_{(11.5)}$	$882 \pm 18.2^{b}_{(12.7)}$	$1120 \pm 16.4^{b}_{(9.4)}$	$1201 \pm 18.2^{a}_{(10.9)}$
PL3	$594 \pm 12.9^{a}_{(12.1)}$	$748 \pm 19.4^{a}_{(13.3)}$	$1043 \pm 17.4^{a}_{(10.9)}$	$1185 \pm 19.3^{a}_{(10.0)}$
Naked-neck	$669 \pm 16.5^{b}_{(14.1)}$	$868 \pm 23.0^{b}_{(19.4)}$	$1145 \pm 20.7^{b}_{(12.5)}$	$1259 \pm 23.0^{a}_{(13.0)}$
RIR:				
B	$692 \pm 14.1^{b}_{(14.5)}$	$978 \pm 21.2^{c}_{(16.1)}$	$1229 \pm 19.1^{c}_{(9.8)}$	$1387 \pm 21.2^{b}_{(10.4)}$
C	$781 \pm 13.2^{c}_{(14.8)}$	$1102 \pm 19.8^{d}_{(13.9)}$	$1301 \pm 17.9^{c}_{(10.4)}$	$1434 \pm 19.7^{b}_{(8.9)}$
WLh Pure avr.	$657 \pm 8.7^{A}_{(11.9)}$	$869 \pm 13.1^{A}_{(14.5)}$	$1135 \pm 11.2^{A}_{(10.1)}$	$1222 \pm 12.4^{A}_{(11.2)}$
RIR Pure avr.	$739 \pm 10.4^{B}_{(15.9)}$	$105 \pm 15.7^{B}_{(16.0)}$	$1267 \pm 13.4^{B}_{(10.5)}$	$1412 \pm 14.9^{B}_{(9.8)}$
Pure vs. cross comparison:				
Parental breed avr.: (PL2 and RIR-B)	$673 \pm 9.0^{P}_{(13.2)}$	$923 \pm 13.8^{P}_{(15.4)}$	$1166 \pm 12.5^{P}_{(10.6)}$	$1280 \pm 16.1^{P}_{(12.9)}$
Breed cross: PL2♂ X RIR-B♀	$865 \pm 12.0^{Q}_{(9.6)}$	$1149 \pm 18.3^{Q}_{(9.9)}$	$1271 \pm 16.7^{Q}_{(8.9)}$	$1398 \pm 21.5^{Q}_{(9.7)}$
Hetrosis (%)	28.47	24.56	8.98	9.17
Parental strain avr.: (RIR-B and RIR-C)	$736 \pm 13.1_{(15.9)}$	$1044 \pm 18.5_{(16.0)}$	$1267 \pm 15.2_{(10.5)}$	$1412 \pm 15.1_{(9.8)}$
Strain cross: RIR-B♂ x RIR-C♀	$739 \pm 19.0_{(17.1)}$	$996 \pm 26.8_{(17.9)}$	$1222 \pm 22.0_{(12.4)}$	$1346 \pm 21.9_{(10.4)}$
Hetrosis %)	0.39	-3.69	3.58	-4.68

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$).
:a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison
WLh Pure avr. refers to average of PL1, PL2 and Naked-neck
RIR Pure avr. refers to average of RIR-B and RIR-C
Figure in the parentheses are the coefficients of variation (%)

Table 3: Means (± SE) of body weights (g) of different genetic groups

Genetic group	BW28	BW32	BW36	BW40
WLh:				
PL1	$1287 \pm 25.8^{b}_{(8.0)}$	$1331 \pm 22.5^{a}_{(7.2)}$	$1451 \pm 27.1^{a}_{(9.7)}$	$1239 \pm 29.3^{a}_{(12.4)}$
PL2	$1180 \pm 20.0^{a}_{(12.5)}$	$1327 \pm 18.1^{a}_{(8.5)}$	$1426 \pm 21.8^{a}_{(8.9)}$	$1235 \pm 23.6^{a}_{(11.5)}$
PL3	$1244 \pm 21.0^{ab}_{(9.9)}$	$1332 \pm 19.2^{a}_{(9.5)}$	$1454 \pm 23.1^{a}_{(10.5)}$	$1265 \pm 25.0^{a}_{(13.0)}$
Naked-neck	$1287 \pm 25.0^{b}_{(13.1)}$	$1311 \pm 23.8^{a}_{(12.4)}$	$1433 \pm 27.5^{a}_{(13.5)}$	$1239 \pm 29.8^{a}_{(15.5)}$
RIR:				
B	$1384 \pm 23.1^{c}_{(12.6)}$	$1543 \pm 21.0^{b}_{(9.1)}$	$1695 \pm 25.3^{b}_{(10.9)}$	$1626 \pm 27.4^{b}_{(12.4)}$
C	$1496 \pm 21.6^{d}_{(9.2)}$	$1637 \pm 19.6^{c}_{(8.4)}$	$1782 \pm 23.6^{b}_{(8.6)}$	$1721 \pm 25.6^{b}_{(10.3)}$
WLh Pure avr.	$1240 \pm 14.3^{A}_{(12.3)}$	$1324 \pm 12.3^{A}_{(9.6)}$	$1435 \pm 14.7^{A}_{(10.6)}$	$1237 \pm 15.9^{A}_{(13.0)}$
RIR Pure avr.	$1444 \pm 17.2^{B}_{(11.5)}$	$1593 \pm 14.7^{B}_{(9.2)}$	$1741 \pm 17.6^{B}_{(10.0)}$	$1677 \pm 19.1^{B}_{(11.6)}$
Pure vs. cross comparison:				
Parental breed avr.: PL2 and RIR-B	$1267 \pm 18.0^{P}_{(14.9)}$	$1419 \pm 6.1^{P}_{(11.6)}$	$1541 \pm 19.28^{P}_{(13.2)}$	$1401 \pm 23.4^{P}_{(18.3)}$
Breed cross: PL2♂ X RIR-B♀	$1423 \pm 23.9^{Q}_{(9.8)}$	$1536 \pm 21.4^{Q}_{(8.8)}$	$1650 \pm 25.7^{Q}_{(8.8)}$	$1521 \pm 31.2^{Q}_{(9.9)}$
Hetrosis (%)	12.26	8.21	7.13	8.51
Parental strain avr.: (RIR-B and RIR-C)	$1444 \pm 18.1_{(11.5)}$	$1593 \pm 16.0_{(9.2)}$	$1741 \pm 18.9_{(10.0)}$	$1677 \pm 20.2_{(11.6)}$
Strain cross: RIR-B♂ x RIR-C♀	$1471 \pm 26.2_{(11.4)}$	$1562 \pm 23.2_{(9.5)}$	$1725 \pm 27.9_{(9.8)}$	$1602 \pm 29.3_{(10.3)}$
Hetrosis (%)	1.89	-1.94	-0.96	4.47

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$).

: a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison

WLh Pure avr. refers to average of PL1, PL2 and Naked-neck
RIR Pure avr. refers to average of RIR-B and RIR-C
Figure in the parentheses are the coefficients of variation (%)

Naked-neck strain has been derived from selected strain PL2. There were no significant differences between the naked-neck and PL2 strains for body weight at various ages with the exception of 28-week body weight implying that naked-neck allele did not influence body weight in the White Leghorn genomic background.

Of the two Rhode Island Red strains, RIR-C had higher body weights than RIR-B at all the ages but the differences were statistically significant at 12, 16, 28 and 32 weeks of age. The two RIR strains used in the present study were obtained from different genetic sources, and presumably, had different selection history.

A comparison of selected White Leghorn pure strains (average body weight of the PL1, PL2 and Naked-neck) with the average of the RIR strains showed that RIR breed had significantly higher body weight than WLh at all the ages in amounts varying between 11.6 and 35.5%. The superiority of RIR over WLh was more conspicuous during laying periods as compared to growing periods. The difference between the WLh and RIR breeds for body weight is well-documented as the RIR is a dual-purpose breed, and hence heavier. Miah *et al* (2002) also reported higher body weights in Rhode Island Red as compared to White Leghorn.

The cross between WLh and RIR (PL2♂ X RIR-B♀) had significantly higher ($p \leq 0.5$) body weight than the parental pure average at all the ages. The magnitude of heterosis was, in general, higher during growing than the laying periods. The higher body weight of cross breds over the parental pure average was not attributable to the maternal effect mediated via maternal egg size since the egg weight of

RIR was lower than WLh. Despite significant difference in body weights between RIR-B and RIR-C, their cross RIR-B♂ x RIR-C♀ did not show any significant differences from the parental average. The magnitude of heterosis depends on the level of dominance, and the degree of genetic diversity between the strains/breeds involved (Falconer 1996). Since breeds are likely to differ from each other genetically more than the strains, higher degree of heterosis observed between WLh and RIR than between RIR-B and RIR-C is as per the theoretical expectation.

4.2 EGG PRODUCTION TRAITS

Egg production of each pullet was recorded from the onset of production upto 44 weeks of age, and was partitioned into 3 periods viz; from age at sexual maturity (ASM) to 30; 30-36 and 36-44 weeks. The means (±SE) for ASM and egg production traits up to 40 weeks of age are presented in Table 4. Age at sexual maturity (d) did not differ significantly among various strains of WLh and RIR with the exception of the unselected control strain. The control strain matured significantly later than the other strains. The average ASM of pure White Leghorn, selected strains and naked-neck attained early sexual maturity as compared to RIR strains but the differences was statistically non-significant. The breed cross, PL2♂ X RIR-B♀, attained significantly earlier sexual maturity than pure breds. However the RIR strain cross did not differ from the pure strain average. The mean (±SE) for various components of egg productivity during the three different periods are presented in Table 5 to 9.

Table 4: Mean (± SE) of ASM, Egg production traits upto 40 weeks of age of different genetic groups

STRAIN	ASM	EN40	Rate of lay	EW40
Genetic group:				
WLh: PL1	$144.0 \pm 2.11^{a}_{(7.4)}$	$105.3 \pm 2.6^{ab}_{(12.7)}$	$77.5 \pm 1.6^{a}_{(11.0)}$	$55.2 \pm 0.55^{b}_{(5.5)}$
PL2	$138.1 \pm 1.7^{a}_{(7.4)}$	$107.2 \pm 2.1^{b}_{(14.2)}$	$75.6 \pm 1.3^{a}_{(12.9)}$	$55.4 \pm 0.44^{b}_{(5.1)}$
PL3	$152.3 \pm 1.8^{b}_{(9.8)}$	$97.2 \pm 2.2^{a}_{(13.6)}$	$76.7 \pm 1.3^{a}_{(13.2)}$	$50.8 \pm 0.47^{b}_{(5.5)}$
Naked-neck	$139.8 \pm 2.1^{a}_{(7.2)}$	$106.0 \pm 2.6^{ab}_{(13.1)}$	$75.5 \pm 1.6^{a}_{(9.0)}$	$55.6 \pm 0.56^{b}_{(7.2)}$
RIR: B	$144.1 \pm 1.9^{a}_{(9.9)}$	$102.0 \pm 2.4^{ab}_{(17.7)}$	$75.1 \pm 1.5^{a}_{(14.1)}$	$50.9 \pm 0.51^{a}_{(6.3)}$
C	$145.0 \pm 1.8^{a}_{(8.4)}$	$98.0 \pm 2.3^{a}_{(18.1)}$	$72.4 \pm 1.4^{a}_{(13.6)}$	$51.1 \pm 0.48^{a}_{(6.9)}$
WLh Pure avr.	$140.2 \pm 1.0_{(7.5)}$	$106.3 \pm 1.4^{B}_{(13.5)}$	$76.1 \pm 0.8_{(11.4)}$	$55.4 \pm 0.30^{B}_{(5.9)}$
RIR Pure avr.	$144.6 \pm 1.3_{(9.1)}$	$99.9 \pm 1.7^{A}_{(18.0)}$	$73.7 \pm 1.02_{(14.0)}$	$51.0 \pm 0.36^{A}_{(6.6)}$
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	$140.7 \pm 1.2^{Q}_{(8.9)}$	$105.0 \pm 1.6^{P}_{(15.9)}$	$75.4 \pm 0.9_{(13.4)}$	$53.5 \pm 0.36_{(7.0)}$
Breed Cross PL2♂ x RIR-B♀	$132.1 \pm 1.6^{P}_{(7.5)}$	$116.4 \pm 2.1^{Q}_{(11.3)}$	$78.7 \pm 1.2_{(8.7)}$	$54.8 \pm 0.48_{(5.5)}$
Hetrosis %	-6.05	10.82	4.34	2.42
Parental strain avr. (RIR-B and RIR-C)	$144.6 \pm 1.5_{(9.1)}$	$99.9 \pm 1.9_{(18.0)}$	$73.7 \pm 1.0_{(14.0)}$	$51.0 \pm 0.36_{(6.6)}$
Strain Cross RIR-B♂ x RIR-C♀	$142.8 \pm 2.2_{(11.6)}$	$101.6 \pm 2.8_{(16.8)}$	$74.1 \pm 1.5_{(12.3)}$	$51.2 \pm 0.52_{(6.1)}$
Hetrosis %	-1.27	1.69	0.63	0.39

Figures bearing different superscripts within a column differ significantly $p \leq 0.05$).
: a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison
WLh Pure avr. refers to average of PL1, PL2 and Naked-neck
RIR Pure avr. refers to average of RIR-B and RIR-C

Table 5: Mean (± SE) of egg weight of different genetic groups

	Egg Weight			Ratio		
	30th week	36th week	44th week	EW36/EW30	EW44/EW36	EW44/EW30
Genetic group:						
WLh: PL1	53.7± 0.55[b]	55.2± 0.53[b]	57.9± 0.68[c]	1.027	1.04	1.07
PL2	53.2± 0.47[b]	55.4± 0.45[b]	58.4± 0.58[c]	1.041	1.05	1.09
PL3	49.4± 0.51[a]	50.7± 0.47[a]	53.9± 0.59[a]	1.026	1.06	1.09
Naked	53.8± 0.59[a]	55.6± 0.55[b]	57.1± 0.76[bc]	1.03	1.02	1.06
RIR: B	48.3± 0.54[a]	50.9± 0.53[a]	54.4± 0.67[ab]	1.05	1.06	1.126
C	48.8± 0.50[b]	51.1± 0.48[a]	53.4± 0.62[a]	1.04	1.04	1.09
WLh Pure avr.	53.5± 0.31[B]	55.4± 0.30[B]	57.9± 0.38[B]	1.03	1.04	1.08
RIR Pure avr.	48.6± 0.37[A]	51.0± 0.36[A]	53.9± 0.46[A]	1.04	1.05	1.1
Pure vs. cross comparison:						
Parental breed avr. (PL2 and RIR-B)	51.1± 0.41	53.5± 0.37[P]	56.9± 0.56	1.04	1.06	1.11
Breed Cross PL2♂xRIR-B♀	52.4± 0.55	54.8± 0.48[Q]	55.9± 0.56	1.04	1.02	1.06
Hetrosis %	2.54	2.42	-1.75			
Parental strain avr. (RIR-B and RIR-C)	48.6± 0.37	51.0± 0.36	53.9± 0.49[Y]	1.04	1.05	1.1
StrainCross RIR-B♂xRIR-C♀	48.1± 0.56	51.2± 0.52	51.8± 0.71[X]	1.06	1.01	1.07
Hetrosis %	1.02	0.39	-3.89			

Figures bearing different superscripts within a column differ significantly $p \leq 0.05$).
: a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison
WLh Pure avr. refers to average of PL1, PL2 and Naked-neck
RIR Pure avr. refers to average of RIR-B and RIR-C

Table 6: Means (±SE) for egg number and rate of lay of different genetic groups

Egg Number (EN)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Cumulative to 44 wks**
Genetic group				
WLh: PL1	46.8± 1.76^{b}	36.8± 0.59^{b}	43.9± 0.88^{b}	123.1±3.26
PL2	48.7±1.51^{b}	36.9± 0.49^{b}	45.5± 0.76^{b}	128.7±2.71
PL3	39.8± 1.62^{a}	36.4± 0.52^{b}	44.9± 0.76^{b}	118.0±2.87
Naked-neck	49.9± 1.89^{b}	35.2±0.61^{ab}	43.1±0.98^{ab}	119.2±3.26
RIR: B	50.1± 1.74^{b}	34.0± 0.59^{a}	42.8±0.86^{ab}	121.6±3.14
C	46.3± 1.60^{b}	33.6± 0.53^{a}	40.3± 0.80^{a}	119.2±2.96
WLh Pure avr.	48.4± 1.00	36.4± 0.32^{B}	44.4± 0.46^{B}	123.1±3.1
RIR Pure avr.	48.0± 1.20	33.8± 0.39^{A}	41.5± 0.55^{A}	120.4±2.1
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	49.3± 1.09^{P}	35.7±0.35^{P}	44.3± 0.54	125.71±1.70^{P}
Breed Cross PL2♂xRIR-B♀	57.7± 1.44^{Q}	36.9±0.47^{Q}	45.0± 0.69	138.1±2.40^{Q}
Hetrosis %	17.03	3.36	1.58	9.76
Parental strain avr. (RIR-B and RIR-C)	48.0± 1.30	33.8± 0.43	41.5± 0.58	120.4±2.1
Strain Cross RIR-B♂xRIR-C♀	50.0± 2.00	33.7± 0.62	42.5± 0.83	122.1±3.2
Hetrosis %	4.16	-0.29	2.4	1.66
Rate of Lay (%)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Age\Stage Pooled**
Genetic group				
WLh: PL1	70.4± 1.82	87.7± 1.40^{b}	78.4± 1.57^{b}	78.8± 1.10^{b}
PL2	68.0± 1.56	87.9± 1.18^{b}	81.3± 1.35^{b}	79.0± 0.94^{b}
PL3	70.82± 1.68	86.8± 1.24^{b}	80.2± 1.37^{b}	79.4± 0.98^{b}
Naked-neck	69.5± 1.95	83.8±1.46^{ab}	77.0±1.76^{ab}	76.8± 1.18^{ab}
RIR: B	73.6± 1.80	81.1± 1.40^{a}	76.5±1.55^{ab}	77.0± 1.09^{ab}
C	69.1± 1.66	80.1± 1.28^{a}	72.0± 1.43^{a}	73.7± 1.00^{a}
WLh Pure avr.	69.31± 1.70	86.7± 0.77^{B}	79.3± 0.83^{B}	78.3± 0.60^{B}
RIR Pure avr.	71.41± 1.75	80.5± 0.91^{A}	74.1± 0.98^{A}	75.2± 0.72^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	51.1± 0.41	85.1± 0.85^{P}	79.2± 0.97	78.2± 0.68^{P}
Breed Cross PL2♂xRIR-B♀	52.4± 0.55	88.0± 1.21^{Q}	80.4± 1.24	80.7± 0.89^{Q}
Hetrosis %	2.54	3.29	1.51	3.19
Parental strain avr. (RIR-B and RIR-C)	71.2± 1.19	80.5± 1.02	74.1± 1.04	75.2± 0.67
Strain Cross RIR-B♂xRIR-C♀	71.4± 1.82	80.3± 1.48	75.8± 1.49	76.0± 0.99
Hetrosis %	0.28	-0.24	4.99	1.06

Figures bearing different superscripts within a column differ significantly (p≤0.05).

EM (Kg)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Cumulative to 44 wks**
Genetic group				
WLh: PL1	2.50± 0.08^{bc}	2.03± 0.03^{d}	2.53± 0.04^{cd}	7.12 ± 0.08^{cd}
PL2	2.58± 0.07^{c}	2.04± 0.02^{d}	2.66± 0.04^{d}	7.51 ± 0.09^{d}
PL3	1.96± 0.08^{a}	1.84± 0.02^{bc}	2.41± 0.04^{bc}	6.37 ± 0.06^{a}
Naked-neck	2.68± 0.09^{c}	1.95± 0.03^{cd}	2.46± 0.05^{bc}	6.80 ± 0.08^{bc}
RIR: B	2.42± 0.08^{bc}	1.73± 0.03^{ab}	2.32± 0.04^{ab}	6.61 ± 0.05^{ab}
C	2.24± 0.07^{ab}	1.71± 0.02^{a}	2.14± 0.04^{a}	6.36 ± 0.05^{a}
WLh Pure avr.	2.58± 0.05^{A}	2.01± 0.01^{B}	2.57± 0.02^{B}	7.12 ± 0.08^{B}
RIR Pure avr.	2.34± 0.06^{B}	1.72± 0.02^{A}	2.22± 0.03^{A}	6.48 ± 0.08^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	2.51± 0.05^{P}	1.91± 0.02^{P}	2.51± 0.03	7.15 ± 0.10^{P}
Breed Cross PL2♂xRIR-B♀	3.02± 0.07^{Q}	2.02± 0.03^{Q}	2.51± 0.04	7.72 ± 0.09^{Q}
Hetrosis %	18.72	5.75	0	1.11
Parental strain avr. (RIR-B and RIR-C)	2.32± 0.06	1.72± 0.02	2.22± 0.02	6.48±0.05
Strain Cross RIR-B♂xRIR-C♀	2.40± 0.09	1.72± 0.03	2.19± 0.04	6.32±0.07
Hetrosis %	3.44	0	-1.35	-0.02
EMFD (g\d)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Age\Stage Pooled**
Genetic group				
WLh: PL1	37.7± 0.95^{b}	48.3± 0.76^{d}	45.3± 0.87^{cd}	43.7± 0.63^{d}
PL2	36.1± 0.81^{ab}	48.7± 0.63^{d}	47.5± 0.75^{d}	44.0± 0.54^{d}
PL3	34.9± 0.87^{ab}	44.0± 0.66^{bc}	43.0± 0.76^{bc}	40.7± 0.56^{bc}
Naked-neck	37.3± 1.02^{ab}	46.5± 0.79^{cd}	43.9± 0.97^{bc}	42.5± 0.68^{cd}
RIR: B	35.7± 0.94^{ab}	41.2± 0.76^{ab}	41.1± 0.86^{ab}	39.3± 0.63^{ab}
C	33.58± 0.86^{a}	40.7± 0.69^{a}	38.3± 0.79^{a}	3.7.5± 0.57^{a}
WLh Pure avr.	36.99± 0.52^{B}	48.02± 0.41^{B}	45.92 $\pm0.48^{B}$	43.5± 0.35^{B}
RIR Pure avr.	34.56 ± 0.63^{A}	40.98± 0.51^{A}	39.77± 0.57^{A}	38.3± 0.42^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	35.98± 0.64^{P}	45.62 ± 0.54^{Q}	44.90± 0.60	42.0± 0.43^{P}
Breed Cross PL2♂xRIR-B♀	38.65± 0.85^{Q}	48.22± 0.72^{P}	44.91± 0.77	43.9± 0.56^{Q}
Hetrosis %	7.42	5.69	0.02	4.52
Parental strain avr. (RIR-B and RIR-C)	34.56± 0.57	40.98± 0.49	39.77± 0.52	38.36± 0.35

Strain Cross RIR-B♂xRIR-C♀	34.25± 0.87	41.10± 0.71	39.25± 0.75	38.29± 0.52
Hetrosis %	-0.89	0.29	-1.3	-0.18

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$).

Table 7: Means (±SE) for EM and EMFD for different genetic groups

Table 8: Means (±SE) for EPE and EINDEX of different genetic groups

EPE (g/Kg)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Age\Stage Pooled**
Genetic group				
WLh: PL1	1.92 ± 0.06^{bc}	1.40 ± 0.02^{d}	1.91 ± 0.04^{cd}	1.74 ± 0.03^{d}
PL2	2.07 ± 0.05^{c}	1.44 ± 0.02^{d}	2.03 ± 0.03^{d}	1.84 ± 0.03^{d}
PL3	1.53 ± 0.06^{a}	1.28 ± 0.02^{c}	1.79 ± 0.03^{c}	1.53 ± 0.03^{c}
Naked-neck	2.07 ± 0.07^{c}	1.38 ± 0.03^{cd}	1.86 ± 0.04^{c}	1.76 ± 0.03^{d}
RIR: B	1.67 ± 0.06^{ab}	1.04 ± 0.02^{b}	1.41 ± 0.04^{b}	1.38 ± 0.03^{b}
C	1.46 ± 0.05^{a}	0.97 ± 0.02^{a}	1.23 ± 0.03^{a}	1.22 ± 0.03^{a}
WLh Pure avr.	2.03 ± 0.03^{B}	1.14 ± 0.06^{B}	1.95 ± 0.02^{B}	1.79 ± 0.02^{B}
RIR Pure avr.	1.56 ± 0.04^{A}	1.00 ± 0.01^{A}	1.31 ± 0.02^{A}	1.29 ± 0.02^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	1.90 ± 0.04^{P}	1.27± 0.02	1.76 ± 0.03^{Q}	1.64 ± 0.03^{Q}
Breed Cross*PL2♂xRIR-B♀	2.05 ± 0.05^{Q}	1.23± 0.03	1.59 ± 0.04^{P}	1.62 ± 0.02^{P}
Hetrosis %	7.89	-3.14	-9.65	-1.21
Parental strain avr. (RIR-B and RIR-C)	1.56± 0.04	1.00± 0.01	1.31± 0.02	1.29± 0.02
StrainCross*RIR-B♂xRIR-C♀	1.59± 0.06	1.00± 0.02	1.33± 0.03	1.30± 0.03
Hetrosis %	1.92	0	1.52	0.77
EINDEX (g/d/Kg)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Age\Stage Pooled**
Genetic group				
WLh: PL1	28.8 ± 0.71^{c}	33.5 ± 0.68^{c}	34.1 ± 0.74^{cd}	32.0 ± 0.45^{d}
PL2	29.0 ± 0.61^{c}	34.4 ± 0.57^{c}	36.3 ± 0.64^{d}	33.1 ± 0.38^{d}
PL3	27.3 ± 0.66^{bc}	30.4 ± 0.60^{b}	32.0 ± 0.65^{c}	29.9 ± 0.40^{c}
Naked-neck	29.03 ± 0.77^{c}	32.8 ± 0.71^{bc}	33.2 ± 0.83^{c}	31.6 ± 0.48^{cd}
RIR: B	24.6 ± 0.71^{b}	24.8 ± 0.06^{a}	25.2 ± 0.73^{b}	24.9 ± 0.44^{b}
C	21.9 ± 0.65^{a}	23.1 ± 0.62^{a}	22.0 ± 0.68^{a}	22.3 ± 0.41^{a}
WLh Pure avr.	$28.99 \pm .392^{B}$	33.73 ± 0.385^{B}	34.8 ± 0.42^{B}	32.4 ± 0.25^{B}
RIR Pure avr.	23.22 ± 0.471^{A}	23.89 ± 0.474^{A}	23.5 ± 0.49^{A}	23.5 ± 0.30^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	27.17± 0.48	30.45± 0.54	31.53 ± 0.67^{Q}	29.6 ± 0.34^{Q}
Breed Cross PL2♂xRIR-B♀	26.25± 0.64	29.36± 0.72	28.51 ± 0.85^{P}	28.0 ± 0.44^{P}
Hetrosis %	-3.38	-3.57	-9.57	-5.4
Parental strain avr. (RIR-B and RIR-C)	23.22± 0.39	23.89± 0.35	23.50± 0.39	23.5± 0.32
StrainCross RIR-B♂xRIR-C♀	22.68± 0.61	23.93± 0.52	23.79± 0.57	23.4± 0.22
Hetrosis %	2.32	0.16	1.23	-0.42

Figures bearing different superscripts within a column differ significantly ($p \le 0.05$).

Comparison of the two pure strains of WLh viz; PL1 and PL2 indicated the absence of significant difference between the two strains for egg number (EN), egg weight (EW), rate of laying (ROL), egg mass (EM), egg mass per functional day (EMFD), egg production efficiency (EPE), efficiency index (EINDEX), egg weight percentage (EWTP).

The mean (±SE) for various feed efficiency traits of different genetic groups during the three different periods are presented in Table 9 to 10. Feed consumption was significant different between the two strains during the 30-36 weeks period though PL2 had higher feed consumption during all age periods with the exception of 36-44 weeks period. There was no significant difference for feed efficiency per dozen eggs (FEDZ) but for feed efficiency per egg mass (FEEM) significant difference was observed for ASM to 30 weeks period only.

The control line, PL3 has been derived from the selected strain PL2. The selected strain PL2 had significantly higher egg number than the control during the first phase of laying period though numerically it had higher egg production in each of the three periods. The cumulative egg production upto 40 weeks of age of the PL2 was significantly higher than of the control line. The rate of lay and cumulative egg production upto 44 weeks of age of the selected strain PL2 and the control was similar (Table 6). The genetic improvement in egg number upto 40 weeks of age though combined selection in PL2 has thus resulted from a reduction in age at sexual maturity. Since reduction in ASM below certain age may not be desirable, further selection for improvement of egg number in PL2 should be based on either the rate

Table 9: Means (±SE) for EWTP and Feed consumption of different genetic groups

EWTP (%)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Age\Stage Pooled**
Genetic group				
WLh: PL1	4.11± 0.07^{bc}	3.82± 0.05^{c}	4.77± 0.09^{c}	4.22± 0.05^{d}
PL2	4.29± 0.06^{c}	3.91± 0.05^{c}	4.85± 0.07^{c}	4.33± 0.04^{d}
PL3	3.87± 0.06^{b}	3.51± 0.05^{b}	4.31± 0.07^{b}	3.89± 0.04^{c}
Naked-neck	4.20± 0.08^{c}	3.92± 0.06^{c}	4.71± 0.10^{c}	4.24± 0.05^{d}
RIR: B	3.41± 0.07^{a}	3.06± 0.05^{a}	3.40± 0.08^{a}	3.29± 0.05^{b}
C	3.18± 0.06^{a}	2.89± 0.05^{a}	3.10± 0.08^{a}	3.06± 0.04^{a}
WLh Pure avr.	4.21± 0.04^{B}	3.88± 0.03^{B}	4.79±0.05^{B}	4.27± 0.02^{B}
RIR Pure avr.	3.29± 0.05^{A}	2.97± 0.04^{A}	3.24± 0.06^{A}	3.16± 0.03^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	3.91± 0.06^{Q}	3.56± 0.04^{Q}	4.22± 0.08^{Q}	3.89± 0.03^{Q}
Breed Cross PL2♂xRIR-B♀	3.56± 0.08^{P}	3.34± 0.06^{P}	3.70± 0.10^{P}	3.53± 0.05^{P}
Hetrosis %	-8.95	-5.61	-12.32	-9.25
Parental strain avr. (RIR-B and RIR-C)	3.29± 0.05	2.97± 0.03	3.24± 0.04	3.16± 0.02
StrainCross RIR-B♂xRIR-C♀	3.20± 0.08	2.99± 0.04	3.27± 0.06	3.15± 0.04
Hetrosis %	-2.73	0.67	0.92	-0.31
FC (g)				
	Up to 30 wks	**30-36 wks**	**36-44 wks**	**Age\Stage Pooled**
Genetic group				
WLh: PL1	109.7± 2.65^{ab}	112.2±.94^{cd}	115.5± 3.51^{a}	112.3± 1.89^{abc}
PL2	117.4± 2.27^{b}	124.8± 2.46^{e}	112.7± 3.03^{a}	118.5± 1.61^{c}
PL3	107.8± 2.44^{a}	104.9±2.59^{bc}	112.8± 3.06^{ab}	108.4± 1.68^{a}
Naked-neck	109.2± 2.84^{ab}	119.0±3.06^{de}	128.0± 3.92^{bc}	118.2± 2.03^{c}
RIR: B	110.4± 2.62^{ab}	108.2±2.94^{bcd}	129.7± 3.46^{c}	115.8± 1.87^{bc}
C	105.1± 2.41^{a}	97.7± 2.67^{a}	130.7± 3.20^{c}	110.6±1.72^{ab}
WLh Pure avr.	112.8± 1.52^{B}	119.4± 1.66	117.50±2.07^{A}	116.5± 1.08^{B}
RIR Pure avr.	107.6± 1.83^{A}	102.4± 2.05	130.32±2.46^{B}	112.9± 1.31^{A}
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B))	114.4± 1.74^{P}	117.9± 2.08	120.1± 2.21^{P}	117.4± 1.19^{P}
Breed Cross PL2♂xRIR-B♀	120.4± 2.31^{Q}	124.6± 2.74	138.9± 2.82^{Q}	127.8± 1.56^{Q}
Hetrosis %	5.24	5.68	15.65	8.85
Parental strain avr. (RIR-B and RIR-C)	107.6± 2.01	102.4± 2.30^{X}	130.3± 2.58	112.9± 1.51
StrainCross RIR-B♂xRIR-C♀	107.6± 3.08	112.1± 3.34^{Y}	134.1± 3.70	118.0± 2.23
Hetrosis %	0	9.47	2.91	4.51

Figures bearing different superscripts within a column differ significantly p≤0.05).

Table 10: Means (± SE) for FEDZ and FEEM of different genetic groups

FEDZ (Kg)				
	Up to 30 wks	30-36 wks	36-44 wks	Age\Stage Pooled
Genetic group				
WLh: PL1	1.89± 0.06[ab]	1.54± 0.04[ab]	1.78± 0.06[ab]	1.74± 0.04[ab]
PL2	2.13± 0.05[b]	1.72± 0.04[b]	1.68± 0.05[a]	1.85± 0.03[b]
PL3	1.88± 0.06[a]	1.45± 0.04[a]	1.73± 0.05[ab]	1.68± 0.03[a]
Naked-neck	1.92± 0.07[ab]	1.73± 0.05[b]	1.98± 0.07[bc]	1.87± 0.04[b]
RIR: B	1.83± 0.06[a]	1.61± 0.04[ab]	2.06± 0.06[c]	1.83± 0.03[b]
C	1.87± 0.06[a]	1.47± 0.04[a]	2.21± 0.06[c]	1.84± 0.03[b]
WLh Pure avr.	2.00± 0.03[B]	1.67± 0.02[B]	1.79± 0.03[A]	1.82± 0.02
RIR Pure avr.	1.85± 0.04[A]	1.53± 0.03[A]	2.14± 0.04[B]	1.83± 0.02
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	2.00±0.04	1.67± 0.03	1.85± 0.04[P]	1.84± 0.02[P]
Breed Cross PL2♂xRIR-B♀	1.98±0.04	1.70± 0.04	2.09± 0.05[Q]	1.92± 0.03[Q]
Hetrosis %	-1	1.79	12.97	4.34
Parental strain avr. (RIR-B and RIR-C)	1.85± 0.04	1.53± 0.04[X]	2.14± 0.04	1.83± 0.03
StrainCross RIR-B♂xRIR-C♀	1.85± 0.04	1.74± 0.06[Y]	2.15± 0.07	1.91± 0.04
Hetrosis %	0	13.72	0.46	4.37
FEEM (Kg)				
	Up to 30 wks	30-36 wks	36-44 wks	Age\Stage Pooled
Genetic group				
WLh: PL1	2.95± 0.10[a]	2.33± 0.07[a]	2.56± 0.09[ab]	2.62± 0.06[a]
PL2	3.35± 0.09[b]	2.58± 0.06[ab]	2.41± 0.08[a]	2.79± 0.05[ab]
PL3	3.18± 0.09[ab]	2.39± 0.06[ab]	2.68± 0.08[ab]	2.74± 0.05[a]
Naked-neck	2.97± 0.11[ab]	2.59± 0.07[ab]	2.90± 0.10[bc]	2.81± 0.06[ab]
RIR: B	3.18± 0.09[ab]	2.64± 0.07[b]	3.15± 0.09[cd]	2.99± 0.06[b]
C	3.18± 0.09[ab]	2.40± 0.06[ab]	3.45± 0.08[d]	3.00± 0.05[b]
WLh Pure av.	3.12± 0.05	2.51±0.04	2.58± 0.05[A]	2.74± 0.03[A]
RIR Pure avr.	3.18± 0.07	2.50±0.05	3.31± 0.06B	2.99± 0.04[B]
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	3.27±0.06	2.60± 0.04	2.73± 0.06[P]	2.87± 0.03
Breed Cross PL2♂ x RIR-B♀	3.15±0.08	2.59± 0.06	3.12± 0.08[Q]	2.95± 0.05
Hetrosis %	3.66	-0.38	14.28	2.78
Parental strain avr. (RIR-B and RIR-C)	3.19± 0.11	2.50±0.07	3.45± 0.11	2.99± 0.07
StrainCross RIR-B♂ x RIR-C♀	3.18± 0.07	2.81±0.11	3.31± 0.07	3.15± 0.04
Hetrosis %	-0.31	12.4	-4.05	5.35

Figures bearing different superscripts within a column differ significantly p≤0.05).
: a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison
WLh Pure avr. refers to average of PL1, PL2 and Naked-neck
RIR Pure avr. refers to average of RIR-B and RIR-C

of lay or egg number from 30 to 40 weeks of age. Strain PL2 had significantly higher egg weight during the three age periods than the control implying that moderate selection practiced for egg weight during 36-40 weeks of age concomitantly led genetic improvement at all the ages. Strain PL2 also had significantly higher value for EM, EPE, EINDEX, EWTP, during three-age period. For EMFD, FC and FEDZ, PL2 had higher value during all age period except up to 30, 30-36, 36-44 week age period respectively; these observations showed that selection for egg number and egg weight has resulted in genetic improvement in all the components of egg productivity.

The naked-neck and the PL2 strain did not differ significantly from each other for some production traits but for some trait EM, EPE, EINDEX, FC, FEDZ, FEEM, they showed significant difference during 36-44 weeks of age period only.

Of the two RIR strain, RIR-B had better performance for derived egg production traits as compared to RIR-C. RIR-B had significantly higher performance for EPE; EINDEX during all age period except up to 30 weeks and 30-36 weeks age period respectively, while for some traits like EM, EWTP and FC, RIR-B had higher value up to 30 weeks and on stage pooled basis. Actual feed consumption (g) obtained in present study for White Leghorn and Rhode Island Red strains is in accordance with that reported by Sazzad (1992) and Huque *et al* (1999). Rizzi and Cheiricato (2005) had also reported the improvement of egg production with advancement of age in four lines of laying chicken.

A comparison of selected White Leghorn pure strain (average of the PL1, PL2 and Naked-neck) with the average of the RIR strain, showed significant differences for production traits during all age periods. Pure WLh had higher egg production and ROL except up to 30 weeks age period as compared to pure RIR. Pure RIR had higher EW, BW, and EM during all the age periods. Pure WLh had higher EMFD, EPE, and EINDEX than the pure RIR during all age periods. Among feed efficiency traits FC, FEDZ and FEEM, pure WLh had higher performance up to 30 weeks and 30-36 weeks age period, later on pure RIR showed higher performance.

The comparisons in between breed cross PL2♂ X RIR-B♀, cross; in general, showed higher performance than the parental average but difference was not consistent during all the age periods. During 36-44 weeks of age period, no differences for EN, ROL, EM and EMFD were observed between them.

Parental strain average RIR-B and RIR-C had non-significant difference than their cross RIR-B♂ x RIR-C♀ for egg production derived traits but in case of feed efficiency traits, cross had significantly higher FC, FEDZ and FEEM during 30-36 weeks age period.

4.3 EGG QUALITY AND COMPOSITION TRAITS

Egg quality and composition traits for different stocks were evaluated at 30, 36 and 44 weeks of age. The means (± SE) of exterior egg traits at different ages are presented in Table11. On genetic-group pooled basis, the egg weight increased from 30 weeks (51.5g) upto 44 weeks of age (55.5g). The differences between the ages were

Table 11: Mean (±SE) of different exterior egg trait of different genetic groups at different age

Age (wk)	Egg weight (g)	Egg length (cm)	Egg width (cm)	Shape index (%)	Shell colour	Specific gravity
30	$51.1±0.21^{a}_{(7.80)}$	$5.47±0.01^{a}_{(3.98)}$	$3.97±0.006^{a}_{(3.38)}$	$72.8±0.16_{(4.27)}$	2.89±0.004	$99.81±0.33^{b}_{(6.38)}$
36	$53.63±0.22^{b}_{(7.82)}$	$5.54±0.01^{b}_{(4.15)}$	$4.04±0.007^{b}_{(3.30)}$	$72.9±0.17_{(4.53)}$	2.81±0.048	$99.63±0.34^{b}_{(6.13)}$
44	$55.51±0.23^{c}_{(7.89)}$	$5.60±0.01^{c}_{(4.19)}$	$4.06±0.007^{b}_{(3.47)}$	$72.6±0.17_{(4.50)}$	2.78±0.050	$97.62±0.35^{a}_{(7.38)}$
Genetic group:						
WLh: PL1	$55.65±0.37^{B}_{(6.45)}$	$5.59±0.02^{C}_{(3.85)}$	$4.10±0.011^{C}_{(2.95)}$	$73.44±0.29^{CD}_{(4.79)}$	$1.04±0.084^{A}$	$96.01±0.60^{A}_{(5.67)}$
PL2	$55.79±0.32^{B}_{(7.37)}$	$5.68±0.01^{D}_{(3.66)}$	$4.05±0.009^{B}_{(3.06)}$	$71.37±0.24^{A}_{(4.06)}$	$1.0±0.070^{A}$	$98.97±0.50^{CD}_{(6.81)}$
PL3	$51.62±0.33^{A}_{(7.85)}$	$5.48±0.01^{B}_{(3.93)}$	$3.97±0.010^{A}_{(3.14)}$	$72.53±0.25^{BC}_{(3.99)}$	$1.05±0.074^{A}$	$100.59±0.52^{D}_{(6.08)}$
Naked	$55.85±0.39^{B}_{(6.96)}$	$5.71±0.02^{D}_{(3.54)}$	$4.08±0.012^{BC}_{(3.02)}$	$71.48±0.30^{AB}_{(4.29)}$	$1.24±0.087^{A}$	$98.63±0.62^{BCD}_{(6.44)}$
RIR: B	$51.24±0.35^{A}_{(8.84)}$	$5.48±0.02^{AB}_{(3.90)}$	$3.98±0.014^{A}_{(4.06)}$	$72.74±0.27^{C}_{(4.48)}$	$5.69±0.079^{B}$	$96.61±0.57^{AB}_{(6.78)}$
C	$51.17±0.34^{A}_{(8.22)}$	$5.41±0.01^{A}_{(3.86)}$	$3.99±0.012^{A}_{(3.51)}$	$73.84±0.26^{D}_{(4.17)}$	$5±0.076^{C}$	$98.36±0.55^{BC}_{(7.40)}$
Age x group Interaction	**NS**	**NS**	**NS**	**NS**	**NS**	*
WLh Pure avr.	$55.76±0.18^{Y}_{(6.99)}$	$5.66±0.10^{Y}_{(3.78)}$	$4.07±0.006^{Y}_{(3.06)}$	$72.02±0.16^{X}_{(4.55)}$	$1.08±0.050^{X}$	$97.99±0.33_{(6.53)}$
RIR Pure avr.	$51.20±0.22^{X}_{(8.52)}$	$5.44±0.12^{X}_{(3.93)}$	$3.98±0.006^{X}_{(3.77)}$	$73.31±0.20^{Y}_{(4.38)}$	$5.33±0.062^{Y}$	$97.52±0.44_{(7.17)}$
Age x group Interaction	**NS**	*	**NS**	**NS**	**NS**	**NS**
Pure vs. cross comparison:						
Parental breed avr. (PL2 and RIR-B)	$53.79±0.23_{(9.03)}$	$5.59±0.01_{(4.18)}$	$4.02±0.007^{P}_{(3.62)}$	$71.97±0.18^{P}_{(4.36)}$	$3.06±0.124^{Q}$	$97.93±0.37^{P}_{(6.90)}$
Breed Cross PL2♂xRIR-B♀	$54.67±0.32_{(6.65)}$	$5.56±0.02_{(3.33)}$	$4.06±0.01^{Q}_{(3.12)}$	$73.11±0..25^{Q}_{(4.36)}$	$2.28±0.167^{P}$	$101.12±0.51^{Q}_{(6.21)}$
Age x group Interaction	**NS**	**NS**	**NS**	**NS**	**NS**	*
Hetrosis %	1.6	-0.53	0.9	1.58	-25.49	3.25
Parental strain avr. (RIR-B and RIR-C)	$51.20±0.23_{(8.52)}$	$5.44±0.012^{N}_{(3.93)}$	$3.98±0.008_{(3.77)}$	$73.31±0.19_{(4.38)}$	$5.33±0.090^{M}$	$97.52±0.40^{M}_{(7.17)}$
StrainCross RIR-B♂xRIR-C♀	$50.61±0.33_{(7.89)}$	$5.38±0.017^{M}_{(3.55)}$	$3.97±0.012_{(3.36)}$	$73.94±0.27_{(3.93)}$	$5.69±0.135^{N}$	$101.08±0.58^{N}_{(5.93)}$
Age x group Interaction	*	*	**NS**	**NS**	**NS**	**NS**
Hetrosis %	-1.15	-1.1	0.25	0.85	6.75	3.65

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$) ; a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison; WLh Pure avr. refers to average of PL1, PL2 and Naked-neck; RIR Pure avr. refers to average of RIR-B and RIR-C; Figure in the parentheses are the coefficients of variation (%)

statistically significant ($p \leq 0.05$). The increase in egg weight from 30 to 44 weeks of age may be partially due to the increase in body weight between these ages. Egg length and width also increased significantly with age. However, shape index did not depict any significant change. Egg shell colour also did not change with age of birds. Egg specific gravity (SG), which is the indirect measure of egg shell quality, remained unchanged from 30 to 36 weeks of age but declined significantly thereafter. The decrease in egg specific gravity may be partially due to the increase in egg weight during the corresponding period and partially to increased rate of lay during the later period. The birds may not be able to mobilize sufficient calcium for high egg production. Ledur *et al* (2002) had also reported increase in egg quality traits with advancement of age. Premavalli and Vishwanathan (2004) had also reported significant increase in egg weight, shape index and yolk colour, while egg specific gravity, shell thickness, Haugh Unit Score (%) and yolk index decreased significantly with advancement of age.

The differences between the two selected strains of White Leghorn, PL1 and PL2 were non-significant for egg weight. But PL2 had higher length whereas PL1 had higher width of eggs. Shape index of PL1 was significantly higher than that of PL2 implying that eggs of PL2 were rounder. Egg shell quality, as determined by specific gravity, of PL2 was significantly better than of PL1. The egg production and egg weight of strains PL1 and PL2 were similar. But the existence of differences between these two strains for specific gravity indicates that

a part of variability between the two strains for specific gravity is independent of the variation in egg production and egg weight.

The selected strain PL2 had significantly higher egg weight but lower length, width and shape index than its unselected counterpart, the control line (PL3). The specific gravity of PL2 was lower than the control but the difference was non-significant. Thus selection for egg production and egg weight has led to increase in egg weight, length and width but declined shell quality. There were no differences between the naked-neck and the PL2 strains for any of the exterior egg quality traits. Of the two RIR strains RIR-C had significantly higher shape index but lower tinting of eggs as compared to RIR-B. Differences for egg weight; length and width among layer strains were also reported in studies by Singh *et al* (2000) and Fayeye *et al (*2005).

The age X strain interaction was significant for egg specific gravity (Fig.1). A comparison of WLh with RIR showed that WLh had significantly higher egg weight but lower shape index. The yolk colour and specific gravity did not differ between the breeds. The egg weights observed in this study are in fair accord with those reported by Bish *et al*, 1985; Brah *et al*, 2002; Sethi *et al*, 2003; Brah and Chaudhary 2004 and Jilani *et al,* 2005 for White Leghorn and Rhode Island Red strains. Specific gravity as a measure of egg shell quality had been reported in the studies by Brah and Chaudhary 2004; Devi and Reddy 2005. Brah and Chaudhary (2004) had observed significant strain differences for the egg specific gravity while Devi and Reddy (2005) had observed

non-significant strain differences for the egg specific gravity among White Leghorn strains.

The cross between WLh and RIR did not differ significantly from the parental average for egg weight and egg length. However, shape index and specific gravity of the cross were better than the average of the parental breeds implying significant and desirable hetrosis. The cross between the RIR strains had significant superiority over the strain average for egg shell colour and specific gravity.

The means (±SE) for albumen quality and yolk quality are presented in Table 12. The albumen height and HUS increased significantly from 30 to 36 weeks of age but declined significantly from 36 to 44 weeks of age. Higher values of Haugh Unit Score (%) at 36 weeks indicated that the eggs have good albumen quality during the mid period of laying but as the production increased with age, the albumen quality started deteriorating. The absence of age x genetic group interaction for albumen quality meant that different genetic groups showed the same magnitude of change with age. The coefficients of variation for albumen height were more than for HUS. This may be because albumen height is also influenced by egg weight but HUS is independent of egg weight. There were significant age differences in height and diameter of yolk as well as for yolk index. Yolk height increased consistently from 30 to 44 weeks of age. This increase in yolk height with age corresponded with the concomitant increase in weight, length and width of eggs. Yolk diameter increased significantly from 30 to 36 weeks but not thereafter. The increase in yolk

Table 12: Mean (±SE) for interior egg quality trait of different genetic groups at different ages

Age (wk)	Albumen height	HUS(%)	Yolk height(mm)	Yolk diameter(mm)	Yolk index(%)
30	6.8±0.053^{b} $_{(17.22)}$	84.4± .32^{b} $_{(8.08)}$	15.96±0.03^{a} $_{(4.44)}$	36.4±0.07^{a} $_{(3.83)}$	43.84±0.07^{a} $_{(6.14)}$
36	7.0±0.055^{c} $_{(14.96)}$	85.3±0.33^{b} $_{(7.28)}$	17.08±0.04^{b} $_{(4.53)}$	36.8±0.07^{b} $_{(3.73)}$	46.41±0.07^{b} $_{(5.86)}$
44	6.2±0.057^{a} $_{(13.88)}$	79.3±0.34^{a} $_{(7.30)}$	17.40±0.04^{c} $_{(4.34)}$	36.9±0.07^{b} $_{(3.53)}$	47.15±0.07^{c} $_{(5.59)}$
Genetic groups:					
WLh: PL1	7.1±0.09^{D} $_{(16.68)}$	84.7±0.61^{C} $_{(8.46)}$	16.62±0.09^{ABC} $_{(5.59)}$	36.7±0.01^{BC} $_{(3.73)}$	45.28±0.13^{BC} $_{(7.04)}$
PL2	6.6±0.08^{BC} $_{(15.67)}$	81.6±0.52^{B} $_{(8.21)}$	16.81±0.07^{BC} $_{(5.48)}$	37.0±0.01^{CD} $_{(4.07)}$	45.43±0.11^{BCD} $_{(6.84)}$
PL3	6.0±0.087^{A} $_{(15.55)}$	79.4±0.54^{A} $_{(7.87)}$	16.46±0.08^{A} $_{(6.39)}$	36.8±0.01^{BC} $_{(3.33)}$	44.72±0.12^{AB} $_{(6.78)}$
Naked	6.8±0.103^{CD} $_{(15.81)}$	83.4±0.64^{BC} $_{(7.89)}$	16.50±0.09^{AB} $_{(5.54)}$	37.3±0.01^{D} $_{(2.92)}$	44.23±0.13^{A} $_{(6.39)}$
RIR: B	6.4±0.09^{B} $_{(15.61)}$	82.3±0.58^{B} $_{(8.01)}$	16.83±0.08^{BC} $_{(5.26)}$	36.4±0.01^{AB} $_{(3.93)}$	46.23±0.12^{CD} $_{(5.89)}$
C	6.9±0.09^{D} $_{(15.64)}$	85.5±0.56^{D} $_{(7.41)}$	16.90±0.08^{C} $_{(5.21)}$	36.2±0.01^{A} $_{(3.87)}$	46.68±0.12^{D} $_{(5.97)}$
Age x group Interaction	**NS**	**NS**	*	*	*
WLh Pure avr.	6.8±0.05 $_{(16.39)}$	83.08±0.32 $_{(8.36)}$	16.62±0.03^{X} $_{(5.58)}$	37.4±0.01^{Y} $_{(3.77)}$	44.43±0.06^{X} $_{(6.87)}$
RIR Pure avr.	6.7±0.06 $_{(16.09)}$	83.97±0.39 $_{(7.93)}$	16.87±0.04^{Y} $_{(5.24)}$	36.3±0.01^{X} $_{(3.91)}$	46.47±0.07^{Y} $_{(5.95)}$
Age x group Interaction	**NS**	**NS**	**NS**	*	*
Pure vs. cross comparison:					
Parental breed avr. (PL2 and RIR-B)	6.55±0.06^{P} $_{(15.68)}$	81.9±0.36^{P} $_{(8.13)}$	16.82±0.05^{P} $_{(5.38)}$	36.7±0.01 $_{(4.08)}$	45.83±0.08^{P} $_{(6.51)}$
Breed Cross PL2♂xRIR-B♀	6.87±0.08^{Q} $_{(15.62)}$	83.7±0.49^{Q} $_{(7.94)}$	17.26±0.07^{Q} $_{(5.15)}$	36.8±0.01 $_{(3.56)}$	46.90±0.10^{Q} $_{(5.89)}$
Age x group Interaction	**NS**	**NS**	**NS**	**NS**	**NS**
Hetrosis %	4.88	2.2	2.61	5.17	-2.55
Parental strain avr. (RIR-B and RIR-C)	6.74±0.06 $_{(16.09)}$	83.97±0.37 $_{(7.93)}$	16.87±0.05 $_{(5.24)}$	36.3±0.01 $_{(3.91)}$	46.47±0.07 $_{(5.95)}$
StrainCross RIR-B♂xRIR-C♀	6.86±0.09 $_{(15.06)}$	84.97±0.54 $_{(7.15)}$	16.79±0.08 $_{(5.89)}$	36.4±0.01 $_{(3.64)}$	46.12±0.11 $_{(5.80)}$
Age x group Interaction	**NS**	**NS**	*	**NS**	**NS**
Hetrosis %	1.78	1.19	-0.47	0.27	0.74

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$) ; a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison; WLh Pure avr. refers to average of PL1, PL2 and Naked-neck; RIR Pure avr. refers to average of RIR-B and RIR-C; Figure in the parentheses are the coefficients of variation (%)

height without a concomitant increase in yolk diameter is reflected in a significant increase in yolk index with age.

The WLh selected strains, PL1 and PL2, despite having similar egg production and egg weight, differed significantly from each other for albumen quality with PL2 having poorer albumen quality as measured by albumen height and HUS. Yolk height, diameter and yolk index of the two strains were, however, similar. The selected strain PL2 had significantly better albumen quality and yolk height than its unselected counterpart, the control strain (PL3). Selection for egg production and egg weight in PL2 has thus resulted in desirable genetic changes in albumen quality and yolk height as correlated responses.

A comparison of the naked-neck line with PL2 showed that naked-neck allele did not affect any of the albumen and yolk quality traits. Of the two strains of RIR, strain RIR-C had significantly better albumen quality though egg weight and egg production of both the strains were similar. The presence of significant between-strain differences within breeds for albumen quality despite the lack of differences in egg production and egg weight between the strains indicated that a portion of the variation between strains for albumen quality is independent of egg production and egg weight. The two RIR strains did not differ for yolk quality traits. The age x genetic group interaction was significant for yolk traits (Fig. 2 to 4). The observed differences between WLh and RIR were non-significant for albumen quality but were significant for yolk traits. The differences between WLh and RIR were in consistent for different attributes of yolk.

The cross between WLh and RIR had significantly better albumen quality than the average of parental strains. The breed cross, PL2♂ x RIR-B♀, had significantly higher yolk height and yolk index than the parental average. The cross between the strains RIR-B and RIR-C did not differ from the respective pure average.

Ashraf *et al* (2003) compared egg quality characteristics of Lyallpur Silver Black (LSR) with Rhode Island Red (RIR) breeds of poultry in Pakistan and reported non-significant differences in Haugh Unit Score (%) and yolk index.

Shell thickness declined from 30 to 44 weeks of age (Table 13). Among all the strains PL3 has thickest shells (39.38mm) while RIR-B had thinnest shells (36.9mm). There were non-significant differences between PL2 and control for shell thickness while PL2 differed significantly from PL1. Pure While Leghorn strains had better shell thickness as compared to RIR strains. Devi and Reddy (2004) had also compared the shell thickness of White Leghorn strains with the control line to study the effect of selection and had observed significant strain differences for shell thickness.

Mean for absolute weight of different egg components are presented in Table 13. The increase in egg weight with age occurred due to significant increase in weight of yolk, albumen and shell. The coefficients of variation did not show any change with age. There were significant differences among genetic groups for yolk weight, albumen weight and shell weight. Strain PL2 had higher yolk weight, albumen weight and shell weight as compared to the unselected control line

Table 13: Mean (±SE) for weight of different egg components for different genetic groups at different ages

Age (wk)	Egg weight(g)	Yolk weight(g)	Albumen weight(g)	Shell weight(g)	Yolk colour
30	$51.06\pm0.21^{a}_{(7.80)}$	$14.21\pm0.06^{a}_{(7.69)}$	$32.10\pm0.17^{a}_{(10.22)}$	$4.73\pm0.03^{a}_{(9.92)}$	3.23 ± 0.033^{a}
36	$53.63\pm0.22^{b}_{(7.82)}$	$15.69\pm0.06^{b}_{(7.34)}$	$32.87\pm0.17^{b}_{(10.05)}$	$5.05\pm0.03^{b}_{(10.82)}$	2.10 ± 0.034^{b}
44	$55.51\pm0.23^{c}_{(7.89)}$	$15.96\pm0.06^{c}_{(7.69)}$	$34.23\pm0.18^{c}_{(10.28)}$	$5.31\pm0.03^{c}_{(10.55)}$	11.31 ± 0.035^{c}
Genetic groups:					
WLh: PL1	$55.65\pm0.37^{B}_{(6.45)}$	$15.33\pm0.12^{BC}_{(8.73)}$	$35.26\pm0.28^{B}_{(8.14)}$	$5.06\pm0.05^{BC}_{(9.92)}$	5.30±0.38
PL2	$55.79\pm0.32^{B}_{(7.37)}$	$15.53\pm0.10^{C}_{(8.11)}$	$35.0\pm0.23^{B}_{(8.16)}$	$5.2\pm0.04^{D}_{(10.13)}$	5.53±0.32
PL3	$51.62\pm0.33^{A}_{(7.85)}$	$14.81\pm0.11^{A}_{(8.52)}$	$31.80\pm0.24^{A}_{(9.52)}$	$5.0\pm0.04^{B}_{(10.62)}$	5.27±0.33
Naked	$55.85\pm0.39^{B}_{(6.96)}$	$15.46\pm0.13^{BC}_{(7.04)}$	$35.17\pm0.30^{B}_{(9.02)}$	$5.21\pm0.05^{CD}_{(11.13)}$	4.90±0.39
RIR: B	$51.24\pm0.35^{A}_{(8.84)}$	$15.02\pm0.12^{AB}_{(10.43)}$	$31.55\pm0.26^{A}_{(10.46)}$	$4.65\pm0.05^{A}_{(11.64)}$	5.57±0.36
C	$51.17\pm0.34^{A}_{(8.22)}$	$15.17\pm0.11^{ABC}_{(10.43)}$	$31.18\pm0.25^{A}_{(9.43)}$	$4.81\pm0.45^{A}_{(12.30)}$	5.33±0.35
Age x group Interaction	**NS**	*	**NS**	**NS**	**NS**
WLh Pure avr.	$55.76\pm0.18^{Y}_{(6.99)}$	$15.45\pm0.05^{Y}_{(8.04)}$	$35.12\pm0.15^{Y}_{(8.59)}$	$5.18\pm0.02^{Y}_{(10.5)}$	5.29±0.20
RIR Pure avr.	$51.20\pm0.22^{X}_{(8.52)}$	$15.10\pm0.06^{X}_{(10.31)}$	$31.36\pm0.18^{X}_{(9.96)}$	$4.73\pm0.03^{X}_{(12.11)}$	5.44±0.25
Age x group Interaction	**NS**	*	**NS**	**NS**	**NS**
Pure vs. cross comparison:					
Parental breed avr. (PL2 and RIR-B)	$53.79\pm0.23_{(9.03)}$	$15.30\pm0.08^{P}_{(9.31)}$	$33.49\pm0.19_{(10.68)}$	$4.99\pm0.03^{P}_{(12.30)}$	5.55±0.243
Breed Cross PL2♂xRIR-B♀	$54.67\pm0.32_{(6.65)}$	$15.76\pm0.10^{Q}_{(8.26)}$	$33.61\pm0.26_{(7.99)}$	$5.29\pm0.04^{Q}_{(9.83)}$	5.63±0.327
Age x group Interaction	*	**NS**	**NS**	**NS**	**NS**
Hetrosis %	1.63	3.0	0.35	6.0	1.44
Parental strain avr. (RIR-B and RIR-C)	$51.20\pm0.22_{(8.52)}$	$15.10\pm0.06_{(10.31)}$	$31.36\pm0.18_{(9.96)}$	$4.73\pm0.03_{(12.11)}$	5.44±0.25
StrainCross RIR-B♂xRIR-C♀	$50.61\pm0.33_{(7.89)}$	$14.91\pm0.13_{(9.60)}$	$30.87\pm0.27_{(9.97)}$	$4.82\pm0.04_{(9.00)}$	5.38±0.365
Age x group Interaction	*	*	**NS**	**NS**	**NS**
Hetrosis %	-1.15	-1.25	-1.56	1.9	-1.1

Figures bearing different superscripts within a column differ significantly ($p \le 0.05$) ; a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison; WLh Pure avr. refers to average of PL1, PL2 and Naked-neck; RIR Pure avr. refers to average of RIR-B and RIR-C; Figure in the parentheses are the coefficients of variation (%)

indicating that selection in PL2 for egg weight and egg number has concomitantly lead to the improvement of yolk, albumen and shell contents of the egg also. Naked-neck line did not differ from PL2 for weight of any of the egg-components.

The two RIR strains did not differ from each other for weight of any of the egg components. White Leghorn selected strains had higher yolk weight, shell weight, albumen weight as compared to RIR strains. The cross between WLh and RIR had significantly higher weight of yolk and shell than the parental average but egg weight and albumen weight did not show any significant difference. The cross between RIR strains did not differ from their parental average for weight of any of the egg components. The values of physical egg composition traits obtained in the present study are in accord with the internal and external egg quality traits reported in different strains by various workers (Baumgartner *et al,* 1998; Devi and Reddy 2004; Fayeye *et al* 2005).

The means (±SE) for % yolk, albumen and shell for different genetic groups are presented in Table 14. The % albumen was highest at 30 weeks but declined from 30 to 36 weeks and remained stable thereafter. The % yolk and shell were lowest at 30 weeks. The changes at other stages in % yolk and shell were inconsistent.

The differences between WLh strains, PL1 and PL2, were significant for % shell and shell thickness. The superiority of PL2 over PL1 for these attributes of shell quality and strength is in concord with difference observed for specific gravity between the two strains. The

Table 14: Mean (±SE) for % yolk, albumen shell and shell thickness of different genetic groups at different ages

Age (wk)	% Yolk	% Albumen	%Shell	Shell thickness(mm)
30	27.93±0.10^{a} (7.6)	62.77±0.12^{b} (3.72)	9.29±0.04^{a} (7.67)	37.19±0.14^{a} (7.19)
36	29.36±0.10^{c} (6.98)	61.19±0.11^{a} (3.52)	9.43±0.04^{ab} (7.89)	38.53±0.15^{b} (7.86)
44	28.85±0.11^{b} (7.32)	61.55±0.12^{a} (3.72)	9.59±0.04^{b} (8.34)	40.14±0.14^{c} (6.60)
Genetic groups:				
WLh: PL1	27.59±0.19^{A} (7.65)	63.29±0.20^{B} (3.78)	9.10±0.07^{A} (8.56)	37.51±0.27^{AB} (7.40)
PL2	27.87±0.12^{A} (5.90)	62.68±0.17^{B} (2.94)	9.44±0.05^{B} (7.39)	38.97±0.22^{C} (6.31)
PL3	28.76±0.16^{B} (7.07)	61.54±0.18^{A} (3.47)	9.69±0.06^{C} (6.74)	39.38±0.23^{C} (6.84)
Naked	27.76±0.20^{A} (7.19)	62.88±0.21^{B} (3.44)	9.34±0.07^{AB} (7.93)	39.02±0.28^{C} (8.14)
RIR: B	29.37±0.18^{BC} (7.69)	61.53±0.19^{A} (3.92)	9.09±0.06^{A} (8.21)	36.90±0.25^{A} (8.06)
C	29.68±0.17^{C} (6.82)	60.90±0.19^{A} (3.73)	9.41±0.06^{B} (9.39)	37.91±0.24^{B} (9.00)
Age x group Interaction	**NS**	**NS**	**NS**	**NS**
WLh Pure avr.	27.76±0.09^{X} (6.83)	62.92±0.10^{Y} (3.38)	9.31±0.03 (8.04)	38.54±0.14^{Y} (7.51)
RIR Pure avr.	29.53±0.11^{Y} (7.26)	61.20±0.13^{X} (3.86)	9.26±0.04 (9.03)	37.42±0.17^{X} (8.68)
Age x group Interaction	**NS**	**NS**	**NS**	**NS**
Pure vs. cross comparison:				
Parental breed avr. (PL2 and RIR-B)	28.53±0.12 (7.27)	62.17±0.12^{Q} (3.52)	9.29±0.04^{P} (7.96)	38.05±0.17^{P} (7.74)
Breed Cross PL2♂xRIR-B♀	28.87±0.16 (6.87)	61.43±0.17^{P} (3.33)	9.68±0.05^{Q} (6.69)	39.94±0.23^{Q} (7.08)
Age x group Interaction	**NS**	**NS**	**NS**	**NS**
Hetrosis %	1.19	-1.19	4.19	4.96
Parental strain avr. (RIR-B and RIR-C)	29.53±0.11 (7.26)	61.20±0.13 (3.86)	9.26±0.04^{M} (9.03)	37.42±0.18 (8.68)
StrainCross RIR-B♂xRIR-C♀	29.52±0.19 (7.92)	60.92±0.21 (4.09)	9.54±0.07^{N} (7.32)	38.33±0.27 (6.84)
Age x group Interaction	**NS**	**NS**	**NS**	**NS**
Hetrosis %	-0.03	-0.45	3.02	2.43

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$) ; a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison; WLh Pure avr. refers to average of PL1, PL2 and Naked-neck; RIR Pure avr. refers to average of RIR-B and RIR-C; Figure in the parentheses are the coefficients of variation (%)

selected strain PL2 had significantly higher proportion of yolk and shell but lower proportion of albumen than its unselected counterpart, the control line. The naked-neck line did not differ from PL2 for any of the % egg components as well as for egg shell thickness. Of the two RIR strains, strain RIR-C had significantly higher % shell and shell thickness. The RIR breed had significantly higher % yolk than WLh, which was due to the lower % albumen of RIR. The shell thickness of WLh was significantly better than RIR.

The eggs of cross between WLh and RIR had significantly higher % shell than the parental average but the % albumen of cross was lower than pure average. The cross exceeded the pure in shell thickness. The RIR strain cross (RIR-B♂ x RIR-C♀) yielded significant superiority over the pures for % shell only. Devi and Reddy (2004) have done comparative evaluation of selected White Leghorn strains for egg quality traits and had reported significant strain differences for shell weight %.

4.4 IMMUNOCOMPETENCE TRAITS

Immune Response to Sheep Red Blood Cells: Immune response to Sheep Red Blood Cells (SRBC) was measured in terms of Haemagglutination titre (HA) at 0, 5 and 10 days post-primary inoculation (PPI) of SRBC. Haemagglutination titre gives indication of the total antibodies. The mean (±SE) for antibody (HA) titre are given in Table 15. The presence of natural antibody was evident in all the genetic groups. The mean titre on day 0 averaged to be 0.20±0.06 pooled over all the groups. The mean titers at day 0 varied from 0.13±0.06 to 0.25±0.06 across groups

but the differences among genetic groups were statistically non-significant. Kundu *et al* (1999a) also did not find significant differences among divergent chicken stocks for natural antibodies.

All groups showed HA titre increased from day 0 onward, and attained highest values on day 10 (Fig. 5). Of the WLh strains, the control line, PL3, exhibited the highest response on day 5 and 10 post inoculation. The lowest response was observed for the selected strain PL2, while the naked-neck had intermediate response. Of the Rhode Island Red strains, RIR-C showed higher response than RIR-B strain at day 5 and 10 post inoculation.

The HA titre on day 10, showed significant higher response in WLh (3.83±0.13) as compared to the RIR (3.24±0.15) and pure average PL2 and RIR-B, HA response was lower than cross of these two strain but there was no significant difference between pure average RIR-B and RIR-C strain and their cross.

The peak titre of antibodies was obtained 4-9 days post primary inoculation of Sheep Red Blood Cells in many studies by various workers (Vanderzipp and Leenstra, 1980; Ubosi *et al*, 1985; Martin *et al*, 1989; Miller *et al*, 1992).

The comparison of strains for immune response to Sheep Red Blood Cells had been done by various workers (Martin *et al,* 1989; Benda *et al,* 1990; Kundu *et al,* 1999a, 1999b; Msoffe *et al,* 2001) and they had reported significant differences between various breeds and strains for total, MER and MES titre.

Table 15: Means (±SE) for Total (HA) titre of different genetic groups at 0, 5 and 10 days post-primary inoculation

Genetic group	day 0	day 5	day10	%Non - responder
WLh :				
PL2	0.13±0.06	2.12±0.21	$3.43±0.20^{ab}$	17.2
PL3	0.17±0.06	2.93±0.22	$4.16±0.22^{b}$	28.3
Naked-neck	0.21±0.07	2.67±0.25	$3.97±0.24^{b}$	22.7
RIR :				
B	0.17±0.06	2.61±0.23	$3.00±0.20^{a}$	18.9
C	0.24±0.06	2.88±0.22	$3.52±0.26^{ab}$	12.5
WLh pure avr,	0.16±0.04	2.55±0.13	$3.83±0.13^{B}$	22.8
RIR pure avr.	0.21±0.01	2.74±0.15	$3.24±0.15^{A}$	16.0
Pure vs. cross comparison:				
Parental breed avr. PL2 and RIR-B)	0.25±0.06	2.80±0.20	$4.05±0.21^{Q}$	8.7
Breed cross PL2♂xRIR-B♀	0.15±0.04	2.36±0.15	$3.22±0.16^{P}$	18.1
Parental strain avr. RIR-B and RIR-C	0.21±0.05	2.87±0.22	3.06±0.22	7.5
Strain cross RIR-B♂xRIR-C♀	0.20±0.06	2.74±0.16	3.24±0.16	16.0

Figures bearing different superscripts within a column differ significantly ($p \leq 0.05$).

:a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison

WLh Pure avr. refers to average of PL2, PL3 and Naked-neck

RIR Pure avr. refers to average of RIR-B and RIR-C

Response to Phytohaemagglutinin (PHA-P): *In vivo* cell mediated immune response to mitogen, phytohaemagglutinin (PHA-P) was used as an indicator of general cellular immune responsiveness. The response to phytohaemagglutinin and various measurements obtained are presented in Table 16. After PHA-P inoculation the foot web thickness was highest in PL3 (Control) strain. Increase in foot web thickness due to PBS and PHA was highest in the control line and naked-neck respectively. The response calculated as difference between increase in foot web thickness due to PBS and PHA-P, was highest in strain PL3 (0.82 mm) followed by PL1, PL2, Naked, RIR-C and RIR-B. There were significant differences between strains for response to phytohaemagglutinin. The poorest response was obtained in RIR-B strain (0.62 mm). Thus the selection in White Leghorn strain resulted in reduced *in vivo* cell mediated immune response to PHA-P.

Cell mediated response to PHA-P was higher in White Leghorn selected (0.77 mm) strain as compared to RIR (0.63 mm) thus RIR had lower *in vivo* cell mediated immune responsiveness compared to White Leghorn selected strains. The *in vivo* cell mediated immune response to mitogens is studied by various workers (Parmentier *et al,* 1998a; Haunshi and Sharma 2002; Reddy *et al,* 2005). In between PL2 and RIR-B and their cross PL2♂ x RIR-B♀ and RIR-B and RIR-C and their cross RIR-B♂ x RIR-C♀, cross showed non-significant difference for foot index value.

Table 16: Means (±SE) of Foot Index, PBS and PHA response of different genetic groups

Genetic group	PBS response	PHA response	Foot Index
WLh :			
PL1	0.14±0.02	0.94 ± 0.04^{b}	0.80 ± 0.04^{cd}
PL2	0.15±0.02	0.91 ± 0.03^{b}	0.77 ± 0.03^{bcd}
PL3	0.15±0.02	0.97 ± 0.03^{b}	0.82 ± 0.03^{d}
Naked-neck	0.21±0.02	0.88 ± 0.04^{ab}	0.67 ± 0.04^{abc}
RIR :			
B	0.15±0.02	0.75 ± 0.04^{a}	0.60 ± 0.04^{a}
C	0.20±0.02	0.84 ± 0.04^{ab}	0.64 ± 0.04^{ab}
WLh Pure avr,	0.17±0.02	0.93 ± 0.02^{A}	0.77 ± 0.02^{A}
RIR Pure avr.	0.16±0.01	0.79 ± 0.03^{B}	0.62 ± 0.03^{B}
Pure vs. cross comparison:			
Parental breed avr. PL2 and RIR-B	0.15 ± 0.02^{Q}	0.85±0.03	0.71±0.03
Breed cross PL2♂xRIR-B♀	0.20 ± 0.02^{P}	0.86±0.04	0.66±0.04
Parental strain avr. RIR-B and RIR-C	0.17±0.02	0.79±0.03	0.62±0.03
Strain cross RIR-B♂xRIR-C♀	0.15±0.02	0.76±0.05	0.60±0.04

Figures bearing different superscripts within a column differ significantly ($p\leq0.05$).
:a, b, c, d for strain comparison; A, B for breed comparison and P, Q and X, Y for pure vs. breed/strain cross comparison
WLh Pure avr. refers to average of PL1, PL2 and Naked-neck
RIR Pure avr. refers to average of RIR-B and RIR-C

4.5 GENETIC DIVERSITY

Genetic diversity among pure strains of White Leghorn, Rhode Island Red and their cross was evaluated using fourteen microsatellite loci. The observed and the expected number of alleles (according to Hardy Weinberg equilibrium) and the range of allele frequencies for all the fourteen microsatellite markers are presented in Table 17. With the exception of MCW-111 and ADL-268 in PL2, all the loci studied exhibited polymorphism in all the populations with the overall observed and expected number of alleles being 7.71 and 5.00, respectively. Marker MCW-104 showed highest polymorphism across all the populations with observed average number of alleles being 14, while MCW-111 showed least polymorphism with only 3 alleles. The average allele number in different strains is consistent with what has been recommended (at least 3-4 alleles in a population) for estimation of genetic diversity and genetic distances by microsatellite analysis (Wimmers *et al,* 2000).

MCW-104 was highly polymorphic in strains PL2 and cross (PL2♂ X RIR-B♀); and in RIR-B strain LEI-234 (8.0) was highly polymorphic. Average number of observed alleles in strains PL2, breed cross (PL2♂ X RIR-B♀); and RIR-B were 4.25, 3.78 and 4.57, respectively.

Expected number of alleles across all strains was highest for MCW-104 (10.34) and lowest for MCW-111 (2.57). Mean expected number of alleles was highest in RIR-B and was lowest in cross PL2♂ X RIR-B♀.

Table 17: Observed and expected number of alleles in different populations

Locus	Observed feature	Overall	PL2	RIR-B	PL2 X RIR-B
MCW-14	Na Ne	6.00 4.05	3.00 2.56	3.00 2.66	3.00 2.57
MCW-67	Na Ne	11.0 5.4	2.00 1.23	7.00 5.76	5.00 4.45
MCW-183	Na Ne	11.0 4.25	5.00 3.07	7.00 4.59	5.00 2.64
LEI-166	Na Ne	7.00 4.37	5.00 4.26	4.00 3.56	5.00 3.90
ADL-268	Na Ne	6.00 4.41	0.00 0.00	6.00 5.14	2.00 1.60
MCW-206	Na Ne	7.00 4.68	5.00 3.21	2.00 1.60	4.00 3.26
MCW-104	Na Ne	14.00 10.34	6.00 4.37	7.00 5.64	8.00 6.48
MCW-123	Na Ne	5.00 3.23	3.00 2.57	2.00 2.00	3.00 2.45
MCW-330	Na Ne	6.00 3.94	2.00 1.27	4.00 2.81	4.00 3.26
MCW-165	Na Ne	8.00 5.33	5.00 3.06	6.00 4.00	4.00 3.07
MCW-248	Na Ne	6.00 4.80	5.00 4.26	3.00 2.66	4.00 3.66
MCW-111	Na Ne	3.00 2.57	0.00 0.00	3.00 2.39	2.00 1.60
MCW-34	Na Ne	5.00 4.14	4.00 2.90	2.00 1.93	3.00 2.57
LEI-234	Na Ne	13.00 8.56	6.00 4.37	8.00 4.84	1.00 1.00
Mean	Na Ne	7.71 5.00	4.25 3.09	4.57 3.54	3.78 3.04

Where **Na** – Observed number of alleles,
Ne – Effective number of alleles

The heterozygosity values in different strains are presented in Table 18. The observed heterozygosity was zero for all microsatellite loci studied except for MCW-104 and LEI-234, which had observed heterozygosity of 0.20 and 0.13 across all strains, respectively. These heterozygosity estimates are consistent with those reported by Crooijmans *et al* (1996) for layer chickens. Expected heterozygosity across the strains ranged from 0.62 (MCW-111) to 0.91 (MCW-104) while average heterozygosity varied from 0.31 (MCW-111) to 0.81 (MCW-104). Vanhala *et al* (1998) obtained expected heterozygosity in the range of 0.38 to 0.67 in eight chicken lines viz; three White Leghorn hybrids, three Finnish landrace, a Rhode Island Red and a broiler hybrid line with all the expected heterozygosity being greater than observed.

Locus	Feature	Overall	PL2	RIR-B	PL2XRIR-B

Highest expected heterozygosity was observed; in strain RIR-B for loci MCW-67 and in strain PL2 the expected heterozygosity was highest for LEI-166, MCW-104, MCW-248 and LEI-234 (Table 18). Mean observed heterozygosity across all strains was 0.02 and it was highest in strains RIR-B. All the fourteen loci studied were 100% polymorphic in RIR-B; while in PL2, 85.71% loci were polymorphic. The low values of mean heterozygosity observed for different strains could be attributed to the long history of selection that different strains had undergone for some economic traits.

MCW-14	Ho He Avg H	0.00 0.77 0.61	0.00 0.65 0.61	0.00 0.71 0.61	0.00 0.65 0.61
MCW-67	Ho He Avg H	0.00 0.83 0.59	0.00 0.20 0.59	0.00 0.86 0.59	0.00 0.83 0.59
MCW-183	Ho He Avg H	0.00 0.77 0.69	0.00 0.69 0.69	0.00 0.80 0.69	0.00 0.64 0.69
LEI-166	Ho He Avg H	0.00 0.78 0.74	0.00 0.81 0.74	0.00 0.80 0.74	0.00 0.77 0.74
ADL-268	Ho He Avg H	0.00 0.79 0.39	0.00 0.00 0.39	0.00 0.84 0.39	0.00 0.42 0.39
MCW-206	Ho He Avg H	0.00 0.80 058	0.00 0.73 0.58	0.00 0.42 0.58	0.00 0.74 0.58
MCW-104	Ho He Avg H	0.20 0.91 0.81	0.11 0.81 0.81	0.25 0.85 0.81	0.22 0.89 0.81
MCW-123	Ho He Avg H	0.00 0.71 0.56	0.00 0.65 0.56	0.00 0.66 0.56	0.00 0.62 0.56
MCW-330	Ho He Avg H	0.00 0.76 0.51	0.00 0.20 0.51	0.00 0.67 0.51	0.00 0.74 0.51
MCW-165	Ho He Avg H	0.00 0.82 0.69	0.00 0.69 0.69	0.00 0.78 0.69	0.00 0.70 0.69
MCW-248	Ho He Avg H	0.00 0.80 0.70	0.00 0.81 0.70	0.00 0.71 0.70	0.00 0.76 0.70
MCW-111	Ho He Avg H	0.00 0.62 0.31	0.00 0.00 0.31	0.00 0.60 0.31	0.00 0.42 0.31
MCW-34	Ho He Avg H	0.00 0.77 0.58	0.00 0.70 0.58	0.00 0.53 0.58	0.00 0.66 0.58
LEI-234	Ho He Avg H	0.13 0.90 0.52	0.11 0.81 0.52	0.18 0.83 0.52	0.00 0.00 0.52
Mean	Ho He Avg H	0.02 0.79 0.59	0.01 0.65 0.63	0.03 0.72 0.59	0.01 0.63 0.59
No. of polymorphic loci		14	12	14	13
% of polymorphic loci		100%	85.71%	100%	92.86%

Table 18: Observed (Ho), expected (He) and average (Avg H) heterozygosity of all loci in different strains

The genetic distances and genetic identity between different strains as per Nei (1978) are presented in Table19. The genetic distance was highest (2.015) between PL2 and RIR-B strains as both these strains had been developed from different genetic sources while the genetic distance was smallest (0.47) between PL2 and cross strains. The Rhode Island Red selected strains RIR-B and cross (PL2♂ X RIR-B♀) had genetic distance of 1.024.

The χ^2 -square values for testing the Hardy Weinberg equilibrium of allele frequencies of the different strains (Table 20) revealed significant differences between the observed and expected frequencies for most of the loci studied across all the strains. The Hardy Weinberg disequilibrium in different strains is indication of influence of forces like migration, mutation or selection.

Table 19: Nei’s genetic distance (below diagonal) and genetic identify (above diagonal) for all population

Population	PL2	PL2♂ X RIR-B♀	RIR-B
PL2	***	0.6233	0.1333
PL2♂ X RIR-B♀	0.4727	***	0.359
RIR-B	2.0149	1.0244	***

The dendrogram based on Nei’s (1978) genetic distance method UPGMA modified from NEIGHBOR procedure of PHYLIP version 3.5 (Fig.6) showing the genetic diversity among these strains, grouped PL2 and cross (PL2♂ X RIR-B♀) under cluster 1 and RIR-B under cluster 2.

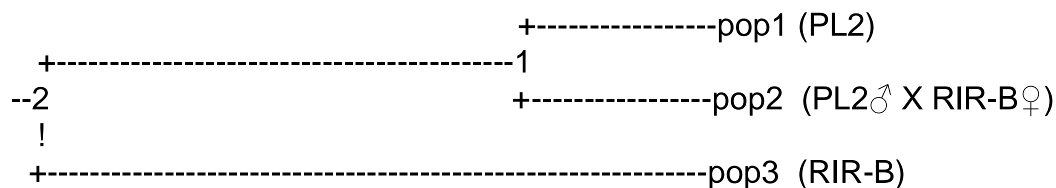
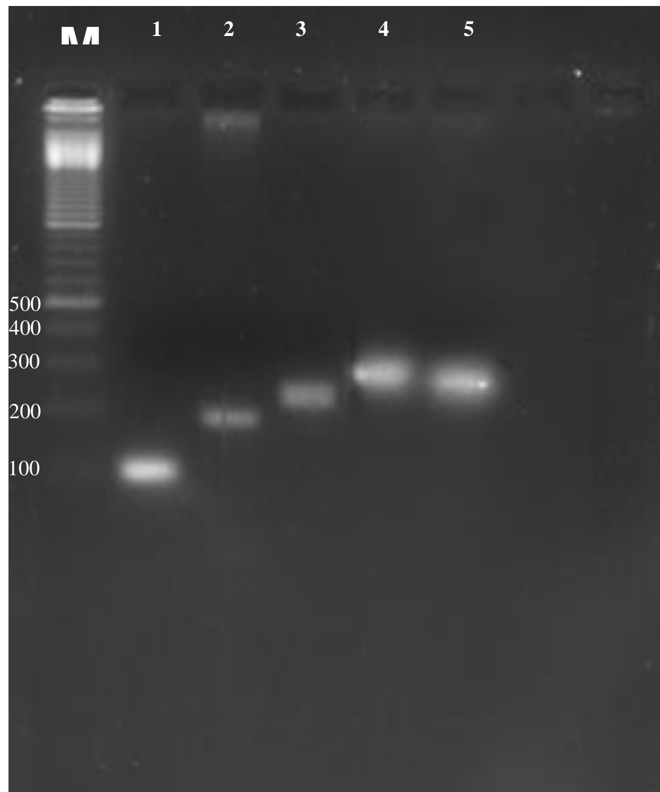


Fig. 6: Dendrogram Based on Nei's (1978) Genetic distances between different strains

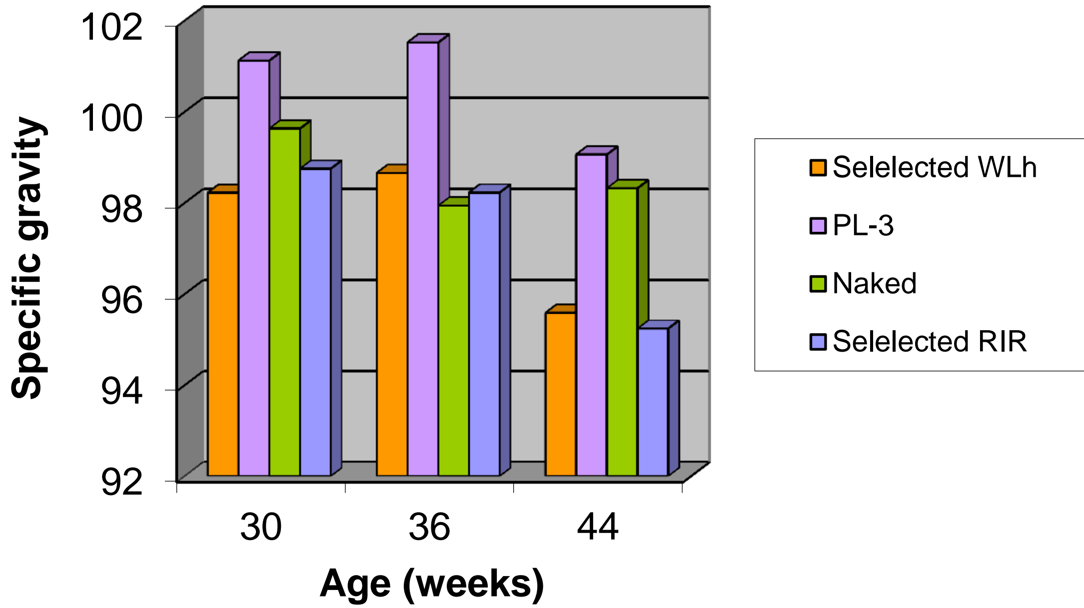
Table 20: χ^2 -square values for testing the Hardy Weinberg equilibrium of allele frequencies of the different strains

Locus	PL2	RIR-B	(PL2♂ X RIR-B♀
MCW-14	21.6	14.66	21.6
MCW-67	17.06	113.6	49.33
MCW-183	96.72	153.01	90.4
LEI-166	57.86	28	69.6
MCW-206	60.57	7.2	35.46
MCW-104	57.86	101.9	81.66
MCW-123	21.6	4	23.11
MCW-330	17.06	54.66	35.46
MCW-165	96.72	100.44	56.44
MCW-248	57.86	14.66	41.6
MCW-34	40.57	6.4	18.93



M= 100 base pair ladder
1= MCW0111Microsatellite PCR product (106 bp)
2= MCW0020 Microsatellite PCR product (189 bp)
3= MCW0034 Microsatellite PCR product (257 bp)
4= LEI0234 Microsatellite PCR product (295 bp)
5= MCW0103 Microsatellite PCR product (286 bp)

Plate 3: Agrose gel electrophoresis for the identification of PCR product using molecular weight marker



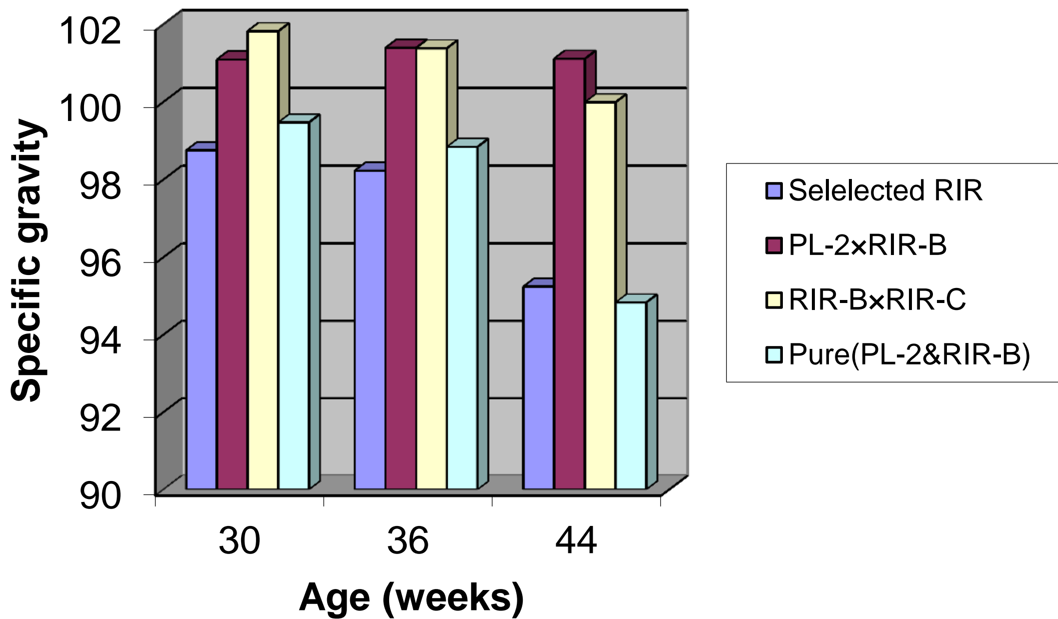
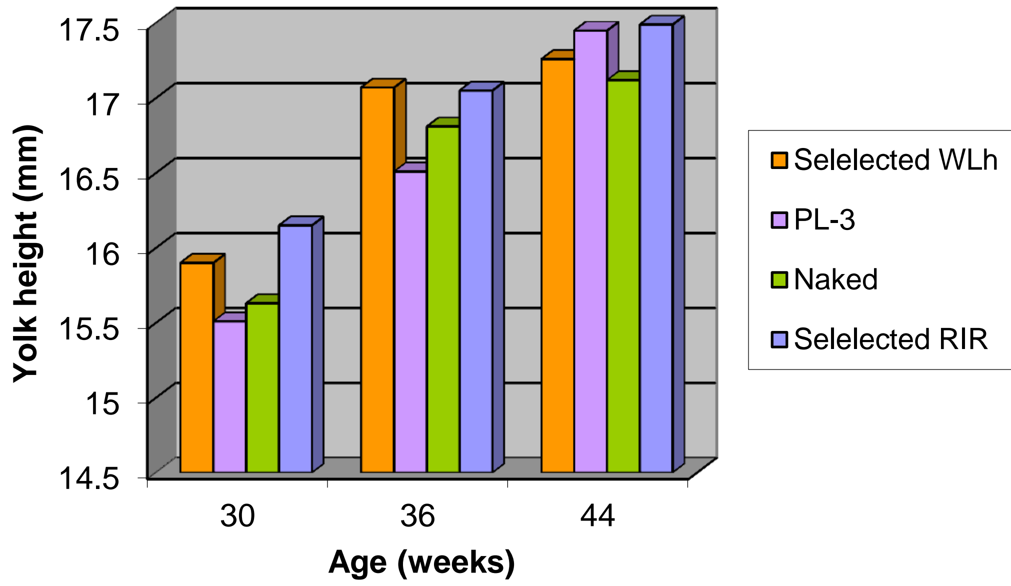


Fig. 1: Egg specific gravity in different strains showing age x strain interaction



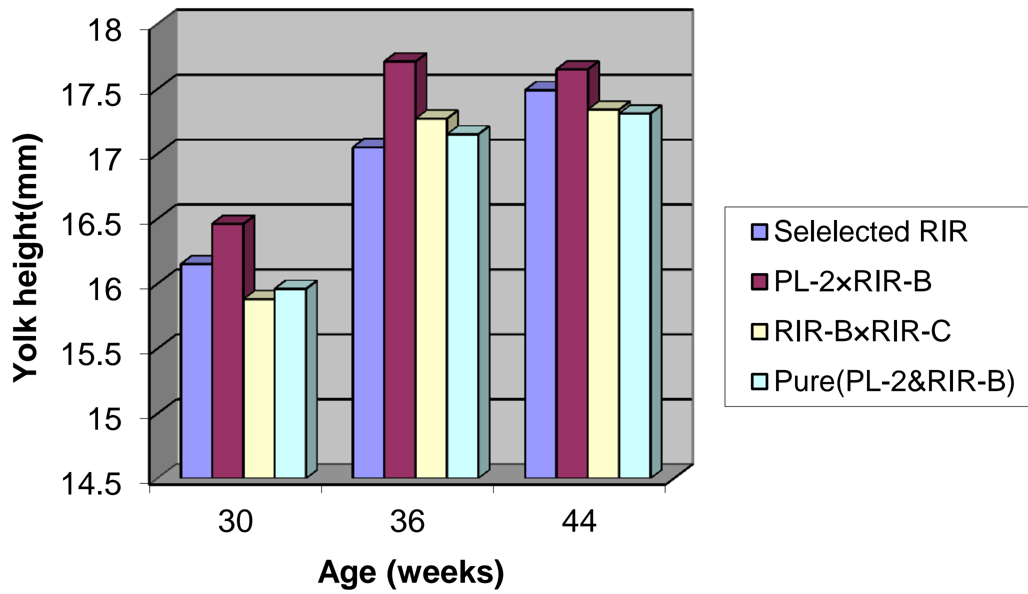
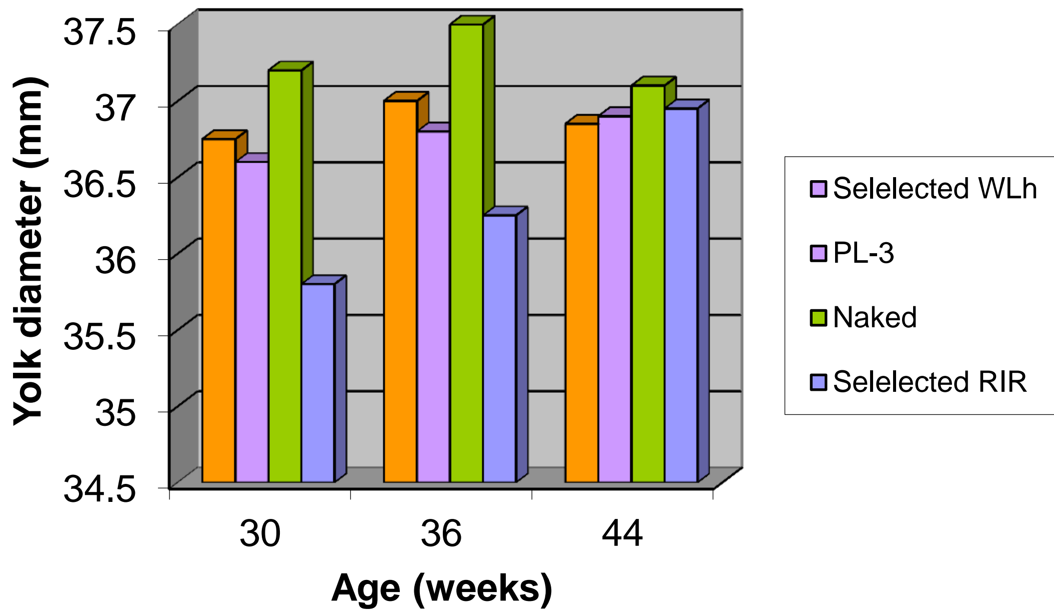


Fig. 2: Yolk height in different strains showing age x strain interaction



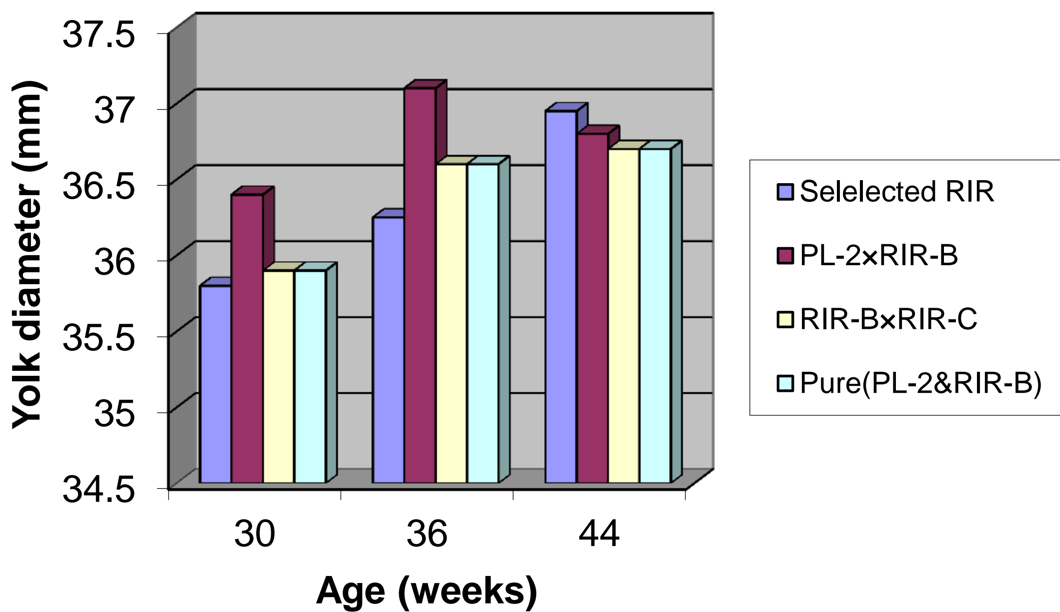
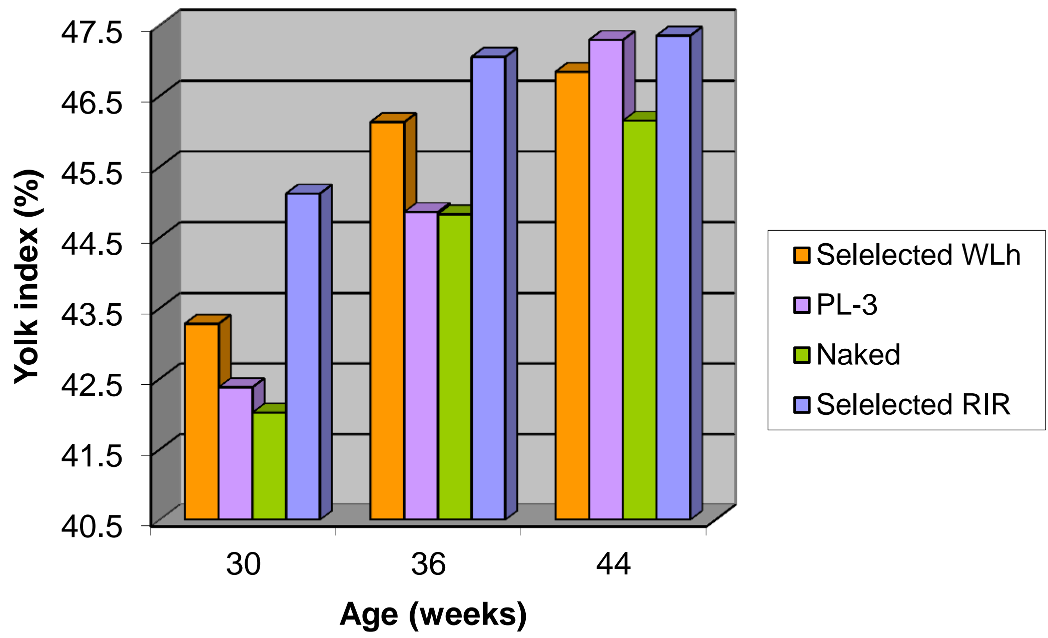


Fig. 3: Yolk diameter in different strains showing age x strain interaction



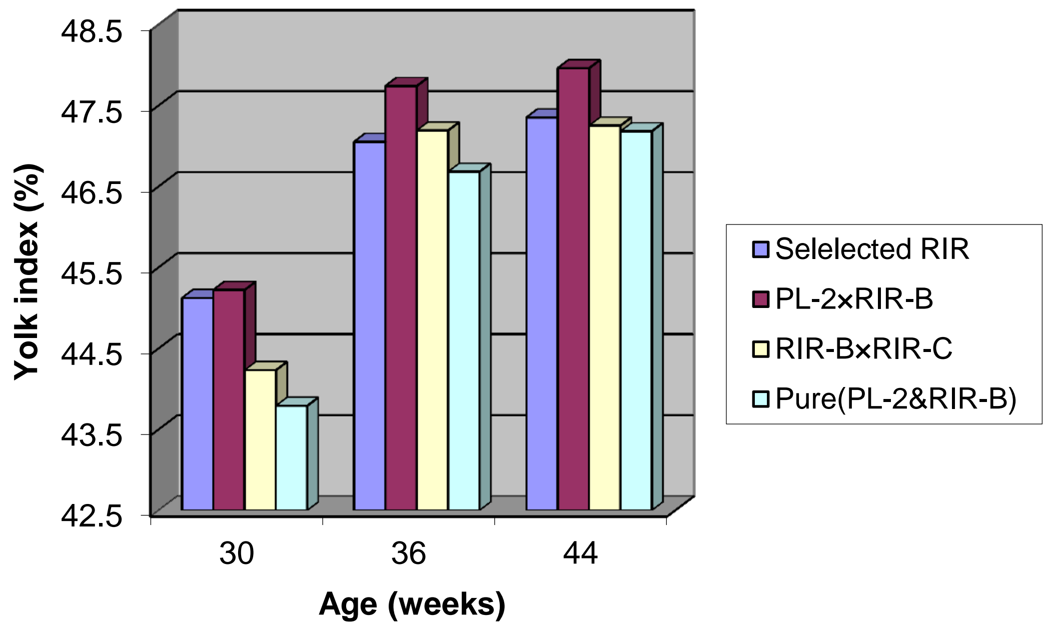


Fig. 4: Yolk index in different strains showing age x strain interaction

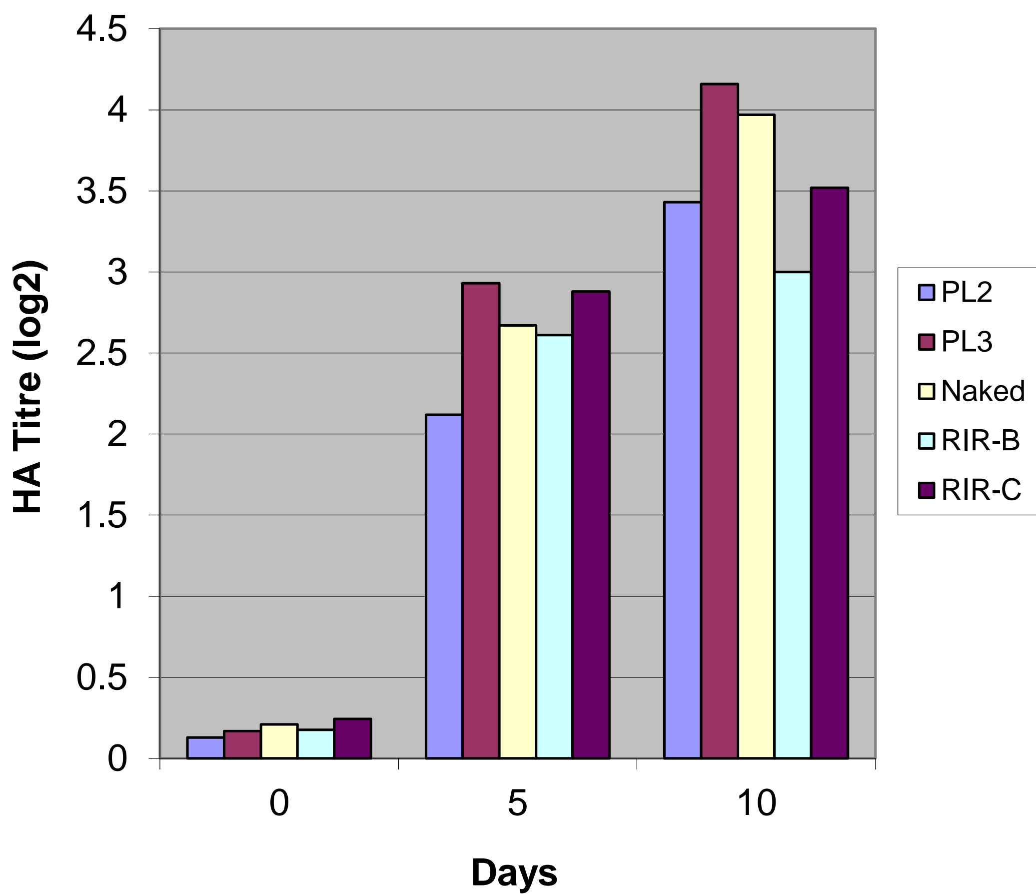


Fig. 5: Antibody response in different strains at different days post primary injection

CHAPTER – V

SUMMARY AND CONCLUSIONS

The present study was conducted on two selected strains, a control line, a naked-neck strain of WLh; two strains of RIR and their crosses with the objective to evaluate growth, egg production and quality traits, immunocompetence and genetic diversity among the strains using microsatellite markers.

Of all the strains, RIR-C had highest body weight. Selected WLh, PL1 and PL2 did not differ for body weight significantly, while both had higher body weight than the control at 12, 16 and 20 weeks of age but thereafter control tended to maintain higher body weight. RIR-C had higher body weight than RIR-B due to difference in genetic sources and selection history. Breed cross PL2♂ X RIR-B♀ had higher body weight than parental pure average at all the ages. The magnitude of heterosis was higher during growing than the laying period.

The egg production was recorded from the onset of production upto 44 weeks of age. The control strain matured significantly later than the other strains and breed cross PL2♂ X RIR-B♀ attained earlier sexual maturity. PL2 had higher egg number than the control during the first phase of laying period. PL2 had higher egg weight, egg mass, egg production efficiency index than the control during all the three age periods.

RIR-B had better performance for the egg production traits as compared to RIR-C. Pure WLh (average of selected strain PL1, PL2 & Naked neck) had higher EMFD, EPE and EINDEX than pure RIR during all age periods. Breed cross PL2♂ X RIR-B♀ showed higher performance than the parental average but not in all the age periods.

Egg quality and composition traits for different genetic groups were evaluated at 30, 36 and 44 weeks of age. The egg weight, length, width, yolk

height, yolk diameter, yolk index, % shell, shell thickness, yolk, albumen and shell weight increased from 30 to 44 weeks of age while specific gravity, albumen height, HUS, % albumen declined with age.

PL2 had significantly higher egg weight but lower length, width and shape index than the control line, PL3. Of the two RIR strains, RIR-C had higher shape index. Pure WLh showed higher egg weight, but lower shape index than pure RIR strains. Control PL3 had higher specific gravity among all the groups but specific gravity showed age x genetic group interaction. PL2 had better albumen quality and yolk height than the control, PL3, line. The two RIR strains did not differ for yolk quality traits. The age x genetic group interaction was significant for yolk traits.

Breed cross PL2♂ X RIR-B♀ had better albumen and yolk quality than parental average. Among all the strains PL3 had thickest shell while RIR-B had thinnest shell. PL2 had higher yolk weight, shell weight as compared to unselected control line. The breed cross had significantly higher weight of yolk and shell than the parental average.

Immune response to Sheep Red Blood Cells was measured as total titre at 0, 5 and 10 days post primary inoculation. The presence of natural antibody was evident in all the genetic groups. All groups showed an increase in HA titre upto 10 days post immunization. Of the WLh strains, the control line (PL3) showed highest response on day 5 and 10 post primary inoculation. Of the two RIR strains, RIR-C showed higher response than RIR-B strain at day 5 and 10 post inoculation. Breed cross had higher response than the parental average.

The response after PHA–P inoculation, foot index was highest in strain PL3. The poorest response was obtained in RIR-B strain. Breed and strain cross differed non-significantly(p>0.05) from their parenteral average.

Genetic diversity was evaluated among the selected strains PL2, RIR- B and their cross by using 14 microsatellite markers. All the loci exhibited polymorphism in all the populations except MCW-111, ADL-268 in PL2 strain. Marker MCW-104 showed highest polymorphism.

The observed heterozygosity was zero for all loci except for MCW-104 and LEI-234. All the 14 loci were 100 % polymorphic in RIR-B, while in PL2,

85.71% loci were polymorphic. The dendrogram based on Nei (1978) grouped PL2 and cross PL2♂ X RIR-B♀ under cluster 1 and RIR-B under cluster 2.

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APPENDIX- I

REAGENTS FOR DNA ISOLATION

I. 2.7% EDTA for blood collection

2.7 g of EDTA (molecular weight 292.25g) in 100 ml of ddH_2O.

II. Lysis buffer for poultry

10mM Tris HCl (pH 8.0)

100mM Sodium Chloride

1mM EDTA (pH 8.0)

0.5 % SDS

III. 0.5 M EDTA-1000 ml pH-8.0

EDTA disodium salt (molecular weight 372.24) – 186.12g in 800 ml of ddH_2o.

Add NaOH pellets to adjust the pH (approximately 20g NaOH). EDTA dissolves only at pH 8.0. Make the volume to 1000 ml. Dispense into aliquots and sterilize by autoclaving.

Functions of EDTA – 1.Chelates Mg^{++} ions
2. Protects from nucleases
3. Makes plasma membrane more fragile.

IV. DNA Extraction buffer-500ml

1M Tris (pH 8.0): 5ml

5M NaCl: 40 ml

0.5M EDTA (pH 8.0): 2 ml

Distilled water upto 500 ml

Autoclave in batches of 100 ml and store at room temperature

V. 1M Tris (pH 8.0)

(a) Using Tris HCl

Tris HCl -157.6 g

Distilled water upto 1000 ml.

Adjust pH to 8.0 with NaOH pellets.

Autoclave in 100 ml batches.

(b) Using Tris Base.

Dissolve 121.1 g of Tris Base in 800 ml of ddH_2O. Adjust the pH to desired value by adding concentrated HCl. Allow the solution to cool to room temperature before making final adjustments to the pH. Adjust the volume of the solution to 1 L with ddH_2O. Dispense into aliquots and sterilize by autoclaving.

pH – HCl

8 – 42 ml

7.6 – 60 ml

7.4 – 70 ml

If the 1 M solution has a yellow colour, discard it and obtain better quality Tris. The pH of Tris solutions is temperature-dependent and decreases approximately 0.03 pH units for each 1°C increase in temperature. For example, a 0.05 M solution has pH values of 9.5, 8.9 and 8.6 at 5°C, 25°C and 37°C, respectively.

VI. 5M NaCl - 1000 ml

Dissolve 292.2g of NaCl in 800 ml of H_2O. Adjust the volume to 1000 ml. Dispense into aliquots and sterilize by autoclaving

VII. 10% Sodium Dodecyl Sulfate (SDS)

(*also called Sodium Lauryl Sulfate)*

Dissolve 100 g of electrophoresis grade SDS in 900 ml of tdH_2O. Heat to 68°C to assist dissolution. Adjust the pH to 7.2 by adding a few drops of concentrated HCl (generally not needed). Adjust the volume to 1litre with H_2O. Wear a mask when weighing SDS and wipe down the weighing area and balance after use because the fine crystals of SDS disperse easily. There is no need to sterilize 10% SDS. Dispense into aliquots.

Functions of SDS -1. Helps in cell membrane lysis,

2. Acts as a catalyst.

VIII. Proteinase ‘K’:

Weigh empty eppendorf, add approximately 20 mg of Proteinase ‘K’ and add 1000 µl (1 ml) of TDW. Store in freezer after proper mixing.

Proteinase *‘K’*

(1) Stock solution - 20 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

(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- 1.Digestion of proteins

IX Equilibration of phenol

Most batches of commercial liquefied phenol are clear and colorless and can be used in molecular cloning without redistillation. Occasionally batches of liquefied phenol are pink and yellow, and these should be rejected and returned to the manufacturer. Crystalline phenol 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.

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.

Before use, phenol must be equilibrated to a pH > 7.8, because DNA will partition into the organic phase at acid pH.

1. 100 g of phenol i.e. crystalline phenol was melted at 65°C and distilled (which takes around ½ - 1 hr).Liquefied phenol should be stored at –20°C.As needed remove phenol from the freezer and then melt at 68°C
2. 8-hydroxyquinolone to a final concentration of 0.1% was added. It is an antioxidant, partial inhibitor of RNAse and a weak chelator of metal ions. In addition, its yellow color provides convenient way to identify the organic phase.
3. Equal volume of 0.5 M Tris HCl pH 8.0 (at room temperature) was added, stirred for ½ hr and kept overnight.
4. Next day supernatant was removed, 0.1 M Tris pH 8.0 was added, stirred for ½ hr and placed back in the refrigerator.
5. 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.
6. Saturation with 0.1 M Tris is given in the morning and the pH tested after decanting some amount of supernatant Tris. The pH of phenolic phase can be checked with indicator paper and should be 8.0.If it is not 8.0, repeat the above steps till pH is obtained.

7. After the phenol is equilibrated and the final aqueous phase (supernatant) has been removed add 0.1 volume of 0.1 M Tris Hcl pH 8.0 (10 ml for 100ml phenol) containing 0.2% 2-Mercaptoethanol. The phenol solution may be stored in this form under 100 mM Tris HCl (pH 8.0) in a light tight bottle at 4°C for period upto 1 month.

Note: Phenol prepared with 8-hydroxyquinolone can be stored in brown bottle for 1 month

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 -

1. Prevents oxidation of phenol
2. Yellow colour provides convenient way to identify the organic phase

X Phenol: Chloroform: Isoamyl alcohol (25: 24: 1)/ (50: 48: 2).

Add 50 ml of Tris Saturated phenol to 50 ml of 24: 1 Chloroform: Isoamyl alcohol. Store at 4°C in brown 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: 1.Reduces foaming during extraction

XI. Chloroform: Isoamyl alcohol (24:1).

Add 96 ml of chloroform to 4 ml of Isoamyl alcohol. Store at 4°C in brown bottle.

XII. TE buffer- pH 8.0 for DNA.

XIII. 70% ethanol

For 20 ml

14ml of ethanol
6 ml of TDW
Chill it at 4°C.

XIV. 3 M sodium acetate (pH 5.2)

Dissolve 408.1 g of sodium acetate in 800 ml of H_2O. Adjust the pH to 5.2 with glacial acetic acid. Adjust the volume to 1 litre with H_2O. Dispense into aliquots and sterilize by autoclaving.

Function of 3M sodium acetate- 1. Precipitates DNA

XV. PBS, pH 7.4

NaCl- 8 g
KCl – 0.2 g
Na_2HPO_4 – 1.44 g
KH_2PO_4 - 0.24 g
H_2O-800ml

Adjust the pH to 7.4 with HCl. Add H_2O to 1L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 minutes at 15 lbs/sq inch, Store at room temperature.

XVI. 2-Mercaptoethanol (2ME)

Usually obtained as a 14.4 M solution.

Store in dark bottles.

APPENDIX- II

REAGENTS FOR PCR

1. Reconstitution of dNTP's

Working solution of dNTP mix with 10mM of each dNTP from 100mM of each dNTP stock

10µl 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. The effective concentration of each dNTP becomes 10mM in the mix.

$$N_1V_1 = N_2V_2$$

$$10mM \times V_1 = 200\mu M \times 50\mu l$$

(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 300µM in 50µl reaction 1.5µl of working solution is added.

Working solution of 2.5mM of each dNTP from 10mM 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.

$$N_1V_1 = N_2V_2$$

$$20 \text{ pM X V1} = 0.4 \text{ µM X } 50\text{µl}$$

$$V_1 = (0.4 \text{ µM X } 50\text{µl}) /20 \text{ pM}$$

$$= 1\text{µl}$$

Similarly if 1.5µl is added the effective concentration becomes 0.6µM in 50µl reaction.

$$N_1V_1 = N_2V_2$$

$$20 \text{ pM X V1} = 0.6 \text{ µM X } 50\text{µl}$$

$$V_1 = (0.6 \text{ µM X } 50\text{µl})/ 20 \text{ pM}$$

$$= 1.5 \text{ µl}$$

3. $MgCl_2$

1µl of 25 mM is added for a 50 µl reaction to get an effective concentration of 0.5 mM.

$$N_1V_1 = N_2V_2$$

$$25 \text{ mM X V1} = 0.5 \text{ mM X } 50\text{µl}$$

$$V_1 = 1\text{µl}$$

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.

APPENDIX-III

REAGENTS FOR AGAROSE GEL ELECTROPHORESIS

1. Agarose

2. TBE (Tris; boric acid; EDTA) buffer (5X)

Tris base	54g
Boric acid	27.5 g
0.5 M EDTA	20ml

Adjust pH to 8.0

3. 6 X loading dye for Agarose gel

	Type-1	Type-2
Bromophenol blue	0.25%	0.25%
Xylene Cyanol	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%.

APPENDIX-IV

REAGENTS FOR DENATURING POLYACRLAMIDE GEL ELECTROPHORESIS

1. Polyacrylamide Solution

Reagents	***Quantity in 100 ml***
Acrylamide	5.7 g
Bis- Acrylamide	0.3 g
Urea	42 g
10X TBE	10 ml
deionised water	40 ml

The polyacrylamide solution was heated to dissolve the contents and then cooled. It was filtered through Whatman filter paper and stored in amber coloured bottle till use at 4□C. 10% Ammonium Per Sulphate (500 ml) and TEMED (50 ml) were added to polyacrylamide solution just before setting of the gel.

2. SSCP Stop solution or loading dye for D -PAGE gel

	Type-1	**Type-2**
Bromophenol blue	0.25%	0.25%
Xylene cyanol	0.25%	-
Sucrose in DW	40%	40%

Mix and store at 4°C

3. **10 % Ammonium per Sulphate:** 0.05 g of Ammonium per Sulphate in 500 µl of distilled water.

4. 10% Glacial acetic acid (Fixing and stop solution)

20 ml of glacial acetic acid in 200 ml of tdH_2o

5. Staining solution (0.1 % $AgNO_3$). 0.2g of $AgNO_3$ in 200 ml of tdH_2O, Add 200□l of 37% formaldehyde 5 minutes before use.

6. Developing solution (0.028 M Na_2CO_3)

6g of Na_2Co_3 in 200ml of tdH_2O, keep it in refrigerator, add 200 µl of 37% formaldehyde 15 minutes before use.

APPENDIX- V

COMPOSITION OF OTHER BUFFERS AND SOLUTIONS USED

1. Composition of Alsever solution

S.No.	Constituent	Quantity
1	Dextrose	20.5 g
2	Sodium Chloride	4.2g
3	Citric Acid	550mg
4	Tri sodium acetate	8g
5	Distilled water	1000ml

Autoclave at 10 lbs for 10 min

2. Composition of Phosphate buffer saline (PBS)

S.No.	Constituent	Quantity
1	Sodium Chloride	8g
2	Potassium Chloride	0.2g
3	Sodium biphosphate	1044g
4	Potassium biphosphate	0.24g
5	Distilled water	800ml

Adjust ph to 7.4 with HCl and make volume to 1000 ml. Autoclave and store at room temp.

VITA

Name of the Student	:	**Dr. SATYENDRA PAL SINGH**
Father's Name	:	Sh. Benami Singh
Mother's Name	:	Smt. Anar Devi
Nationality	:	Indian
Date of Birth	:	03.05.1980
Permanent Home address	:	HNo. 507, Paschimpuri colony, Shastripuram Road, Sikandra, Agra-282007

EDUCATIONAL QUALIFICATION

Bachelor Degree	:	B.V.Sc. & A.H.
University and Year of Award	:	G.B. Pant Agricultural University, Agri. & Technology, Pantnagar-2005
OGPA/OCPA/%Marks	:	8.33/10.00
Master's Degree	:	M.V.Sc. (Animal Breeding and Genetics)
University and Year of Award	:	Guru Angad Dev Veterinary and Animal University, Ludhiana – 2007
Awards/ Distinctions/ Fellowships/ Scholarships	:	University Merit Scholarship during M.V.Sc.