

**WELFARE BEHAVIOR AND PERFORMANCE OF LAYING
HENS AT DIFFERENT STOCKING DENSITIES AND FLOCK
SIZE IN CALIFORNIA AND FURNISHED CAGES**

T H E S I S

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BY

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Enrollment No. V/16/418

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2021

DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled “**WELFARE BEHAVIOR AND PERFORMANCE OF LAYING HENS AT DIFFERENT STOCKING DENSITIES AND FLOCK SIZE IN CALIFORNIA AND FURNISHED CAGES**” or part thereof has not been submitted for any other degree or diploma of any University, nor the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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ABBREVIATIONS

%	:	Percent
⁰ C	:	Degree Celsius
≤	:	Less than or equal to
₹	:	Rupees
EU	:	European Union
ELISA	:	Enzyme-linked Immunosorbent Assay
PCA	:	Prevention of Cruelty to Animals
G.S.R	:	General Statutory Rules
W.P.(C)	:	Writ Petition (Civil)
OIE	:	Office International des Epizooties
M ²	:	Square Meter
Cm ²	:	Square Centimeter
USA	:	United States of America
UK	:	United Kingdom
FAWC	:	Farm Animal Welfare Council
LD	:	Low Density
HD	:	High Density
HDEP	:	Hen Day Egg Production
CPPE	:	Cost of production per egg
NSB	:	Non-specific bindings
g/b/d	:	Gram per bird per day
FCR	:	Feed Conversion Ratio
LBW	:	Live Body Weight
BWG	:	Body Weight Gain
MEC	:	Modified and Enriched Cages
MFC	:	Medium Furnished Cages

CC	:	Conventional Cage
<	:	Less than
>	:	Greater than
GA	:	Get Away
PLC	:	Plastic cages
ME	:	Metabolizable Energy
FC	:	Furnished Cage
ANOVA	:	Analysis of Variance
Kg	:	Kilogram
Cm	:	Centimeter
Mm	:	Millimeter
<i>et al</i>	:	Ethically all
SFC	:	Small Furnished Cages
LFC	:	Large Furnished Cages
Fig	:	Figure
<i>g/egg</i>	:	Gram per Egg
G	:	Gram
FI	:	Feed Intake
Log ₂	:	Logarithms
BW	:	Body Weight
Wk	:	Week
WLH	:	White Leghorn
1 st	:	First
2 nd	:	Second
3 rd	:	Third
SU	:	Space Utilization
Vs	:	Versus
sq. inches	:	Square inches

HU	:	Haugh unit
KBD	:	Keel Bone Deformation
SA	:	Space Allowance
V Sc	:	Visual Scoring
H Sc	:	Hands-on Scoring
rpm	:	Revolutions per minute
HA	:	Hemagglutination Assay
HI	:	Hemagglutination Inhibition
PBS	:	Phosphate Buffered Saline
NaCl	:	Sodium Chloride
KCl	:	Potassium chloride
KH ₂ PO ₄	:	Potassium dihydrogen phosphate
Na ₂ HPO ₄ .2H ₂ O	:	Disodium hydrogen phosphate
μl	:	Microlitre
SD	:	Stocking Density
FS	:	Flock Size
v/v	:	Volume per volume
LB+	:	Lohmann Brown Plus
H/L	:	Heterophil/Lymphocyte
ACTH	:	Adrenocorticotropic Hormones
pg/ml	:	Picograms per mililitre
ng/ml	:	Nanograms per mililitre
SRBC	:	Sheep Red Blood Corpuscles
SEM	:	Standard Error Mean
IgG	:	Immunoglobulin
NDV	:	Newcastle Disease Virus
SE	:	Standard Error
CCTV	:	Closed Circuit Television

DVR	:	Digital Video Recording
GC-MS	:	Gas Chromatography Mass Spectrometry
H & E	:	Haematoxylin and Eosin
Hr	:	Hours
i.e.	:	That is
CPPE	:	Cost of Production Per Egg
SU	:	Space Utilization
RE	:	Recurring Expenses

INTRODUCTION

Poultry meat and egg is the cheapest and most common source of protein consumed at the global level. Amongst the livestock sector, poultry has emerged as the most efficient sub-sector in providing protein to the world because of the unparalleled growth provided through advances in genetics, nutrition and husbandry practices. The poultry sector worldwide and in particular India has undergone massive transformation in structure and operation from a mere backyard activity into a major commercial agri-based industry over a period of four decades. India ranks 3rd in egg production and 4th in chicken meat production in the world. About 3.4 million tons (74 billion) of eggs are produced from 260 million layers and 3.8 million tons of poultry meat is produced from 3000 million broilers per annum in India. (Chatterjee and Rajkumar, 2015). In spite of rapid growth, setbacks like rising cost of feed, emergence of new or reemerging of existing diseases, fluctuating market price of egg and broilers, new welfare measures of the bird need to be addressed to make this enterprise a sustainable one. The major objective of poultry production in any scenario is to provide safe and healthy animal protein to the ever-growing population, overcoming the challenges cropping up on its sustainability. In the recent years, issues relating to animal welfare and environmental pollution by poultry units have been of increasing concern (Craig and Swanson, 1994). Welfare of poultry has become an important issue since EU banned cage rearing of birds. (Chatterjee and Rajkumar, 2015)

In parallelism, Indian animal welfare activist increasingly asserted that rearing of high producing layer birds in cages, in the intensive system of rearing resulted in some of the welfare and health issues which are not visible in the extensive rearing of native birds. Ultimately the activist thought the welfare of the birds raised in cages is compromised. The space provided in cages to commercial layer birds is not sufficient to stand, lie down and turn around without touching each other's and sides of enclosure (Chatterjee and Haunshi,2015).There is evidence that birds undergo a series of morphological, physiological, and

behavioral changes because of the use of battery cages that increases stress in hens due to an overcrowded, barren environment, which can inhibit the hens from performing certain natural behaviors and reduce bone quality (Nicol 1987; Tauson 1999). The restriction of space in the cages or by the size of the flock relative to form stable social relationship hampers the laying birds to exhibit their full behavioral repertoire.

On March 2, 2017, the Law Commission of India received a reference from the Ministry of Law and Justice asking the Commission to conduct a detailed study on the existing laws and international practices on the transport and housekeeping of poultry birds. The Animal Welfare Board of India advised the Government of India and the State Governments to issue suitable directives to poultry farmers to prohibit the use of battery cages in egg production, so that poultry farms keeping egg laying hens adhere to the provisions of the Prevention of Cruelty to Animals (PCA) Act, 1960 and not confine birds in cages. In response, The Gazette of India published a draft of the space allowance for conventional colony enclosures of egg-laying hens for commercial layer production on 29th April 2019 by Ministry of Agriculture and Farmers welfare under G.S.R. 335(E). It indicated that the floor space per bird shall not be less than 550 sq. cm and each cage should accommodate preferably a minimum of 6-8 birds, thus ensuring reasonable space for laying hens for lying down, standing up, flapping wings, turning around and access to feed and water. The use of battery cages is now raising a considerable debate pertaining to the relative effect of the practice on hen well-being. The issue has been raised up to such level that the matter is pending at Honorable High Court, New Delhi (W.P. (C) 9056/2016 & connected matters).

World Organization for Animal Health (OIE) defines: Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behavior and is not suffering from unpleasant states such as pain, fear, and distress. Disease prevention and veterinary treatment, appropriate shelter, management, nutrition,

humane handling and humane slaughter are the prerequisite for good animal welfare. India is a member of World Organization for Animal Health (OIE) and follows the OIE guiding principles on animal welfare which include the universally recognized —Five Freedoms, published in 1965 to promote the right to welfare of animals under human control. According to this concept, an animal's primary welfare needs can be met by providing: Freedom from hunger, malnutrition and thirst; Freedom from fear and distress; Freedom from physical and thermal discomfort; Freedom from pain, injury and disease; and Freedom to express normal patterns of behaviour.

Universally for housing the laying birds, farmers can opt battery cages, that are small enclosures with welded wire mesh and sloping floors; intensive floor systems where the birds are kept on litter (bedding material) so that they have freedom to move within the poultry house and the free-range systems where the layers have access to an outdoor run also. However, the battery cage is the most economical way to produce eggs (Horne, 2006) and the best option to prevent disease occurrence (Hulzenbosch, 2006). Apart from European Union who have advocated for cage free raising of layers in the recent years, only Australia and New Zealand have some commercial non-cage systems. In all other countries, farmers mainly keep layers in cage systems. Under the floor housing system the EU directives have suggested a layer bird has to be provided with a minimum space of 1100cm² and before, the floor space per bird provided was 550cm². Presently in USA layers get 430cm² spaces but in other countries including India hens are kept in cages with space allowance of 300 to 400 cm² which is much lower than the world average.

Modern-day egg producers have increased the net income by utilizing available housing facilities at best capacity by maximizing the number of birds per cage consequently decreasing the cage space allowance per bird. Producers reduce the bird space with the assumption that an increase in total egg production per housing unit increases the profit. The perception, however started to change in the last decade with animal welfare issues receiving more publicity. Although the fact which cannot be ignored is that the estimated production cost of eggs are high

significantly high when the area per bird in cage is increased as in the enriched cages (750cm²), German enriched cages (800cm²) or floor systems (1100cm²).

Laying hens are primarily kept in battery cages (also called conventional cages). For performing most of the natural behaviors in the cages, on an average, hens (Hy-Line W-36) required a mean area of 563 (\pm 8) cm² to stand, 1,316 (\pm 23) cm² to turnaround, 318 (\pm 6) cm² to lie down, and 1,693 (\pm 136)cm² to wing flap. Requirement for mean cage height for standing is 34.8 (\pm 1.3) cm, for turning 38.6 (\pm 2.3) cm, and 49.5 (\pm 1.8) cm for wing flapping. However, the space requirements of hens kept in multiple-hen groups in cage or non-cage systems cannot be based simply on information about the space required for local movement by a single hen. At the same time, due consideration should be given for the tendency of hens in a flock to synchronize their behaviours (Mench and Blatchford, 2014).

Considerable debate on the use of battery cages pertaining to the relative effect of the practice on hen well-being is going on. Display of locomotor, body maintenance, and thermoregulatory behaviors is greatly curtailed in conventional cages and to an extent is determined by the size of the cage and stocking density. It is observed that rebound levels of wing flapping, tail wagging, and stretching occur when hens are moved to a large space after several weeks of confinement in a small area, with the intensity of rebound of some behaviors being correlated with the duration of confinement, indicating that hens do not fully acclimatize to prolonged, severe spatial restriction (Nicol,1987). It has been observed that when birds are maintained at higher stocking densities or at low space allocation there is increased rubbing of the birds plumage against cage walls and other hens when moving across the cage resulting in plumage damage and thus reducing the thermoregulatory capacity. High density can also make it more difficult to gain access to food and water because other hens block the path, especially in deep cages with food at the front and water at the back.

In an attempt to reduce housing, equipment and labor cost per pen, commercial layer producers often increase the number of birds per pen. However, it is evident from literature that high stocking densities can have a detrimental

effect on the economics and welfare of poultry production. At higher stocking densities high mortality, greater incidence of leg problems, and disturbed resting behavior in birds was observed by Hall (2001). Slower growth rate and fewer eggs, with higher mortality at high density was mentioned by Deaton (1983). Significant reduction in feed consumption among birds reared at high stocking density was indicated by Wells (1972). Leeson and Summers, 1984; Carey, 1987, reported heavier eggs to be produced by birds kept at high stocking density than birds at low stocking density. Higher stocking densities have been associated with higher levels of feather pecking in laying hens, in different systems (Nicol *et al.*, 1999).

Furnished cage systems is an attempt to provide an enriched environment (i.e., facilities) to meet the needs of hens while maintaining small group size to minimize social stress. Furnished cages are equipped with perches, dustbath and nesting areas, to increase opportunities for the hens to exhibit natural behaviors (Appleby *et al.*, 2002). Additionally, it is observed that hens in furnished cages perform more behavioral changes or posture changes, and more preening behavior (Pohle and Cheng, 2009). So, the furnished cages are used as an alternative system to the conventional cage system. The group size of recent furnished cages is large, for example 16 hens or 40 hens per cage (Weitzenburger *et al.*, 2005). Furnished cages with larger flock are beneficial for the layers as by providing a larger total cage area, the layers would have more space for exercise, and also reducing the egg production cost per hen. But larger group size increases the risks for aggressive interactions, feather pecking and cannibalism. A small group size offers the benefit of a low incidence of aggressive interactions. For a developing nation, welfare in terms of cage rearing, free range systems, organic farming which would lead to increase in the cost of production and thus could be an unaffordable options for the masses.

Bird preferences for space are complex and confounded by interactions between group sizes and stocking density. The aim of the current study was to examine the differential effects of stocking density flock size with and without

furnished cages on the behaviour of laying hens under commercial conditions with the following objectives:

Objectives

1. To study the effect of different stocking density of commercial laying hens on egg production, egg quality, immune response, economics, welfare and behavioral changes in conventional California cage rearing system
2. To study the interaction effect of stocking density and flock size of laying hens on egg production, egg quality, immune response, economics, welfare and behavioral changes in conventional California cage system.
3. To study the comparative laying hens performance in conventional California cage and modified/furnished conventional California cages (with facility of perch, scratch pad and nesting area) to assess the egg production, egg quality, immune response, economics, welfare and behavioural changes.

REVIEW OF LITERATURE

Floor space allowance per bird and group size per given space affect layer bird production performance, welfare and overall profitability. Increasing population density of birds decreases floor space allowance and increases number of birds in group size; an increase in stocking density is economical to the producers, but it has raised welfare concerns by various activists throughout the world. Studies on the subject of population density effects on layer production parameters have shown conflicting results and many authors have tried to provide an environment by modifying and furnishing the cages whereby the birds can exhibit most of the behavioral activities like perching, pecking, wing flapping, scratching and self-preening. Therefore, the present study was undertaken to evaluate the best possible space and group size for commercial layers to provide best possible welfare from layer farmer and welfare activists' standpoint of view. The effect of stocking density, flock size and furnished cages on the various parameter like production, behavior and welfare has been reviewed and presented in this chapter.

2.1 BACKGROUND

Commercial layer birds throughout the world are mostly maintained in cages for the ease of operation. Welfare activist believes that birds in cages are deprived of the opportunity to perform their full behavioral repertoire. This stance is still encapsulated in guidelines for the protection of animals such as the 'Five Freedoms', laid down by the UK's Farm Animal Welfare Council (FAWC, 1992, 1993). The approach has, however been criticized by several authors because the genotype, environment and experience of captive, domesticated animals may differ considerably from those of their ancestors or modern free-ranging conspecifics. Secondly, the approach does not attempt directly to quantify the value that animals place on specific environmental resources. Nevertheless, the behavioral repertoires of wild or free-ranging animals in semi-natural environments may be a crude indicator of the kinds of behaviors that might be deprived in captive conditions and the types of resources that allow expression of

these behaviors. The public demands assurances that animals used for food are raised humanely. Conventional cages for egg laying hens do not meet all hen's behavioural needs, but cages have potential advantages such as improved production and good health because of scientific management practice as compared to non-cage systems. Providing amenities to cages might allow hens to reap some of the advantages of conventional cages and also better to meet their behavioural needs. Various authors have tried to explore a different cage condition that would benefit the producers as well the activist.

2.2 Effect of stocking density, flock size and furnished cages on Production performance of commercial layers

2.2.1 Effect on Egg Production:

Craig *et al.*, (1986) carried out an experiment to observe egg production performance of laying hens' at floor Vs different cage system for White Leghorns for a 40 week period starting from 22 weeks of age. The different housing condition provided with litter floor space allowance of 2900 cm²/hen, and cage with single hen (space allowance of 929cm²/ bird); cage with 04 hens (space allowance of 464cm²/hen) ; 06 hens per cage (space allowance of 310cm²/bird). A total of twelve hens were taken as an experimental unit or replication within each housing environment. It was observed that laying housing condition (pen Vs cages with different stocking density of 2900 Vs 929, 464 and 310cm²/hen) had no influence on egg production during 40 week of experiment. Although hens in 06-bird cages appeared to be slightly depressed in egg production during the first 10-week period relative to the hens of other environment, the difference was not significant. The same study continued for number of generations at the same place and it has indicated that hens in large group size, high density cages are consistently performing inferior as compared to birds kept in floor pens and single-hen cages. The findings indicated that increasingly inferior performance over long laying periods is associated with apparent stress.

The egg production of laying hens under the different flock sizes, space allowance per bird and modified cage system have been examined and published

by various authors. Mench *et al.*, (1986) studied the effect of cage and floor pen management on production of 30 week age laying hens by keeping them in different stocking density of single cages (S) with only one bird occupying with space allowance of 1394cm²; Low density cages(LD) with two birds space allowance of 1394cm² per bird; a high density cage (HD) with two bird occupation floor space of 697cm² per bird and in floor pen (P) with 25 birds with floor space of 1394cm² per bird. The hen-day egg production (HDEP) for the different groups for S, LD, HD and P were 45.8, 38.3, 38.8 and 47.1% respectively. It was observed the HDEP was significantly greater in penned and individually caged hens when compared with Low density and High density treatment groups. It was concluded that the birds maintained in group on pen system as well single bird occupied same space in cage system performed significantly ($P < 0.05$) better HDEP as compare to low and high density cage system, although higher density housing produces the overall economic benefit depending on feed cost and egg price.

Davami *et al.*, (1987) reported increased population density result in a significant decline ($P < 0.01$) in percent egg production when maintained in two group size of seven and five birds thus allowing a stocking density 420 cm² and 300cm² per bird respectively. As per feeder space the flock size of 5 birds had two more treatment, 5S having a feeder space of 8.6cm per hen and 5N (5 birds per cage) had 12cm of feeder space per hen. The third treatment 7N (7 birds per cage) with flock size of 7 bird had same feeder space 8.6cm per bird as in 5S (Restricted feeder space of 8.6cm per hen). The egg production % were 85.1, 77.3 and 67.1 respectively for 5N, 5S and 7N respectively. Egg production decreased 7.8% by decreasing feeder space from 12 to 8.6 cm per hen and by reducing floor area by 120cm² per hen egg production declined by 10.2%.

Craig and Milliken (1989) observed the productivity of White Leghorn hens in different stocking density of 348 (high), 464 (medium) and 580 (low) cm²/bird and in flock size of four and eight hens for each of the stocking densities. It was recorded that stocking density affected the rate of lay, daily egg mass and percentage survival but not group size. Data was collected from a 50-week laying

period subdivided into five 10-week period. Hens in cages having different flock size of four or eight birds per cage did not differ with egg production traits, the hen-day rate (after 50 % production) was 70.5 and 68.4 % , respectively for four and eight birds per cages. Density, expressed in terms of area per hen had significant effect on rate of lay recorded at 64, 71.8 and 72.6 % for high, medium and low stocking density. Hens in medium and low density had significantly ($P < 0.005$) better rate of production than in high density cages. There was no detectable effect at low and medium densities because of group size, but cages holding four hens were superior in rate of lay. Non-linear effects of density was evident from this study.

Appleby *et al.*, (1993) recorded no difference in egg production in layers raised till 72 weeks of age with a space of $675\text{cm}^2/\text{hen}$ for conventional cages and an additional 375 to $480\text{cm}^2/\text{hen}$ space for perches, dust baths and nest boxes in experimental cages (3 treatment). There were 3 sub treatments for the experimental cages with Litter (L) in nest boxes and dust baths, Artificial turf (A) and Plastic rollway (P). Overall in the experimental cages with facility for nest boxes, perch and sand baths, 90% of eggs were laid in nest boxes, 3 % in dust baths and 6% on the floor. The distribution between sub-treatments differed for egg laid in nest boxes with 86 % in L1 (high nest boxes and low dust bath position) and 83% in L2 (low nest boxes and high dust bath position); 92% in P1 (eggs were prevented from rolling away) and 90% in P2(eggs rolled away); 99% in A1(eggs were prevented from rolling away) and 94% in A2 (allowing eggs to roll away).

Lee and Moss (1995) conducted four experiments on population density on White Leghorn pullets raised in cages (30.5 X 50.8 cm) with density of one, two, three and four birds per cage for Treatment 1, 2, 3, and 4. They observed that treatment with highest density level that is 4 birds per cage produced the lowest percentage hen-day egg production than birds in the lowest density level (1 bird cage). Though HDEP % is generally higher for birds in single-bird cages than birds in multiple-bird cages, placing one bird is obviously not economically advantageous when considering labor and equipment costs per cage.

Abrahamsson and Tauson (1997) observed the effect of group size in furnished cages along with conventional cages on the production of Leghorn laying hens from 20th week until 80th week. The different groups were 5, 6, 7 and 8 hens per cage in the furnished cages with facility for perch, nest boxes and sand-bath. The furnished cage was 50 cm deep and 45cm high at the rear and the width differed as per the group size 5, 6, 7 and 8 to 60, 72, 84 and 96 cm, respectively giving a bird space of 600cm² cage floor area per bird excluding nest and sand-bath. No significant effects of group size within the furnished cage model could be registered on production (HDEP) of 83.5, 83.7, 82.7, 82.9 % for the bird in size of 5, 6, 7 and 8. Production in the conventional cage (84.8%) was not different from that in the furnished cage.

For the eggs laid in nesting area, on an average, 0.6% of all eggs in the furnished cage were laid in the sand and 86% in the nest. There was no effect of group size on nested eggs and eggs laid on sand. The rolling-out efficiency of eggs laid outside the nest and sand-bath area was 95%. There was an effect of group size in egg rolling out efficiency from the nests ($P < 0.01$), with better efficiency in the larger cages. Hence, in the 8-hen cage 93% of the eggs rolled out into the egg cradle and in the 5-hen cage only 85% rolled out.

Tauson (1998) recorded similar egg mass for conventional and furnished cages when kept birds in furnished cages i.e. a) Modified and enriched small cages (MEC) including nests, perches and dust baths for two strains - light-white hybrids (LSL) with 5 birds and medium-heavy hybrids (ISA-Brown) with 4 birds and b) Get Away (GA) with 15 birds of the same hybrids. The controls were maintained in two commercial cage models: c) Conventional cages with 4 birds (C); and d) Conventional plastic cages with solid side and slatted rear partitions with 3 birds (PLC). Cage floor space per bird was 600 cm² in MEC and for GA 850 cm² which was about 40% larger than Conventional. Stocking density in PLC was 720 cm² maintained as per the Swedish Directive effective in 1989 requiring that one hen be removed from existing cages at 480 cm² to achieve the stipulated 600 cm² per bird. The average for the two hybrids in MEC for egg mass and

mortality (22.3 kg and 2.6%) was similar to the conventional cages (21.8 to 21.9 kg and 4.9 to 5.8%), and better than the GA (20.2 kg and 8.6%).

It was seen that fully furnished cages for small groups of birds (<10 per cage) may compete well with conventional cages as far as production and mortality are concerned reported by Tauson (1998). Also, the smaller group size probably, had more stable rank order, improved hygiene due to the presence of perches and the greater ease of inspection, are benefits of MEC compared with GA cages.

Appleby *et al.*, (2002) studied the production parameters of laying hens for a 3year trial period in conventional cages (2500cm² area and 38cm high in the rear) and furnished cages (5000cm² in area, 50cm high in the rear) with perches, dust-bath and nest hollows. The treatment groups were C- conventional cages, F- furnished cages with roll away nests, FD- Furnished cages with dust bath and H- furnished cages with 2 nests hollow at the side, one in front of the other and the cages were furnished with 4 to 8 ISA Brown hens per cage. Cages were stocked with 4 to 8 birds per cage, resulting in varied allowances of cage area, feeder and perch per bird. Conventional cages with 4 birds had a space allowance of 625cm²/bird, 5 birds -500cm²/bird; F and FD cages with 5 birds had a space allowance of 1000cm²/bird, 7 hens-715cm²/bird, 8 hens-625 cm²/bird; H nests with 4 birds had space allowance of 1250cm²/bird,5 hens-1000cm²/bird and 7 hens- 715cm²/bird. Egg production was not significantly affected by cage designs, however, within each cage design , rate of lay was higher with fewer birds per cage (P< 0.05) from a minimum of 81% in F cages with 7 birds to a maximum of 84.9% in FD cages with 5 birds in the 1st year of lay. In the 2nd year of lay, production was 88% in C Cages with no nest, perches and density of 4 birds per cage. In Furnished cages treatments, rate of lay was significantly affected by nest design: 85% with a front rollaway nests and 88% with two nest hollows (P<0.01); number of perches- 89% with one perch and 85% with two (P<0.01);and number of birds per cage-87% with 5 birds, 84% with 8 birds (P<0.01); and 87% with standard food trough and 84% with an elevated food trough (P<0.001). Rate of

lay in the 3rd year did not vary significantly between cage designs ranging from 85 to 88%.

Anderson *et al.*, (2004) housed two commercial strains, Hy-Line W-36 and DeKalb XL, by maintaining 6 hens/layer cages at 2 stocking densities (361 and 482 cm² /bird) with 2 replications each per strain/density combination. The high-density treatment contained 24 hens/ replication and the low-density treatment contained 18 hens/ replication for a total of 168 hens. It was observed that cage density impacted the percentage HDP, which was greater ($P < 0.01$) for hens provided a density of 482 cm² /bird (82.3%) than for hens provided a density of 361 cm² /bird (77.4%). This density effect resulted in an increased ($P < 0.05$) production of daily egg mass in the hens housed at the low density.

Onbasilar and Aksoy (2005) investigated the effects of cage floor and cage density on stress parameters of laying hens using compact-type battery cages, with three floors. Hens were allocated as one, three or five hens in each of 18 cages to obtain three different cage density groups of 1968, 656 and 393.8 cm² floor area per hen, respectively. The same number of cages with different cage density were allocated to three different battery floors (first floor =top, second=middle, third=bottom) systematically. Increasing stocking density had a negative effect on egg production percentage 94.1%, 89.3% and 78.5% for 1968, 656, and 393.8cm² per hen space respectively. Cage floor position did not affect hen–day egg production.

Vits *et al.*,(2005) observed the production and welfare of laying birds from 2 different lines Lohmann brown and white maintained in 3 different type of furnished cages-Aviplus, Eurovent 625a and Eurovent 625A with a space allowance of 750 cm² per hen equipped with perches, a nest box, a dust bath and devices to shorten the claws. The perches were incorporated parallel to the front of the cage. Only in the groups of 10 hens in the Eurovent 625A system were there another rectangular perch. The claw-shortening devices were different in the 3 furnished cage types. In the Aviplus system it consisted of either two abrasive blocks per cage or a perforated metal plate. In the Eurovent 625a system one abrasive block per cage was installed, whereas the system Eurovent 625A

contained adhesive stripes. The group size of birds were 10 and 20 birds for Aviplus and Eurovent 625A; and in group of 40 and 60 birds for Eurovent 625a. The results for the different group sizes in each housing system showed the highest egg production per average hen housed (89.4%) was found in the groups of 20 hens in the Aviplus system. Egg production among the furnished cages per average hen housed (88.9%) and feed consumption of (112.3 g) were higher among hens housed in the Aviplus system than in the other systems.

Benyi *et al.*, (2006) maintained 2 Hyline strains at different stocking densities of 1100,733 and 550cm²/hen by keeping 2, 3 or 4 birds per cage. The hens housed in pairs i.e., 1100 cm²/hen space allowance produced significantly (P < 0.05) more eggs 85% HDEP and had a higher egg output as compared to birds maintained at 733 and 550cm²/hen space of 73 and 70% HDEP respectively. Hens that were housed 3 and 4 per cage did not differ significantly in egg production and egg output. The hens housed in the highest stocking density that is 4 hens per cage which was provided with a floor space of 550 cm² per bird had a reduced egg production, egg output but utilized feed more efficiently than birds housed with 2 to 3 birds per cage (1100cm² and 733cm²/bird space).Egg production showed a significant (P<0.05) interaction between strain and stocking density.

Jalal *et al.*, (2006) have given four space allowance 342,413, 516 and 690cm²/hen that were fed 3 different levels of metabolizable energy (ME-2800,2850 and 2900Kcal of ME/kg) for 15 weeks period to a commercial laying hen strain. It was noticed that egg production and egg mass significantly improved for the hens housed at 690cm²/hen across all energy levels in comparison to other spaces provided to birds. The egg production declined as much as 10.1% (86.19 Vs 76%) as the number of hens per cage was increased from 3 to 6. There were no interaction effects of ME with the various stocking density except for the body weight change. Hens housed at 690cm²/hen had significantly (P<0.05) greater ME efficiency of egg production than hens housed at other cage space.

Karkulin (2006) checked the production and egg quality parameters of laying hens housed in conventional cages and enriched cages. In the conventional

cages 5 birds were maintained with a stocking density of 550cm² per hen and in enriched cages 22 hens were kept equipped with facility of perches, nest box and litter areas that meet the Directive 1999/74 EC criteria. The hen-day production was recorded daily during the 303 days laying period. Very little appreciative result or rather non-significant result were obtained for hen - day production (88.72%) which was reached by hens in enriched cages, that was in average a little bit more compared to hens housed in conventional cages (87.57%). Hens housed in conventional cages laid lower count of eggs at the beginning of the laying period compared to hens in enriched cages, but the differences were erased in the next month. This development in conventional cages signalizes that hens reached sexual maturity a bit later compared to hens in enriched cages.

Wall and Tauson (2007) studied the effect of 3 different perch arrangements in small group furnished cages maintaining eight numbers of laying hens and compared with conventional cages having four number of laying birds. A perch was either fitted across the cage FC- A, providing 12 cm of perch per hen, or 2 perches were installed in a cross FC-B and FC-C, implying 15 cm per hen. The conventional cage (C) measured 48×50×38 cm (width× depth× height) while the furnished cages measured (excluding nest and litter box) 96×50cm (width×depth) and was 45 cm high at the rear. A nest box, 27-cm high in the front, 24-cm wide and 50-cm deep, was positioned at one end of the cage and on top of the nest was a litter box. Production was recorded from 20th week to 80th week of age and found no difference for furnished, FC (A-87.8%, B-88.3% and C-87.6%) and conventional cages (87.9%).

Sarica *et al.*, (2008) studied the effect of space allowance (2000, 1000, 667 and 500 cm² per hen) on egg yield of ISA Brown birds by allocating 1, 2 ,3 and 4 hens per cage of size 40 cm x 50cm for an experimental period of 18 to 53 weeks. Increased space allowance or decreased cage stocking density significantly ($P < 0.5$) enhanced egg production. Hens kept either at 667 cm² or 1000 cm² cage densities produced the same amount of eggs (212 and 211 numbers) while those kept at 500 cm² space allowance decreased egg production with a delay in reaching the 50% egg production age by 5 days ($P < 0.05$). There were no

differences between the treatment groups with respect to live weights at 50% production age, which was taken as criteria for sexual maturity. However, live weights of hens at the end of the study were the highest in 2000 cm² space allowance cages ($P < 0.05$). Viability rates were decreased by lower space allowances with the lowest viability at 500 cm²/hen.

Cronin *et al.*, (2009) examined the effects of group size: single and eight hens, on the HDEP and the proportion of nested eggs maintained in cages measuring 1.2m wide, 0.5 m deep and 0.45 m high at the rear. All cages contained a nest box located on the right side which measured 0.24 m wide, 0.5 m deep and 0.27 m high at the front. The floor of the nest box was overlain with 'Astro turf' (0.22 m x 0.37 m x 15 mm thick). Hen Day Egg Production between 19-33 weeks of age did not differ due to group size and was recorded as 95.1% and 92.4% for single and eight bird cage treatments respectively. Group size however, affected the proportion of nest box eggs between 19-33 weeks of age with maximum percentage for cages with single bird (89.9%) when compared to cages having 8-birds (65.6%). It has been indicated that social factors contribute to hens laying outside the nest box.

Pohle and Cheng (2009) examined housing environment (furnished cages vs. battery cages) effects on the well-being of laying hens. One hundred ninety-two day-old, non-beak-trimmed White Leghorn W-36 chicks were reared and randomly assigned into battery cages or furnished cages at 19 weeks of age. The furnished cages had wire floors and solid metal walls, with perches, a dust bathing area, scratch pads, and a nest-box area with concealment curtain. Ten hens were housed per cage, providing a stocking density of 610 cm² of floor space per hen. The battery cages were commercial wire cages containing 6 birds per cage, providing 645 cm² of floor space per hen. It was observed that compared with the hens housed in the battery cages, the hens housed in the furnished cages produced more eggs at 40 week of age ($P < 0.05$). Hens housed in the furnished cages reached a peak egg production earlier than those housed in the battery cages [i.e., 40:50 week of age (furnished cage: battery cage)].

Tactacan *et al.*, (2009) maintained Shaver White hens from 21 to 61 weeks in either conventional battery cages or in enriched cages. The birds in conventional cages (Wide-50.5cm x Deep-55.9 cm x Height-38.1cm) were kept in a density of 5 birds per cage allowing a floor space of 561.9 cm²/hen. The enriched cages (Width -241.3 cm x deep-55.9cm x Height-48.3cm) had 24 hens per cage and offered a space of 642.6cm²/hen with facility for curtained nesting area (58.4cm wide x 25.4 cm deep), scratch pad (48.3cm wide x 20.3cm deep) and perches. The nesting area was separated from other areas by a red curtain composed of plastic strips, through which the birds entered the nest. The overall hen-day egg production was not significantly different between the 2 cage designs. The only significant difference between treatments was observed in period 10, in which birds in conventional cages (88.6%) had higher hen-day egg production than birds in enriched cages (87%). The rate of lay in both treatments over the period of 21 to 61 weeks ranged from 84.5 to 95.9% and was within the range.

Shimmura *et al.*, (2010) conducted to study find the effect of different housing systems on egg productions maintained the 284 birds from 16 weeks of age into one of 6 housing systems: two types of conventional cages small-SC and large-LC; two of furnished cages-Small furnished as SF and Large furnished –LF. The small conventional cage (SC) was a commercial laying cage 23 x 40 x 42 cm (wide x deep x high) at the rear. The cage provided 450 cm² with 12 cm feeder space per hen and one nipple drinker per cage. The large conventional cage (LC) was 31 x 40 x 42 (wide x deep x high) at the rear. The cage provided 600 cm² with 15 cm feeder space per hen and two water nipples per cage. The small furnished cage (SF) was 65 x 46.5 x 47 cm (wide x deep x high) at the rear providing 604.5 cm² per hen. The nest was equipped with a perch, nest box and a dust bath. The large furnished cage (LF) was commercially produced 240 x 625 x 47 cm (wide x deep x high) at the rear. Each cage was provided with a nest, a dust bath, and two perches. The main cage area provided 6583 cm² of space per hen. There was no significant effect of egg production in the different rearing system.

Wall (2011) checked the production performance of layer hybrids from 20 until 72 weeks of age in 4 designs of furnished cages (FC) housing 8 (T8), 10 (T10), 20 (T20) and 40 (T40) layers. The treatments differed in designs of furnished facilities other than the group size. The T8 (121 x 50 x 45 cm; width x depth x height) had a deep and narrow nest box (1,200 cm²) positioned at one end of the cage and reaching all the way to the feed trough in T 10 (121 x 63 x 45 cm; width x depth x height) the nest (1,500 cm²) was located along the rear partition. In T20 (242 x 79 x 45 cm; width x depth x height) the nest (3,600 cm²) was positioned in one end of the cage. Treatment group T40 consisted of two T20 cages combined either by removing the rear partition in the perch area or by supplying the rear partition with 2 popholes, each with a width of 20 cm. There was no difference in egg laying % (hen day) among the different group size in the four different furnished cages of 86.3, 84, 85 and 84.9% for T8, T10, T20 and T40 respectively. The proportion of eggs laid in nest varied with cage design ($P < 0.001$) and use of nests was lower in T20 (84.2%) and T40 (84.2%) than in T8 (96.6%) and T10 (95.7%).

Guo *et al.*, (2012) studied the effect of group size and stocking density on the welfare and performance of Hy-line Brown hens housed in furnished cage system. Three housing system were assigned to them : Control with 4 hens per cage with a space of 398 cm² per hen and 2 furnished houses, small and large group size with perches and nest. In the small furnished cages 21 hens were housed with a space of 586 cm² per bird and in large furnished cages 48 hens were kept with a space of 543 cm² per bird. Laying rate was not significantly affected by the different housing system for control, small and furnished cages (89.85%, 86.85% and 84.78%).

Rajendran *et al.*, (2013) conducted a trial for a four-week period (that is 41-44 weeks of age) with different stocking density to see the influence on egg production and egg weight in commercial White Leghorn layers. The birds were randomly allotted into five different floor spaces of 270, 135, 90, 67.5, and 54 square inches per bird by allowing single, two, three, four and five birds per box respectively by utilizing 5 rows with 30 boxes in the cage house. All the birds

were reared under elevated cage system with the cage dimension of 15-inch front feeding length, 18-inch depth and 15-inch height. They concluded that hen day egg production was significantly higher in birds provided floor space of 270 sq. inch per bird as compared to others spaces provided. Significantly ($P < 0.01$) higher hen day egg production was recorded in birds provided with floor space of 270 sq. inches per bird (90.71%) than other different floor space, followed by 135 sq. inches per bird (88.69 %) and 90 sq. inches per bird (86.99 %) with no significant difference among them during the experimental period.

Meng *et al.*, (2014) investigated the effects of large or small furnished cages on laying hens. Hyline brown hens ($n=360$) were placed into three treatments with six replicates: large furnished cages (LFC=300 cm x 100cm x 100cm), small furnished cages (SFC=120cm x 50 cm x 45cm), and conventional cages (CC=192cm x 33cm x 35cm). The furnished cages were equipped with nests, sand boxes and perches. There was significant difference for egg production, which was lower ($P < 0.05$) for LFC hens-62.86% than for SFC-68.36% and CC-69.86% hens. The furnished cages provide hens with more space, allowing them for more activity. As a result, the higher utilization of energy in large furnished cages led to the lower egg production observed in this study.

Denli *et al.*, (2016) did a study on comparative performance and egg quality of laying hens in enriched and free cages range where eight hundred 19-wk-old Lohmann Brown hens were housed in enriched cages ($n=400$; 16 cages; 25 hens per cage) and in free-range system ($n=400$) to 50 weeks of age. The enriched cages (120 x 55 x 45; length \times width \times height) had wire floors and solid metal walls. In free-range system, hens were housed in a stocking density with six hens per m^2 door and 0.2 hens per m^2 of range area. The hen-day egg production was significantly higher in enriched cages than free range system throughout the experiment ($P < 0.05$) at all age group at week 30 = 91.6% vs 87.4 %, week 40= 94 % vs 90.3 % week 50= 91.1% vs 88.6 %.

Kang *et al.*, (2016) investigated the effects of stocking density on the performance of laying hens maintained in floor pens where four stocking densities, including 5, 6, 7, and 10 birds/ m^2 , were compared in Hy-line Brown

laying hens for a period of 8 weeks. Over the course of the entire experiment (34 to 41 week), hen-day egg production was lower for ($P < 0.01$) 10 birds/m² stocking density than other stocking densities. In this study, laying performance was shown to decline in response to increased stocking density which is attributable to a reduction in the amount of feeding area per hen.

Widowski *et al.*, (2017) studied the effect of two space allowance 520 cm² and 748 cm² and 2 cage size –Smaller furnished cages and Larger Furnished cages which were furnished with curtained nesting area, perches and scratch mat on laying hens. This resulted in 4 group sizes: 40 vs. 28 birds in smaller FC (SFC) and 80 vs. 55 in larger FC (LFC). Data were collected from 20 to 72 weeks of age. There was no effect of cage size ($P = 0.21$) or SA-space allowance ($P = 0.37$) on hen day egg production, egg weight (P Size = 0.90; PSA = 0.73), or eggshell deformation (P Size = 0.14; PSA = 0.053), but feed disappearance was higher in SFC than LFC ($P = 0.005$). Hen day egg production in cages with different space allowances did not change over time ($P = 0.77$). However, there was a significant interaction between cage size and the age of the birds ($P = 0.010$); egg production was higher in Small FC compared to Large during some week early in the production period (week 26 to 27 and 31 to 34).

Mangnale *et al.*, (2018) studied the production performance of layers in different cage type – Battery, California and Enhanced cages with different stocking density. It was observed that in California cages birds allotted with a floor space of 750 cm² had better production as compared to space allowance of 450 and 465 cm². In battery cages, the 450 cm² floor space had better production than 465 and 750 cm² and in enhanced cages the birds reared with 750 cm² of floor space recorded significantly ($P < 0.05$) better production performance. It was concluded that with low stocking density the production performance of layers was found to be better in California and Enhanced cages at 750 cm² per bird floor allowance. However, the incidence of broken eggs was more in both type of cages. In Battery and Enhanced cages, the birds reared with floor space of 450 and 750 cm² per bird recorded higher net profit.

2.2.2 Effect on Feed Consumption and Feed Efficiency:

To see the effect of population size and floor space per bird Davami *et al.*, (1987) maintained birds in two size groups of seven and five birds thus giving a stocking density 420 cm² and 300 cm² per bird respectively. The feed consumption g/hen/day was 126 and 117 for 5S (Restricted feeder space of 8.6 cm per hen) and 7N (7 birds per cage) treatment with difference that were highly significant ($P < 0.0001$). Hens maintained at the lower population density with no restriction of feed trough length 5N (5 birds per cage) consumed 18 g more feed per hen per day and had improved feed conversion over those in the more populated cages (7N).

Lee and Moss (1995) found feed efficiency was poorer ($P < 0.05$) for WLH birds kept with 4 bird per cage as compared to 1 bird per cage. This was due to reduced egg production rates of birds in 4 birds per cage. Correlation coefficient analysis revealed that egg production was negatively correlated with the population density. Feed required to produce a dozen eggs was positively correlated with ($P < 0.01$) population density in three out of the four experiments.

Anderson *et al.*, (2004) observed that the appetitive behavior frequencies, that is food consumption and feed efficiency were similar for both strains and densities and, surprisingly, there were no significant interactions between strain, density, and age when housed two commercial strains, Hy-Line W-36 and DeKalb XL, by maintaining 6 hens/layer cage at 2 stocking densities (361 and 482 cm²/bird) with 2 replications each per strain/density combination.

Benyi *et al.*, (2006) found that stocking density significantly ($P < 0.05$) affect the food intake of birds that were housed in 2, 3 and 4 birds per cage with a space allowance of 1100, 733 or 550cm²/hen, respectively. Hens that were housed 3 birds per cage consumed significantly less feed as compared to treatments where 2 birds were kept per cage. Hens housed in pairs utilized food less efficiently (148.6 g/egg) to produce an egg and a gram of egg than hens housed 3 (122.2g/egg) or 4 (101.7 g/egg) per cage. The feed efficiency, that is gram of feed

to produce per gram of egg was 2.4, 1.9 and 1.7 respectively for birds kept in stocking density of 1100, 733 and 550 cm²/bird.

Cage space had a significant ($P < 0.05$) effect on feed intake of laying hens. Hens housed at 690cm²/hen had greater feed intake than those fed at 342 and 413cm²/hen, but not those housed at 516cm²/hen across all dietary ME levels. This was stated by Jalal *et al.*, (2006) in their study on laying hens to see the effect of bird cage space and dietary metabolizable energy level on production parameters.

Non-significant results were observed for feed consumption and feed efficiency by Karkulin (2006) between systems of hen keeping, which is in enriched and conventional cages. Hen - day consumption of feed was on the same level in both the enriched and conventional cage types. Slightly higher feed consumption per egg produced in conventional cages (132.45g per g of egg) were caused by lower egg production of hens housed in conventional cages than in enriched cages (130.7g per g of egg).

The mean Feed Intake (FI) was different ($P < 0.05$) among the 3 groups of stocking density in broiler breeders as observed by Mtileni *et al.*, (2007), with a reduction in FI as density level increases. Thus, greater stocking densities were associated with significantly lower feed consumption. This may be attributable to increased competition for feeding space among the birds.

Pohle and Cheng (2009) studied comparative effects of furnished and battery cages on performance in white leghorn hens and found feed efficiency was not affected by the furnished and battery cages from 55 to 60 week of age ($P > 0.05$).

Tactacan *et al.*, (2009) conducted an experiment for 40 weeks and each one period comprise of 4 weeks. They reported a significant effect of cage type on feed efficiency (g of feed/g of egg) when birds were maintained in conventional cages (1.97) and enriched cages (2.04) in period 9. The conventional cages had 5 birds per cage allowing a floor space of 561.9cm²/hen and the enriched cages had

24 hens per cage and offered a space of 642.6cm²/hen. It was noted that birds in conventional cages consumed significantly more feeds than birds in enriched cages in experiment periods 1 and 4 but significantly less in period 8 and 9. There was a significant ($P < 0.0001$) period effect for feed consumption. Feed consumption was highest in period 5, remained relatively constant throughout periods 6 to 8 and gradually declined from period 9 to 10.

Guo *et al.*, (2012) observed hens housed in large furnished cages and small furnished cages had a higher feed intake ($P < 0.05$, $F_{2,15} = 4.97$) and poorer feed efficiency ($P < 0.01$, $F_{2,15} = 10.96$) than that of controls, that is conventional battery cages (45.5 x 35 x 37 cm; length x width x height), with four hens per cage. The floor area was 398 cm² per hen.

Hester *et al.*, (2013) on the trial for effect of perches in cages during pullet rearing and laying had observed chickens with access to perches during the pullet phase had used more feed (102.4 Vs 98.4 g/hen/day), and had poorer feed efficiency (1.453 Vs 1.404) during egg laying than control chickens without access to perches as pullets (pullet phase main effect, where $P = 0.001$, 0.001, and 0.02, respectively). During the laying phase, the opposite trend occurred in that control hens without perches had an increase in feed utilization and efficiency that approached significance ($P = 0.06$ and 0.07, respectively).

Meng *et al.*, (2014) observed that layers in the large furnished cages tended to have lower efficiency of feed conversion (i.e. feed: egg ratio) than small furnished cages or conventional caged hens, although large and small furnished caged hens had similar daily feed intake. The possibility of lower feed intake in the Large furnished cages may be attributed to the greater space allowance (750 cm² per hen) which may increase hen's social competition for resources such as high perches or the dust-bathing box. Presumably, if dominant hens occupy a high perch, they are reluctant to give it away; hence, longer occupation of the high perch would mean less time to feed, which in turn, may reduce total daily feed intake.

Hens in enriched cage system consumed significantly lower feed than hens in free-range system at 50 weeks of age ($P < 0.05$) was reported by Denli *et al.*, (2016). They maintained bird from 19th week to 50th week in enriched Vs free range system 103.7 Vs 107.6, 115.2 Vs 118.4 and 118.6 Vs 124.7 g/hen/day for Enriched Vs Free-range. The feed efficiency were also significantly better in the three period in enriched cages compared to free range of value 2.15 vs 2.20, 2.08 Vs 2.10, 2.12 Vs 2.14 at 30, 40 and 50 weeks of age respectively.

2.2.3 Effect on Live Body Weight:

Body weight for various group sizes of 5,6,7 and 8 birds was determined by Abrahamsson and Tauson (1997) for furnished cages (with perch, sand-bath, nesting) and compared body weights of birds reared in furnished with conventional cages. No significant difference between treatments group was found regarding the live weights of birds recorded at 55 weeks of age. The average body weight of the layers was 1.91 kg.

Heckert *et al.*, (2002) raised birds from hatch to 42 d of age with different stocking density of 10, 15, and 20 birds/m² in each pens, with and without the availability of horizontal perches to see the variation among the birds. They observed no significant differences ($P > 0.01$) among the final body weights and body weight gain of the birds at the different rearing densities or perch treatments.

Keeling *et al.*, (2003) recorded the production related traits of layers in different sized flocks kept in four different groups of 15, 30, 60 and 120 from 0 to 40 weeks. The birds were maintained in a stocking density of 5 birds /m² in litter floor pens. Birds were individually weighed at 3, 7, 12, 15 and 18 weeks of age. It was noticed on an average the birds at group size 30 and 120 were lighter than treatment group of sizes 15 and 60 ($P < 0.05$) and this zigzag pattern was consistent at all the ages. The allocation of resources like feeder and floor space was same in all treatment groups, the difference in body weight among group sizes is unlikely to be explained by restricted physical access to resources rather to be variation in the social behavior of birds. Groups of 15 used aggression to establish and maintain stable dominance relationships; while the birds in groups of 60 and 120

had a relatively nonaggressive, tolerant social behavior; and birds in the intermediate groups of 30 experiencing social disruption because the groups were too large for a stable hierarchy to develop but too small for a tolerant social system to occur.

Stocking density significantly affected the final body weight ($P < 0.05$) when birds were given different density of 1100, 733 or 550 cm^2/bird by Benyi *et al.*, (2006) in the study on the effects of stocking density and group size on the performance of white and brown Hyline layers in semi-arid conditions. It was concluded from the experiment that on an average the hens that were housed at 2 or 3 birds with density of 1100 and 733 cm^2/bird had a higher final body weight, consumed more food, gained more body weight than hens housed at 4 bird per cage with a space of 550 cm^2/bird . There were no significant differences in final body weight and body weight change between hens at stocking density of 1100 and 733 cm^2/bird .

Davami *et al.*, (1987) reported mean body weight at 65 weeks of age of hens maintained at flock size of seven hens per cage (7N) was 1620g and were significantly ($P < 0.05$) lower than those in the two treatments with five hens per cage (5N) with feeder space of 8.6 cm per bird (5S-1690 g) and 12 cm per bird (5N-1693g). A comparison of the 5S vs. 7N treatments may demonstrate that additional nutrients used by the hens in treatment 5S were possibly used for egg production, body tissue, and extra movement of hens with only 5 birds per cage.

Onbasilar and Aksoy (2005) indicated differences in body weight, only at the beginning of the experiment, that is time versus cage density and cage floor were not statistically significant. Increasing cage density, from one and three to five hens/cage, resulted in a lower body weight; however, cage floor position had no effect on body weight.

Sarica *et al.*, (2008) found no difference for different stocking densities (500, 667, 1000 and 2000 cm^2/hen) with respect to live weights at 50% production age, which was taken as a criterion of sexual maturity. However, live weights of hens at the end of the study were the highest in 2000 cm^2/hen space

allowance in cage which was significantly different from that of 667 and 500cm²/hen space.

Hester *et al.*, (2013) on the trial for effect of perches in cages during pullet rearing and laying had observed that chickens with access to perches during the pullet phase had heavier BW (1.791 Vs 1.724 kg), during egg laying than control chickens without access to perches as pullets (pullet phase main effect, P =0.001). During the laying phase, the opposite trend occurred in that control hens without perches had greater BW (1.801 Vs 1.714 kg) than hens with perches (P < 0.01).

2.3 Effect of stocking density, flock size and furnished cages on egg quality:

2.3.1 Egg weight and internal egg quality:

Davami *et al.*, (1987) reported eggs from hens with lower group size and higher space 5S (stocking density 420 cm²/b, group size 5 birds and feeder space 12 cm per hen) and 5N (stocking density 420 cm²/b, group size 5 birds and feeder space 8.6 cm per hen) per hen had significantly (P<0.05) lower egg weights when compared with eggs from treatment 7N (stocking density 300cm²/b, group size 7 birds and feeder space 8.6 cm per hen). Higher feed consumption in 5S and 5N treatments leads to the expectation of larger egg size in these groups, which did not develop but it may be possible that extra feed was needed to maintain the larger body size of these groups. No significant (P > 0.05) differences were noted in shell thickness, specific gravity, and Haugh units of eggs produced by hens maintained in the different treatments. These observations indicate that bird density has little effect upon egg quality traits.

Different stocking density of 348 (high), 464 (medium) and 580 (low) cm²/bird and in flock size of four and eight hens for each of the stocking densities had no any effect on egg weight as observed by Craig and Milliken (1989). The egg weights recorded for high, medium and low stocking density was 58.1, 57.6 and 57.6 g and for flock size of four and eight birds the egg weights were 57.6 and 57.3 with non-significant effect for both flock size and stocking density though egg weights increased throughout the five 10-week periods.

Egg weight averaged for all the weeks of experimental period was not affected by population density as stated by Lee and Moss (1995) in a study conducted on Leghorn layers for population density and layer performance. Only in one out of the four experiments, eggshell thickness was affected (0.387>0.374>0.374>0.364) when birds were kept in stocking density of one, two, three and four in a cage size of 30.58 x 50.8 cm. Also, the albumen height found a significant difference in experiment 3 (8.52>7.92>7.97>7.88) which was lowered ($P < 0.05$) by having more than one bird per cage.

Appleby *et al.*, (2002) maintained birds for 3 years of lay in Conventional-C and Furnished-F cages and reported that in 1st year at 60 weeks of age there was significant effect of cage type on mean egg weight ($P < 0.01$) when the cages were furnished with dust baths, roll-away nest, hollow nest boxes, perches and food troughs at different levels. In 2nd year the effect of stocking density recorded an average egg weight from C cages with 4 bird was 63.4g. It was lower in cages with 5 birds at 63 g and with 8 birds at 62.6 g ($P > 0.05$) which were non-significant. Also non-significant trends for lower egg weight in cages with nest boxes and in those with perches.

Keeling *et al.*, (2003) reported that egg weight was affected by flock size when birds were maintained at group sizes of 15, 30, 60 and 120 from 0 day to 40th week with same stocking density of 5 bird per sq. meter space. The effect of group size on egg weight was consistent ($P < 0.01$) with the different age when egg weights were recorded that is at 24 and 39 week of age. The treatment group with flock size of 30 birds laid smaller eggs ($P < 0.05$) as compared to flock size of 15, 60 and 120. It was concluded from the observation that birds in intermediate group 30 experiencing social disruption because the groups were too large for a stable hierarchy to develop but too small for a tolerant social system to occur. [Such a change in social organization with increasing group size is predicted in theory (Pagel and Dawkins, 1997)].

Onbasilar and Aksoy (2005) reported a difference in egg weight in birds that were kept in different stocking density of 1968, 656 and 393.8 cm² floor area per hen, respectively for hens kept single, three and five hens per cage and floors

(1st, 2nd and 3rd) with respect to time. Egg weight increased significantly with time. Eggs in the first floor were heavier (64g) than the second (62g) or third floor (62g). Increasing cage density from one and three to five hens per cage resulted in a lower egg weight 63.4g, 63.6 and 62.4 g respectively with stocking density between one and three birds per cage has no significant difference. There was an interaction between floor and density. The eggs that were laid on the first floor were heavier than the eggs laid in other floors possibly because the hens in first floor was fresher than others resulting in heaviest eggs. Cage density (1968, 656 and 393.8 cm² floor area per hen) had no effect on egg shape index, egg-shell breaking strength, shell thickness or yolk index. The values of albumen index and Haugh unit were higher in cages containing five hens than in the cages with one or three hen, but some changes were detected in shell thickness, yolk index, and Haugh unit of laying hens with time. None of the interactions of cage density cage floor, cage density period and cage floor period were significant for these egg quality characteristics.

Karkulin (2006) reported that hens housed in enriched cages laid significantly ($P < 0.05$) heavier eggs (63.70g) compared to hens housed in conventional cages (62.77g). Other quality parameters found no significant differences among the cage systems. Egg white and yolk quality was almost unaffected by cage type. Greater differences, but still not significant, were in egg shell quality achieved. Results signalize some negative correlation between egg weight and its shell strength. As the egg is larger, its shell strength declines. This is probably due to larger egg needs of calcium, which is responsible for shell development.

Wall and Tauson (2007) reported no effect on egg weight for 3 different perch arrangements in small group (8 laying hens) of furnished cages (65.1g, 65.1g, and 64.7 g for FC-A, FC-B and FC-C respectively) among themselves and also when compared with conventional cages (65.4g) having four number of laying birds.

Sarica *et al.*, (2008) observed no effect of stocking densities (2000, 1000, 667 and 500 cm² per hen) on the egg quality traits. The external egg quality traits

such as egg weight, specific gravity, shell breaking resistance, shell weight, shell percentage and shell thickness were not affected by stocking densities. However, egg shape indices were different between the treatments and the highest shape index was in 500 cm² treatment ($P < 0.05$). Limiting the space allowance tended to decrease yolk and albumen quality without statistical significance while the numbers of eggs with meat and blood spots were higher in cages of 667 cm² and 500 cm² space allowance per hen.

Pohle and Cheng (2009) observed egg weight was significantly affected by age ($P < 0.05$) but not by the treatments from week 30 to 60 when kept the birds in furnished cages and battery cages. Eggshell thickness was not significantly affected by the treatments from 50 to 60 week of age ($P > 0.05$).

Tactacan *et al.*, (2009) observed that average egg weight did not differ when birds were kept in 2 cage types: Conventional and Enriched with different stocking density of 561.9cm²/hen and 642.6cm²/hen, respectively. The weights of the eggs of birds from conventional cages were higher in period 2, 4 and 6, but these did not affect the average. As expected egg weight increased with hen maturity. A cage x period interaction ($P < 0.01$) was observed during the experiment because of heavier eggs being produced by hens in conventional cages than in enriched cages, with progressive maturity.

Guo *et al.*, (2012) reported no effect of housing – conventional and furnished and effect of flock size in the furnished cages on egg shape index and eggshell thickness.

Rajendran *et al.*, (2013) reported that egg weight was significantly ($P < 0.01$) superior (59.77 g) in the birds provided with floor space of 270 sq. inches per bird -T1 as compared to the other stocking density of 135-T2, 90-T3, 67.5-T4 and 54 sq. inch space per bird space-T5. T5 had the poorest egg weight of 57.09g and was significantly different than T1, T2, T3 and T4 groups. There was non-significant difference in egg weight for stocking density of 135 sq. inches per bird (58.86 g), 90 sq. inches per bird (58.73g) and 67.5 sq. inches per bird (58.18 g) in their trial for cage stocking density in commercial White Leghorn layers.

They concluded that stocking density had a high influence in egg production and egg weight in commercial layers and egg production and egg weight decreased with decrease in floor space.

Hester *et al.*, (2013) on the trial for effect of perches in cages during pullet rearing and laying had observed that chickens that always reared with perches had similar egg production, cracked eggs, egg weight, shell weight, % shell and shell thickness compared to birds that never had perches. For egg traits, only egg weight was affected by previous exposure to perches as pullets; specifically, chickens with access to perches during the pullet phase laid heavier eggs (61.8g) than controls (61.2g) without access to perches. Perch access during egg laying resulted in hens laying eggs with lower egg weights ($P < 0.0001$, 61.0g Vs 62.1g) and shell ($P = 0.006$, 5.180g Vs 5.275g) weights compared with control hens without perches.

Meng *et al.*, (2014) observed that the egg weight tended to be lighter in the large furnished cages (LFC) treatment than in Small furnished cages (SFC) or conventional cages. Eggs produced from LFC and SFC had better HU and albumen height when compared to conventional cages ($P < 0.05$). In LFC, HU and albumen height were numerically higher than in SFC, but was not significant ($P = 0.41$ and $P = 0.54$). Eggshell thickness was significantly ($P < 0.05$), higher in LFC than in CC and egg shell weight was higher in LFC than in SFC ($P < 0.05$). Cage type could not produce any significant effect for eggshell strength ($P = 0.32$) and yolk weight ($P = 0.22$). The lower egg production of the LFC hens (which showed no difference in feed intake from CC hens) might have resulted in thick egg shells. Lower egg production with the same amount of calcium would convert more blood calcium into egg shell, thus making the egg shell thicker.

Denli *et al.*, (2016) reported hens raised in free-range system had greater egg weight than in enriched cages ($P < 0.05$) in the three period (30, 40 and 50 week of age- 59.8 Vs 56.2, 62.8 Vs 60.1 and 64 Vs 62.1 g, respectively). Significant influence of rearing system was obtained on egg shape index at 30 weeks of age, on egg shell thickness throughout the experiment ($P < 0.05$). However, the shape index did not differ between two rearing systems at 40 and 50

weeks of age ($P > 0.05$). The thickest egg shell was recorded in eggs from hens reared in free-range at 40 and 50 weeks of age ($P < 0.05$). Egg quality is important for the economic success of a producer and also consumer appeal. Egg quality may be influenced by several factors including housing regimen and nutritional values. The overall egg internal quality parameters (albumen height and width; and yolk height and width) were not significantly ($P > 0.05$) different between the 2 rearing systems.

Kang *et al.*, (2016) in their investigation on stocking density of laying hens maintained in floors found no significant differences in egg quality among stocking density treatments for eggshell thickness, egg yolk color, and Haugh unit. However, eggshell strength was less ($P < 0.05$) for 10 birds/m² stock density than other stock densities. Eggshell strengths of 4.31, 4.39, 4.41, and 4.11 kg/cm² was measured for the respective stocking densities of 5, 6, 7, and 10 birds/m².

Windowski *et al.*, (2017) observed that egg weights were unaffected by cage size Small and Large Furnished cages ($P = 0.90$) and Space Allowance of 520 cm² and 748 cm² ($P = 0.74$). There was a significant increase in egg weight with age ($P < 0.0001$). Birds in furnished cages had overall average egg weight of 60.0 ± 0.4 g, and those in CC had overall average egg weights of 59.4 ± 0.3 g.

2.3.2 Dirty and cracked eggs

Appleby *et al.*, (1993) recorded low incidence of downgraded eggs in layers raised till 72 weeks of age with a space of 675cm²/hen for conventional cages as compared to furnished cages which had an additional 375 to 480cm²/hen space for perches, dust baths and nest boxes for the 3 furnished- experimental cages. There were 3 sub treatments for the experimental cages with Litter (L) in nest boxes and dust baths, Artificial turf (A) and Plastic rollway (P). There was a non-significant trend for more cracked eggs in experimental treatments (L 1.8%, A 1.1%, P 2.0%) than in treatment Conventional cages (1.0%); most of these cracked eggs were collected from the cage floor and may have been laid from the perches. Similarly, there were more dirty eggs from treatment L (2.3%) than from

others (A 0.4%, P 1.1%, C 0.9%), but not significantly so. These eggs were mostly laid in nest boxes which were contaminated with faeces.

Abrahamsson and Tauson (1997) observed the effect of group size in furnished cages compared with conventional cages on the production proportion of dirty and cracked eggs for laying hens managed in these two housing system for a period of 60 weeks. There was no difference in the cracked egg percentage and dirty egg percentage among the different group sizes allotted for the furnished cages. However there was a lower proportion of cracked eggs (2.6%, $P < 0.001$) and a higher proportion of dirty eggs (5.2 %, $P < 0.01$) in conventional cages as compared to furnished cages. The percentage cracked eggs among the group size of 5, 6, 7 and 8 in furnished cages were 5.4, 6.4, 5.4, and 4.8 % and dirty eggs were 2.8, 3.3, 3.1 and 2.8% respectively.

Increased percentage of cracked eggs was observed by Moinard *et al.*, (1998) in cages with perches and having height 60cm and space allowance of 800 cm²/hen (T7) on comparison to treatment group with same size cage and without perch (T6). Seven different treatment groups were taken for this study on effect of cage area, cage height and perches on feather condition, bone breakage and mortality of laying hens. Treatments differed in space allowance per hen (450, 600 or 800 cm²) and height of the cage (40 or 60 cm), giving six different treatment groups T1(450 x 40), T2 (600 x 40), T3 (800 x 40), T4(450 x 60), T5(600 x 60) and T6 (800 x 60). In the large (800 cm² /hen) and high (60 cm cage) treatment, half of the cages were equipped with perches (20 cm/hen) which was T7 (800 x 60 +P).

Tauson (1998) observed the proportions of cracked and dirty eggs were higher and lower, respectively, in MEC-modified and enriched cages (9.2% and 2.0%) in comparison with Conventional (5.0% and 6.0%) and PLC-Conventional Plastic cages (8.6% and 6.3%). Cracked and dirty eggs were lower in MEC than in the GA (18.6% and 4.9%). The increased proportion of cracked eggs in fully furnished cages may be due to accumulation of eggs outside the nest in the cradle of the cage floor.

Appleby *et al.*, (2002) kept birds in conventional and furnished cages for three years and reported that the number of downgraded eggs (dirty and cracked) was variable with higher downgraded number which was found in furnished cages as they laid eggs in dust bath. At 48 weeks of age there was significant effect of cage type on the percentage of collected eggs that were either cracked or dirty ($P < 0.001$). Only 1.35 % of eggs from the conventional C cages were cracked, compared with 4.5% from H (hollow nest- Furnished) cages. Dirty eggs were also more as the number of birds were increased, but the increase was not significant. There was non-significant effect of cage type on egg quality at either 50 or 72 weeks of age. Also non- significant effect of stocking density was found on any of the egg quality characteristics.

In year 2 and year 3 downgraded eggs were particularly common in dust baths and in cage area of cages with nest box. As a result there were significantly ($P < 0.05$) more second quality eggs in furnished-F cages than in Conventional C cages. Also eggs that were laid at the back of the cages were frequently dirty because of accumulation of droppings. Furnished cages with 2 nest hollows at the side were unsuccessful as only 50% of the eggs laid in the nest hollows, however furnished cages with front rollaway nest at the side had 93% eggs produced in them and some were downgraded. There was a trend for the whole year records to show more downgraded eggs from furnished cages (7.3 to 9.0%) than from C cages (5.6 to 6.7%), although there was no significant overall variation between treatments. As in previous years of 1st and 2nd year increased in number of downgraded eggs with increasing stocking density of birds in third year.

Non-significant differences in percentage of downgraded eggs among the cage systems, conventional and enriched cages were found by Karkulin (2006). There was slightly lower percentage in downgraded egg count in enriched cages (5.99%) as compared to conventional cages (6.32%). In general low amount of downgraded eggs in enriched cages were reached because of the plastic curtain fixed round the nest. This can effectively reduce the rolling out speed of egg from the nest and so lowered the risk of it's breakage lower. Observations suggested that appropriate length of nest curtain can reduce the speed of 65 g heavy egg at

about 20%. While the incidence of dirty eggs was less frequent in enriched cages compared to conventional.

Perch arrangement in the furnished cages had no effect on proportions of cracked eggs was concluded by Wall and Tauson (2007) when different furnished cage were made by different perch design, FC-A, FC-B and FC-C. The proportion of cracked eggs was significantly ($P < 0.001$) lower in the conventional cage-C (2.2%) than in the furnished cage models (3.2, 3.2, 3.6% for FC-A, FC-B and FC-C respectively). However, the dirty eggs were numerically more in conventional cages -7.1 % than in furnished cages of 6.4, 6.3 and 6.5% for FC-A, FC-B and FC-C respectively.

Sarica *et al.*, (2008) reported that during egg collections after 40 weeks of age, the respective cracked and broken eggs amounted to be 2.86%, 3.04%, 3.40%, 3.44% at 2000,1000, 667 and 500 cm² space allowances with the highest proportion of cracked and broken egg at minimum space allowance although differences between the stocking densities were not significant ($P > 0.05$).

Tactacan *et al.*, (2009) carried the study to compare the different indices of production and welfare of birds kept in 2 different caging system. The birds in conventional cages were kept in a density of 5 birds per cage allowing a floor space of 561.9 cm²/hen. The enriched cages had 24 hens per cage and offered a space of 642.6 cm²/hen. The overall percentage of dirty eggs unlike the other egg quality measurements differed markedly between the cage designs ($P < 0.0001$). The percentage of dirty eggs in the conventional cages (2.43 to 5.33% over the 10 periods) was consistently lower throughout the laying cycle as compared to enriched cages (7.18 to 15.52%). There was a significant ($P < 0.05$) period effect for percentage of dirty eggs. However, cage type did not affect the overall percentage of cracked eggs, but the number of cracked eggs significantly ($P < 0.05$) increased with increasing days in laying and was more in second half of the production cycle period 6 to 10 than in first half of the production cycle.

Wall (2011) maintained layer hybrids from 20 until 72 weeks of age in 4 designs of furnished cages (FC), housing 8 (T8), 10 (T10), 20 (T20) and 40 (T40)

numbers of layers and found better the exterior egg quality in T8 (crack -1.9%; dirty -7.5%) compared with T20 (crack -4.7%; dirty -13.6%) and T40 (cracks $P < 0.001, 4.9\%$; dirty $P < 0.01, 13.7\%$). The T10 cage with intermediate levels of cracked (3.1%) and dirty eggs (11.3%) did not differ significantly from any of the other cage designs in exterior egg quality.

Guo *et al.*, (2012) reported Small Furnished cages hens had a higher ($P < 0.05, F_{2,15} = 5.92$) level of egg breakage compared to the control group, that is conventional cages with 4 birds as flock size and 398 cm^2 per bird as stocking density.

Hester *et al.*, (2013) on the trial for effect of perches in cages during pullet rearing and laying had observed that perch access during egg laying resulted in hens laying eggs with a greater percentage of dirty eggs ($P < 0.0001, 3.6\%$ Vs 2.5%) compared with control hens without perches.

The furnished cages-large and small tended to have more broken eggs and dirty eggs than did Conventional cages, the lowest broken eggs and zero dirty eggs were for conventional cage groups, as documented by Meng *et al.*, (2014) in their experiment on effects of large or small furnished cages on performance, welfare and egg quality of laying hens. The tendency to a greater percentage of cracked eggs in furnished cages in this study may be due to poor design of nests or dust bathing boxes.

Free-range system had significantly more dirtier eggs than enriched cage system ($P < 0.05$) in all age group 2.59 vs 5.33% , 2.04 vs 6.72% and 1.88 and 8.41% for week 30, 40 and 50, respectively. Besides, the percentage of cracked eggs was influenced by rearing system and increased with age ($P < 0.05$) that is at week 40 = 0.54 Vs 0.78% and week 50 1.12 vs 0.86% , but not in week 30. The highest percentage of cracked eggs was observed in free-range systems ($P < 0.05$) by Denli *et al.*, (2016).

Percentages of dirty or cracked eggs were not affected by the space allowance or cage size provided for bird. However, the percentage of dirty eggs

was higher at week 69 (59.8 ± 4.0) compared to week 50 (34.2 ± 3.7 ; $P < 0.0001$). There were also more cracked eggs at week 69 ($17.9 \pm 1.5\%$) compared to week 50 ($12.0 \pm 2.0\%$; $P = 0.031$) as found by Windowski *et al.*, (2017).

Li *et al.*, (2017) have investigated the effect of enrichment of cages (Small Furnished cages, SFC and Medium furnished cages, MFC-I, II and III) with perch, nest and dust-bath on the exterior quality of eggs. The Small Furnished Cage had the dimension as per European Union Criteria of 1200 mm wide, 500 mm deep and 450 mm high at the rear with a perch, dust-bath placed on top of the nest. The medium furnished cage I had dimension of 1920 mm wide, 625mm deep and 450mm high, with 2 perches in the front and dust-bath and nest box at the back having same floor allowance as in small furnished cages. The dimensions of MFC-II were 1600 mm wide, 750 mm deep and 700 mm high which had two tiers for greater floor space allowance with 2 perches, nest and a dust-bath. The medium Furnished cages were type III same as in type II, only it had 2 nests in the top tier floor with a dust-bath in between the nests.

MFC-I was found to have more cracked eggs (10.5%, $P < 0.01$) than SFC (1.8%), MFC-II (3.2%) and MFC-III (1.7%). Similarly, the proportion of dirty eggs in MFC-I (37.9%) was significantly higher than in other type of furnished cages (MFC-II: 26.1%, MFC-III: 17.3% and SFC: 10.4%). The dirty eggs in MFC-II was significantly ($P < 0.01$) higher than in MFC-III and SFC. Although the dirty eggs of MFC-III were higher than in SFC, it was not significant. The proportion of dirty and cracked eggs was significantly affected by the Furnished cage type ($P < 0.001$) in that the highest proportion of cracked or dirty egg was found in Medium furnished cages-II and lowest proportion of dirty eggs in Small furnished cages. The result of this experiment indicated that not nest but dust-bath lead to more dirty eggs, more specifically the low position of dust bath in furnished cages.

2.4 Effect of stocking density, flock size and furnished cages on birds welfare:

Numerous welfare indicators for poultry have been suggested in the literature:

1. *Physical indicators*: bone strength, feather condition and claw length.
2. *Physiological indicators*: such as corticosterone level, heterophil: lymphocyte ratio, immune status, body temperature and comb and wattles temperature.
3. *Vocal indicators* incidence of gakel calls.
4. *Behavioural indicators*: feather pecking, activity level (idle or active, type of activity), the incidence of displacement, preening and stereotyped pacing, the tendency to range and walking style.

2.4.1 Physical indicators- Feather Score and Gait Score:

Craig *et al.*, (1986) when kept birds for forty-week period in different environment of pens and cages of different stocking density of 2900, 929, 464 and 310 cm²/hen observed feather damage and loss increased stepwise and significantly with the number of hens kept per cage in the increasing stocking density in cages. Thirty weeks after housing, scores were 8.3, 6.3, and 3.4 for hens in single, 4 and 6-birds per cage respectively. Although the hens in floor pens were not evaluated for feather scores at this time it was evident that most of the birds would have scored 9, i.e., essentially undamaged. The score of 3.4 for hens in 6-birds per cage represents a considerable loss of feathers on the back and top of the wings. Hens in single bird cages tended to have wing and tail feathers that were frayed at the ends from rubbing on cage wires, but feather coverage of the skin was intact. Those birds that were in 4-hen per cage with a stocking density of 464cm²/ bird could be described as moderate (about 10% loss). Feather loss is not so an obvious indicator because it can result from natural cause like seasonal molting. But in this case, the loss of feather was not associated with molting and is regarded as either stressful or potentially stressful because it results in greater heat loss from the body and increase susceptibility to injury of the skin, especially in environments where hens are exposed to the beaks and claws of other birds.

Davami *et al.*, (1987) reported a significant improvement in plumage condition at lower group size bird (five) with score of 16.2 ± 0.25 as compared to 12.5 ± 0.25 for highly populated group (seven birds). The poorer plumage of

densely populated cages can also be caused by abrasion against cage wire and other hens.

Craig and Milliken (1989) observed the feather condition of laying hens in different stocking density of 348 (high), 464 (medium) and 580 (low) cm²/bird and in flock size of four and eight hens for each of the stocking densities. It was reported that increase in stocking density were associated with stepwise adverse influence on feather damage with scores being 7.8, 6.4 and 3.8 which significantly differed for low, medium and high density. Though the flock size of four and eight hens per cage did not have any significant difference in feather score of 6.2 and 5.8 respectively.

Appleby *et al.*, (1993) checked feather condition in layers raised till 72 weeks of age with a space of 675cm²/hen for conventional cages and an additional 375 to 480cm²/hen space for perches, dust baths and nest boxes in experimental cages (3 treatment). There were no significant differences in feather damage birds of conventional cages and furnished cages with perch, dust bath and nest boxes, although there was a tendency for feather damage to be worse in conventional cages of score 5.4 as compared to 3.0 in experimental furnished cages.

Abrahamsson and Tauson (1997) observed the effect of group size in furnished cages and conventional cages for plumage condition of birds at 55 weeks of age. Hens in the furnished cage had better plumage condition ($P < 0.01$), dirtier feet ($P < 0.001$), less toe pad hyperkeratosis ($P < 0.001$), better claw condition ($P < 0.05$) and less rear body wounds ($P < 0.05$) than hens in the conventional cage. The plumage condition was recorded separately on neck, breast, back, wings, tail and around the cloaca. The six plumage scores obtained were added together, implying a minimum total score of 6 and a maximum of 24 points. The plumage scores for furnished cages maintained at flock size of 5,6,7 and 8 were 17,17.2,17.2 and 17.4 respectively which was significantly different from birds in conventional of 15.06. Because no effects on plumage condition or cannibalism were detected between the different group sizes, a group of 8 hens may be considered being below a critical group size in furnished cages, regarding outbreaks of feather pecking or cannibalism.

Effect of perches on layer welfare was evaluated by Barnett *et al.*, (1997) in modified cages. There was improvement in feather condition and cover of the tail feathers in birds in cages with perches than in standard cage, though birds in pen had significant better tail feathers. The presence of a perch increased the length of claws ($P < 0.05$) while the length of the claws was shorter in the pen treatment than both cage treatments ($P < 0.001$). Claw length increased with age ($P < 0.05$).

Moinard *et al.*, (1998) studied the plumage condition of layers at 32, 51 and 70 week old in and at 27, 39, 53 and 67 weeks age in another flock of layers which were kept in 7 different type of cages to study the effect of cage area, cage height and perches on feather condition, bone strength and mortality of laying hens. The treatments differed in area per hen (450, 600 or 800 cm²) and height (40 or 60 cm). Perches with a capacity of 20cm/bird were placed in the half of the cages that were large (800 cm² /hen) with height of 60 cm treatment. The scoring system used was from 1 (denuded) to 4 (perfect plumage) points for 5 different parts of the body: neck, breast, back, wings and tail (Tauson,1998). The maximum total score for a hen with a perfect plumage was therefore 20 points, while the lowest possible score given to the nearly denuded hen was 5 points. The hens were individually inspected and scored by the same person while being held by another. Scores were analyzed separately for flocks 1 and 2. Feather scores were found to be independent of treatment, whether taken totally or for each body part. Mean total values (6 SE) for Flock 1 were 18.8 ± 1.7 at 32 weeks (n=218), 13.5 ± 2.2 at 51 weeks (n=189) and 11.2 ± 2.4 at 70 weeks (n=164). Mean total values (\pm SE) for flock 2 were 19.5 ± 1.2 at 27 weeks (n=218), 16.1 ± 2.8 at 39 weeks (n=205), 12.5 ± 2.1 at 53 weeks (n=187) and 8.5 ± 2.5 at 67 weeks (n=170).

Appleby *et al.*, (2002) observed significant differences ($P < 0.001$) in overall feather damage between treatments and sub-treatments at all age tested when birds were kept in Conventional-C cages and Furnished cages. C- cages tended to be worse than others, with damage to all body parts contributing to this effect. The Furnished treatment group with elevated feed troughs tended to be associated with less damage. It was primarily due to better feathers on the back of

the bird with these troughs, but also had less damage in the neck, wing, abdomen and tail/vent areas. Significant variation ($P < 0.01$) between systems in claw length at each cage was mainly due to cages with elevated troughs in which claws reached about 30 mm by 72 weeks as compared to 25 to 27 mm in other cages.

Claw condition scores were not significantly affected by the cage types. However, feather score was significantly affected by cage type, with the highest (worst) score for CC (Conventional Cages) hens and the lowest score for LFC (Large Furnished Cages) hens ($P < 0.01$). Feather condition score was significantly lower for LFC hens than for SFC hens ($P < 0.05$). Similar to feather score, the highest (worst) gait score was found for CC hens and the lowest for LFC hens. The better gait score for the hens in large furnished cages than for the hens in small furnished and conventional cages may also be due to more walking and activities. The present study showed that feather condition in large furnished cages was better than in small furnished and traditional cages, the poor feather condition of hens in conventional cages is attributable to narrow space and crowded conditions where the hens are unable to preen.

Plumage condition was similar in all variants of cages Modified and enriched-MEC, Get Away- GA and Conventional-C except PLC-Conventional plastic cages, where it was better, probably due to the rear partition with slats and the lower stocking density in a trial by Tauson (1998). Hygiene of the plumage was worse in GA than in all other models and in the aviaries. The birds which were reared in GA and in the aviaries had inferior foot condition due to bumble foot, that was believed to be a defect related to perch design or hygiene. It was observed that the use of perches, keel bone lesions developed in hens in GA and MEC, but of a less severe nature than the birds that were reared in the aviaries. The incidence of wounds due to pecking on the comb and the rear part of the body were more common in the aviaries than in the cages. Use of the Astro-turf (artificial grass) lined nests in MEC was 92 and 65%, and in GA 95 and 75%, in LSL and ISA, respectively.

Stocking density affected walking ability at all ages in broilers reared in pen measured by Sorenson *et al.*, (2000). At 4week of age, birds at 435 cm² per

bird had higher/better Gait scores (GS) than birds at 833 cm² per bird. At 6 weeks of age, birds at 435 cm² per bird had higher scores than birds at 625 cm² per bird, which, in turn, were greater than those of birds at 833 cm² per bird. At 7 weeks of age, birds at 435 and 625 cm² per bird had higher GS than did birds at 833 cm² per bird, and the proportion of birds given a score of 4 or 5 was higher for birds in pens at 435 cm² per bird and 625 cm² per bird than those for birds in pens at 833 cm² per bird. It was concluded for broilers at 4 week of age, leg weakness was a relatively minor problem; few severely lame birds had a gait score (GS) of 4 or 5 at any density. However, 2 week later, the birds had substantially poorer walking ability. A further deterioration was noticed by 7 weeks of age and at all ages, greater leg weakness was seen in the males than females, and the proportion of severely lame birds increased with age of assessment. The effect of stocking density was consistent on Gait score.

Feather and foot damage of laying hens in the study of Appleby *et al.*, (2002) were generally less in furnished cages as compared to conventional cages, also it was more in conventional cages where birds were kept in higher stocking density. There were significant differences ($P < 0.001$) in overall feather damage between treatments and sub-treatments, at all age tested. Conventional cages tended to be worse while furnished cage with elevated food troughs had better feathers not only in the back, but also had less damage in the neck, wing, abdomen and tail/vent areas.

The plumage score of hens were found to be significantly different in densely populated cages with values 16.56, 14.85 and 12.42 at 3-different stocking density of 1968, 656 and 393.8 cm² per hen maintained by Onbasilar and Aksoy (2005). There were no significant differences in plumage score among different cage floors. However, throat skin injuries were found only on the hens maintained on the first floor (5.6% of hens). This may be possibly explained by the difference in light intensity at different cage floor positions. The poorer plumage score of densely populated cages can be caused by abrasion against cage wire or other hens. Higher density appears to cause increased levels of nervousness and feather-pecking activity. Some strains have a greater ability to

adapt to high density environments and this may explain the differences between experiments.

Wall and Tauson (2007) studied the effect of 3 different perch arrangements in small group furnished cages maintaining eight numbers of laying hens and compared the plumage condition with conventional cages having four number of laying birds and found them significantly different ($P < 0.01$) with inferior scoring in furnished cages for the three models (3.32, 3.43 and 3.43) when compared to conventional cages (3.97).

The effects of space allowances on the plumage score at the end of the experiment on different body parts were found significantly different by Sarica *et al.*, (2008). Plumage damage and feather loss occurred on all body parts when hens were kept in a more restricted area ($P < 0.05$). The highest feather losses occurred on the neck in all experimental hens. Plumage scores of neck, chest, tail, back and wings were higher for layers kept at 2000 cm² and 1000 cm² space allowance per hen. In other words, greater plumage damage was determined at high stocking densities.

Feather scores as reported by Tactacan *et al.*, (2009) were similar for enriched and conventional cages reared birds except for the wing region, which was higher ($P < 0.05$) for hens housed in conventional cages, they had better coverage. Birds from conventional and enriched cages were scored for plumage condition at 37 and 61 weeks of age. Plumage condition was scored using a 4-point scoring system for 6 different areas of the body (neck, breasts, back, wings, tail and vent). The average plumage condition for each bird was calculated by adding overall 6 areas to yield a total score ranging from 6 to 24 points. Poorly feathered birds not only present a problem for welfare but also affect overall production economics. Birds with highly deteriorated feather cover can show increased feed consumption due to increased heat loss resulting in higher maintenance energy requirement. In the enriched cages because of greater space available the birds had the opportunity to increase the flapping of their wing which may have increased the possibility of wing abrasion on the bars of the cages. Also, the higher number of birds housed in cages (24) compared to

conventional cages (5) may have increased the feather pecking and have contributed to the observed greater wing deterioration.

Hester *et al.*, (2013) observed when offered perches to birds at different phases pullets and laying phase that perches in laying cages resulted in shorter trimmed claws and improved back feather scores, but had poorer breast and tail feather scores.

Welfare parameters were measured at 32 weeks of age that is nearly at the end of the experimental period by Meng *et al.*, (2014) and included feather condition score, gait score, claw condition score, tonic immobility duration, and body fluctuating asymmetry when compared layers kept in different cage type furnished (large and small) and conventional cages. The feather condition score was assessed on eight parts of the body: head, neck, back, tail, wing, breast, vent and leg. The condition for each part was rated 0–3 as follows: 0, feather perfect with no damage; 1, feather slightly damaged but skin all covered; 2, feather damaged and exposed area of skin was <1 cm by 1 cm; 3, feather damaged and exposed area of skin was >1 cm by 1 cm. Scores for each part were summed; the highest (worst) possible score was 24. The hens that were subjected to test were put into a dark and quiet room to observe and record gait score. The gait score was defined as follows: 0, hens can walk normally and keep good balance; 1, hens have slight gait faults; 2, hens have obvious gait faults, such as lameness; 3, hens can walk only under intense stimulation. Claw score was defined as follows: 0, claw intact, clear with no damage; 1, claw slightly contaminated; 2, claw seriously contaminated; 3, claw damaged and not intact feather score was significantly affected by cage type, with the highest (worst) score for CC hens (4.20 ± 2.04) and the lowest score for LFC hens (0.42 ± 0.52 , $P < 0.01$). Feather condition score was significantly lower for LFC hens (0.42 ± 0.52 , $P < 0.01$) than for SFC hens (1.71 ± 1.16 , $P < 0.05$). Similar to feather score, the highest (worst) gait score was found for CC hens 1.51 ± 1.43 and the lowest for LFC hens 0.11 ± 0.32 .

Li *et al.*, (2016) studied the effects of furnished cage type on behavior and welfare of laying hens. The hens in Conventional cages- CC showed poorer welfare conditions than the furnished cages (Small and Medium Furnished cage,

SFC, MFC), in which the feather condition score, gait score and tonic immobility duration of the hens in CC was significantly higher than SFC, MFC-I, MFC-II, and -III ($P < 0.05$). The KBD-keel bone deformation score was also higher than the other furnished cages, despite the difference not reaching significance. Furthermore, the results showed that claw score of the hens in CC was not different from that in SFC but significantly lower than that in MFC-I ($P < 0.05$). While gait score was not significantly different between the furnished cages ($P > 0.05$). Similarly, no difference was found for KBD score between the furnished cages. Although tonic immobility duration was found higher for the hens in MFC-II than other furnished cages, the difference was not significant.

Hens housed in small cages had higher overall feather cleanliness scores (they were dirtier) than those housed in large cages ($P < 0.0001$); hens housed at low space allowance (SA) had dirtier plumage than those hens housed at higher SA ($P < 0.0001$) regardless of cage size. This was observed by Windowski *et al.*, (2017) in laying hens in an experiment carried from 20 to 72 weeks with two space allowance 520 cm^2 and 748 cm^2 and 2 cage size –Smaller furnished cages and Larger Furnished cages.

The assessment of plumage and integument condition in laying hens provides useful information about the occurrence of feather pecking and cannibalism. Although feather loss and skin injuries can result from mechanical abrasion or clinical diseases, they are valid animal-based indicators for behavioral disorders. This particularly applies to damage on the back and tail region of the hens. Giersberg *et al.*, (2017) tried to evaluate the behavior of dual-purpose breeds (Lohmann Dual, LD) and conventional layer hybrids (Lohmann Brown plus, LB+), and to compare a mere visual assessment (Visual Scoring, VSc), with a method involving the handling of individual animals (Hands-on Scoring, HSc) when the birds were maintained in conventional aviary system. During weekly VSc, the hens' plumage and integument were scored on five body parts. HSc was carried out on seven study days applying the same scoring scale as for VSc. In LB+ hens, minor plumage damage started at 25 weeks and increased to the 71st week. With 99.5% of LB+ showing feather loss to a different extent, the back was

the most severely affected body part. In contrast, only between 4.5% and 7% of LD showed minor feather loss at the end of the study. During week 66 damage to the integument was at peak, with 6% affected LB+. Injuries were found only sporadically in LD hens. Spearman's rho for the comparison of plumages scores given in VSc and HSc was > 0.90 ($P < 0.01$) in both hybrids for most of the tested body regions and weeks, except for the breast or belly region. VSc and HSc were equally valid for detecting skin injuries of all of the body regions ($r_s > 0.86$, $P < 0.01$). Damaging behavior was observed only in the LB+ flocks, though both of the genetic strains were kept under the same conditions.

2.4.2 Physiological indicator-H/L Ratio

The physiological stress response of laying hens maintained at different housing conditions of pen and cages and at different density was studied by Mench *et al.*, (1986). From day old to 30th week the layers were maintained in floor pens and at 30 weeks were assigned to the specific treatment groups of single cages (S) with only one bird occupying floor space of 1394cm²; Low density cages (LD) with two birds and floor space of 1394cm² per bird; a high-density cage (HD) with two bird occupation and floor space of 697cm² per bird and in floor pen (P) with 25 birds with floor space of 1394cm² per bird. Physiological investigation for H/L ratio, blood was collected at 68 weeks of housing and stained with Wright's stain and examined for determination of heterophil to lymphocyte ratio. There was no difference between treatments in heterophil to Lymphocyte ratio which is considered to be one of the most sensitive indicators for stress.

Barnett *et al.*, (1997) evaluated the welfare of layer hens housed in cages modified with perches. Welfare was assessed on the basis of physiological measures of stress heterophil: lymphocyte ratio and corticosterone concentrations 'at rest' and in response to ACTH. Factors examined were cage modification (perches vs. standard cage), upper and lower tier, birds in flock size of 1 or 2 birds/cage and age of commencing of experiment at 35 vs. 60 weeks of age. There were no effect of cage modification on the physiological variables, the H/L ratio ranged between 1.62 to 1.97 over the various factors.

Onbasilar and Aksoy (2005) showed significant effects of cage stocking density on H–L ratios 0.62, 0.57 and 0.95 for stocking density per hen of 1968, 656 and 393.8 cm² respectively although there was no significant cage floor effect on blood parameters. The ratio of heterophils to lymphocytes of the group having 5 hens/cage was higher ($P < 0.01$) than those of groups having 1 or 3 hens/cage. This condition was the result of the elevated concentration of corticosterone in blood circulation, which caused an increase in heterophil count and a decline in lymphocyte count.

Singh *et al.*, (2009) measured the heterophil: lymphocyte ratio to measure stress in 3 commercial laying strains, Lohman White, H&N white, Lohman Brown (LB), and a non-commercial cross between Rhode Island Red (male) and Barred Plymouth Rock (female), kept in conventional cages or floor pens. All chicks were reared in their respective environments, and 450 and 432 pullets were placed at 18 and 7 weeks of age in cages and floor pens, respectively. Blood from 12 hens per strain was taken at 19, 35, and 45 weeks of age in each housing system. A total of 100 heterophils and lymphocytes were counted and their ratio (H/L ratio) was calculated which ranged from 0.16 to 0.33. There was no significant difference for the environment (cage vs floor) and in 2-way interaction of strain x age. In the full model, 2-way interactions between environment and strain, and that between environment and age for the H/L ratio, were significant. In both environments, brown-egg layers (LB and CR hens lay brown eggs) had higher H/L ratios than the white-egg layers (LW and HN hens lay white eggs), with crosses having the highest ratio. In cages, LW hens were observed to have a higher H/L ratio than HN hens, and in floor pens, white-egg layers were not different for the H/L ratio. The ratio was the same in the birds maintained in cages or floor pens for LB, HN, and CR hens, but the LW hens in cages had a higher ratio than those in floor pens. In cages the H/L ratio was highest at week 22 and decreased with age, whereas in floor-pens, the H/L ratio was higher at week 45 than at week 35th. At week 22nd, the ratio was higher in cages than in floor pens, and at week 45th, it was higher in floor pens.

H/L ratio for laying hens housed in conventional and enriched cages were not affected by cage design as reported by Tactacan *et al.*, (2009) in his paper entitled performance and welfare of laying hens in conventional and enriched cages. Differential leukocyte counts were observed to be similar in layers maintained in conventional and enriched cages except for the number of lymphocytes which was higher ($P < 0.05$) in conventional than in enriched cages.

Cotter (2015) examined the H/L ratio to define it as a method of stress/welfare indicator for commercial hens housed conventionally (CC) in aviaries (AV) or in enriched cages (EN) at 18 and 77 weeks of age. The H/L ratio of the hens at 18 weeks was not statistically different between birds raised at conventional cages and in aviaries.

Kang *et al.*, (2016) reported H/L ratio were greater ($P < 0.01$) for 10 birds/m² stocking density than of 6, and 7 birds/m² stocking density. Mean H/L ratio was 0.34, 0.37, 0.37, and 0.52 for the 5, 6, 7, and 10 birds/m² treatments, respectively in their experiment on effect of stocking density on the laying performance, blood parameter, corticosterone, litter quality, gas emission and bone mineral density of laying hens in floor pens.

2.4.3 Physiological indicator - corticosterone

Mashaly *et al.*, (1984) observed the changes in serum corticosterone concentration of laying hens as a response to increased population density. To attain the different stocking density of 516, 387 and 310cm² per bird, the birds were housed in number of three, four and five birds per cage of dimension 30.5cm x 50.8 cm in two different rooms. All birds were bled from brachial vein and blood was collected on weekly basis for 6 weeks to check serum corticosterone by RIA-radioimmunoassay. The serum corticosterone level for the 5-bird group, that is with stocking density of 310 cm² per bird was 2397 ± 106 pg/ml (mean \pm SE), which was significantly ($P < 0.05$) higher than the average for the four-bird group (1853 ± 90 pg/ml), which was significantly ($P < 0.05$) higher than the three-bird group (1654 ± 106 pg/ml). Regardless of the room, it was found that the actual magnitude of the concentration was higher in the five-bird group than in the other

two groups at all periods tested. Therefore, in order to minimize physiological stress more than 387 cm²/bird should be considered when housing layers in cages.

The mean plasma corticosterone levels tested on hens maintained in different flock size in cages and pens were 0.60 ± 0.1, 0.57 ± 0.1, 0.68 ± 0.1 and 1.20 ± 0.1 ng/ml for single hens in cages (S), Low density (LD), High Density (HD) and in pen (P) birds respectively as reported by Mench *et al.*, (1986). It was observed the hens kept in pens had significantly different (P<0.001) corticosterone value as compared to cages and the twice the higher value of corticosterone was observed to be related with trap nesting. When trap nesting discontinued the difference in cage-pen disappeared. Plasma corticosterone levels checked at night time were low and displayed little variability but was significantly higher (P < 0.05) in HD caged hens. The mean values observed at night were 0.35 ± 0.1, 0.23 ± 0.1, 0.56 ± 0.1, and 0.29 ± 0.1 ng/ml for single hens in cages (S), Low density (LD), High Density (HD) and in pen (P) birds respectively.

Craig *et al.*, (1986) have studied the welfare parameters namely corticosteroid, mortality, feather condition and egg production of layers kept in four different laying housing environment. Plasma corticosteroid concentrations present during the first 2 weeks after housing was higher as compared to later after adaptation, taken 3 weeks later. Because all pullets were moved from rearing to the egg laying house environment at one time, it was not possible to determine whether the initially higher level was response to the new housing environment or some other variable such as the onset of egg laying. Housing environments influenced the plasma corticosterone concentrations. Hens in floor pens (2900cm²/hen) and 6 birds per cage (310cm²/hen) did not differ significantly from each other but had levels 12% greater than those of hens in single (929cm²/hen) and 4-hen cage (464cm²/hen). The absence of housing by period (ten-week) interaction suggests that whatever causes were responsible for the differences found between housing environment were present initially and continued throughout the 22 week period. At 45-weeks of age also 6 bird cages and floor-pens did not differ significantly but had corticosterone levels approximately 15%

higher than those in single or 4-bird cage and was consistent with the magnitude of difference between the same groups at 22-week of age. But significant differences in corticosterone were found among the birds in cages when kept at 6 birds/ cage as compared to single bird or four-bird/cage.

Corticosteroid concentration in plasma appears to be so variable among hens treated alike that significance may be detectable only when a relatively large numbers of samples are collected. Corticosteroid concentration in the plasma of hens at low-density floor pens did not differ from those of high-density, larger-group-size cage environment. Hens in relatively spacious floor pens, although have other indications of greater well-being, than birds in high-density cages, but their plasma corticosterone were found higher. So the study had a difficulty in using corticosteroid as indicator of hens general well-being. Not only the plasma corticosteroid fail to indicate the presence of stressful situation, but they also may be misleading by indicating that stressful situation prevail, when they do not as indicated by other criteria of well-being.

Davami *et al.*, (1987) could not find any significant differences in corticosterone concentration of birds maintained in two group sizes of five and seven birds 1855 ± 123.9 Vs 2060 ± 109.9 pg/ml, though there was a trend toward higher concentration in hens maintained at higher population density

With increased corticosterone level the antibody titer tends to decrease and heterophil counts was increased as observed by Post *et al.*, (2003). In order to avoid treatment of animals with physical or psychological stress, Post *et al.*, added corticosterone to the drinking water to serve as a practical alternative and investigated the hormone related stress. Corticosterone provided at dose of 10 or 20mg per liter of water significantly inhibit the specific antibody production against Sheep Red Blood Cell.

Onbasilar and Aksoy (2005) showed significant effects of cage density on plasma corticosterone, serum glucose, cholesterol concentrations, although there were no significant cage floor-top, middle or lower floor effect on blood parameters. When the cage density increased from one to five hens per cage, it

resulted in a significant increase of the plasma corticosterone ($P < 0.01$). During stress conditions, neural impulses come to the hypothalamus and are converted to neuro-humoral factors. Corticotropin-releasing factor stimulates the anterior hypophysis to secrete ACTH, which, in turn, stimulated the adrenal for corticosterone secretion. Therefore, higher corticosterone levels in the 5 hens/cage groups could reflect higher stress conditions.

Different stocking density of broilers in relation to stress and welfare and well-being was studied in an experiment conducted by Thaxton *et al.*, (2005). Research has shown that the concentration of blood corticosterone (CS) is the most sensitive indicator of stress and when the well-being of the chickens is compromised by stressful condition, blood corticosterone will be elevated. In this study, blood CS concentrations were determined on 192 broilers. The highest single CS value recorded was 1,070 pg/mL, and the lowest recorded individual CS value was 340 pg/ml. The mean CS concentration for all 192 birds was 607 with an SEM of 10. Mean CS concentrations for the 8 growers, over all time periods, did not differ at $P \leq 0.05$. The CS levels did not appear to be affected by housing type.

No treatment effect on corticosterone concentrations among the hens ($P > 0.05$) was reported, whereas age effects were found in the hens housed in the battery cages only ($P < 0.05$) by Pohle and Cheng (2009) on their comparative study of White Leghorn layers in battery and furnished cages. In those hens, the corticosterone concentrations were increased with age, with a peak at 60 weeks of age. Early increases in production may be suggestive of reduced stress in furnished cage hens because the hens housed in the furnished cages had lower concentrations of both corticosterone and serotonin compared with the hens housed in the battery cages.

Tactacan *et al.*, (2009) found no change in plasma corticosterone in birds kept in different housing systems - conventional and enriched cages with different stocking densities. In the experiment, conventional cages had 5 birds per cage allowing a floor space of 561.9cm²/hen. The enriched cages had 24 hens per cage

and offered a space of 642.6cm²/hen. Blood samples were collected at 59 and 61 weeks that is before and after ND vaccination.

During the initial (34 to 37 week) and final 4 week of the experiment (38 to 41 week), serum corticosterone was greater ($P < 0.01$) in stocking density of 10 birds/m² than other stock densities that is 5, 6 and 7 birds /m², concluded by Kang *et al.*, (2016) when birds were maintained in floor pens. Further it was reported blood parameters are good indicators of the physiological, pathological, and nutritional status of an animal, and changes resulted in hematological parameters have the potential to be used to elucidate the impact of nutritional factors and additives supplied in the diet.

2.4.4 Immune response:

Mench *et al.*, (1986) observed no differences in immunocompetence with micro-hemagglutination titers to SRBC among treatments groups of different flock size groups of single hen in cages (S-1 bird), Low density (LD-2 bird), High Density (HD-2 bird) and in pen (P-25 birds) birds.

Patterson and Siegel (1998) and Heckert *et al.*, (2002) observed that treatments with different stocking density had no significant effect on hemagglutinin titers to SRBC. Genetical background, use of different suspension of sheep erythrocytes or may be the different cage densities have shown the differences in the Ab titer.

Cage density significantly affected antibody titers (2.22, 2.57 and 1.96 for stocking density of 1968, 656 and 393.8 cm² per hen) as cited by Onbasilar and Aksoy (2005). When the cage density was increased from 3 to 5 hens/cage it resulted in a significant decrease ($P < 0.01$) in the antibody titers to SRBC.

Pohle and Cheng (2009) found plasma IgG concentrations were altered by age but not treatment when layers were maintained in different environment that is battery and furnished cages. In both groups, increased IgG concentrations were found at 60 week of age compared with other observed time points ($P < 0.01$) like at 50 weeks of age.

Antibody production (\log_2 value) against NDV vaccine before and after vaccination for NDV for laying hens housed in conventional and enriched cages were not affected by cage design as reported from the findings of Tactacan *et al.*, (2009). However, as expected a significant increase in antibody titer was observed in both housing system post vaccination.

The ND antibody titer was significantly different between housing systems was reported by Shimmura *et al.*, (2010), when birds were kept in 6 housing systems, which had two types of conventional cages (small: SC; large: LC), two type of furnished cages (small: SF; large: LF), and two type of non-cage systems (single-tiered aviary: SA; free-range: FR). It was observed that antibody titer to be lower in large furnished cages (LF) compared with the other housing systems (SC, 9.4 ± 0.5 ; LC, 9.5 ± 0.5 ; SF, 10.0 ± 0.4 ; LF, 7.8 ± 0.5 ; SA, 9.2 ± 0.3 ; FR, 10.40 ± 0.3 ; $P > 0.05$).

2.4.5 Behavioral effect:

Mench *et al.*, (1986) observed caged birds as compared to pens showed less locomotor activity and more scanning even in less density (LD) which were spacious when birds were maintained in different flock size in different treatment groups of single hens in cages (S-1 bird), Low density (LD-2 bird), High Density (HD-2 bird) and in pen (P-25 birds) birds. It was stated that the visual exploration may have substituted for locomotor exploration of the environment. Decrease in locomotion in high density cages was accompanied by an increase in standing, but not resting. The caged birds in the nest boxes were seen more often to groom and sleep and it appeared as if the nest box served as an area for refuge for the lower-ranking hens.

No any difference was observed for the incidence of maintenance behaviors differ pens and cages but did demonstrate differences within the cage treatment. Grooming was noticed with greater frequency in two-bird cages but did not display the density-related decrease. Feeding was observed to be less frequent in HD than LD cages; the limited space at the feeder probably made it difficult for both hens to feed simultaneously, thus decreasing feeding activity in general.

Indeed, paired HD hens displayed significantly fewer episodes of synchronized behavioral activity than did LD hens (Chi-squared, $P < 0.01$), particularly with respect to feeding (5 and 35% of intervals synchronized, respectively), drinking (7 and 20% of intervals), and resting (4 and 37% of intervals). The total percentage of time for feeding was 33,28,37 and 43 % ; resting was 14,20,20 and 16% ; grooming 16,18,17 and 10%; standing 7,15,5 and 9 % , scanning 6, 10,10 and 12%, locomotor activity 8,1,3,1 % and nesting and laying 10,1,0, and 1 %; feather pecking 1.5,3.2,1.4 and 0.2%; aggressive activity 1.7,1.9, 1.7 and 0% for the birds in Pen (P), High density birds (HD), Low Density (LD) and single bird in cage (S) respectively. There was significant differences for the feather pecking and aggressive activity between single bird in cage with the rest of the treatment ($P < 0.05$). Although the observational technique used in this experiment did not provide an accurate measurement of short-duration behaviors like aggressive head pecking, agonistic behavior was noted to vary in character between treatments, with the majority of encounters in pens consisting of pecks, threats, and fights, whereas those in cages consisted overwhelmingly of pushing, leading to displacement at the feeder and drinker.

Anderson *et al.*, (1989) reported the behavioral adaptation of White Leghorn pullets at different cage densities and flock size with four birds /cage with 464cm² floor area and 10.2 cm feeder space /pullet and six birds /cage with 310 cm² floor area and 5.1 cm feeder space. Video observations of settling -in behaviors were made to determine initial adaptations of pullets to the new cage environment. Group size had significant ($P < 0.05$) effects on the amount of standing, crouching, preening, feeding, and comfort movements during the first 5 days post-housing in layer cages. Hens housed four/cage spent significantly less time standing than hens housed six/ cage. Time spent standing was relatively constant over the first 5 days post-housing with a slight, but significant ($P < 0.05$) decrease during Days 2 and 3, followed by a return to the original level by Day 5 (5.5,4.9,4.9,5.1 and 5.5 for the consecutive 5 days) observed 30second/3-minute period. Significantly ($P < 0.05$, 3.9 and 2.9) greater preening of hens housed four per cage suggests that hens housed six per cage may not have had sufficient space

to perform grooming freely. Preening tended to increase after the 1st day, which may indicate a lower level of stress on the bird.

Feeding activity was significantly more frequent in four-hen cages than in six-hen cages. This may have been the result of availability of feeder space to the different hen populations. Hens in the four-bird cages could feed simultaneously, whereas those in the six-hen cages had to compete for access to the feeder. Means for group size x days post-housing interactions for standing and drinking behaviors showed period of standing of hens in four-bird cages were significantly more frequent on Day 1, then dropped to frequencies lower than those of pullets in six-bird cages on subsequent days. There was no any difference in the levels of drinking activity except for day 1 when significant differences were noticed for group sizes, the hens housed four/cage had more drinking than those housed six/cage.

Appleby *et al.*, (1993) checked the use of cage area in layers raised till 72 weeks of age with a space of 675cm²/hen for conventional cages and an additional 375 to 480cm²/hen space for perches, dust baths and nest boxes in experimental cages (3 treatment). In the daytime, hens in experimental treatments spent approximately two-thirds of the time in the part of the cage in front of the perch, varying from 60 to 65% between sub-treatments of Litter (L) in nest boxes and dust baths, Artificial turf (A) and Plastic rollway (P) with no significant differences. The birds in experimental cages spent about one quarter of the time on the perch and the rest of the time distributed between the back of the cage, the nest box and dust bath. This when compared to the control Conventional cages, birds spent 75% of the time in the front part of the cage and 25% in the rear. At night, most birds in experimental cages roosted on the perch: 94% at 23 week and 90% at 34 weeks.

In experimental cages, birds performed a median of one bout of dust bathing in the sand each afternoon, with a duration of 4 to 7 min. In control cages, a median of 3 bouts of vacuum dust bathing, each only 10s long, was observed on the wire floor. In treatments A and P, the dust bath was too small for birds to turn round or to stretch fully; some individuals were also seen to lie, pecking at the

sand but scratching and dust bathing in the main, wire part of the cage. Birds also sometimes pecked and scratched in dust baths, then left without dust bathing.

Abrahamsson and Tauson (1997) observed the effect of group size in furnished cages with conventional cages on the behavior of birds. No effect of group size on use of perches could be registered. At night, on average 91% of the hens in the furnished cage roosted on the perches. During the day, an average of 28% of the hens used the perch simultaneously, slightly higher at 7 h after lights on (30%) than at 11 h after lights on (26%). Generally, few hens stayed in the sand-bath and nest during the night, but more hens stayed in the sand-bath in the smaller group sizes than in the larger (5 hens, 0.3%; 6 hens, 0.06%; 7 and 8 hens, 0%; $P < 0.05$). An average of 1.7% of the hens stayed in the nest during the night, with no effect of group size. In the dust-bathing behavior study, the average proportion of hens simultaneously visiting the sand-bath, as well as dust-bathing in it, was greater in the small group sizes than in the larger (visiting: 5 hens, 5.2%; 6 hens, 6.5%; 7 hens, 3.3%; 8 hens, 4.1%; $P < 0.05$) (bathing: 5 hens, 1.2%; 6 hens, 1.7%; 7 hens, 0.8%; 8 hens, 0.8%; $P < 0.05$). In the small pilot study of 24 cages at 74 weeks, more dust-bathing behavior was registered on the wire floor (1.8%) than in the sand-bath (0.8%).

There were few main effects of treatment on behavior when perches were provided in the cage by Bennett *et al.*, (1997). The presence of a perch in a cage resulted in significant perching activity which was at a higher frequency than in the floor pens ($P < 0.01$). It might be concluded that the large amount of time that birds spent perching in this treatment (46.5% of observation time) might result in improved feather condition and cover through a reduction in pecking behavior. There was more bird–bird interactions in 2-bird than 1-bird cages both for pecking and being pecked ($P < 0.05$). There was less pecking with age for bird–bird pecking interactions and for pecking between cages.

Moinard *et al.*, (1998) studied effect of cage area, cage height and perches on feather condition, bone strength and mortality of laying hens. Birds housed in the biggest cages showed higher frequencies of walking behavior in the treatment group which had highest space allowance, increased surface area of cage might

have induced increased activity, including abnormal behaviors such as cannibalism.

Tauson (1998) observed eggs laid in the dust-bath can cause problems when given four different cage systems such as Get away (GA), medium Enriched cages (MEC), Conventional and Conventional plastic cages. The average proportion of eggs laid in the dust-bath in the present study was lower in MEC (0.1%) than in GA (1.7%). Age at first opening of the dust-bath in MEC (16 or 26 week) did not affect egg location. Opening at 16 week compared with 26 weeks increased the frequency of birds visiting the bath from 5 to 11% of observations per day between 30 and 70 wk. The use of perches in MEC averaged 96% during the night.

Bilcik and Keeling (1999) examined the feather pecking and aggressive behavior in laying hens maintained at different group sizes (15,30,60 and 120 birds) from day 1 to 40 weeks of age. Observations were focused on pecks received from other pen-mates. Feather pecks were classified as gentle (careful pecks, not resulting in feathers being pulled out and usually without reaction from the recipient bird) and severe (forceful pecks, sometimes with feathers being pulled out and with the recipient bird moving away). Behavioral observations were performed at the age of 22, 27, 32 and 37 weeks. From the total of 410 feather pecks (gentle and severe) observed throughout the 4 observation periods, 76 pecks were targeted towards tail (18.5%), 72 on rump (17.6%), 53 on back (12.9%), 52 on coverts (12.7%), 47 on breast (11.5%), 42 on belly (10.2%), 33 on neck (8.0%), 12 on primaries (2.9%), 8 on head (2.0%), 8 on under-neck (2.0%) and 7 on legs (1.7%). The total number of observed gentle feather pecks (273) received was higher than number of severe pecks (137) received 66.6% vs 33.4% of total feather pecks received, only a small proportion of actual feather damage can be attributed to the gentle type of pecks. Aggressive pecks (367 pecks in total) had an effect on feather condition on the head but not on the rest of the body. It was reported that increasing numbers of aggressive pecks received were associated with decreased body weight and increased feather damage on the head at 27 and 32 weeks of age.

Appleby *et al.*, (2002) observed behaviors of birds in Convention-C and Furnished-F cages, specially comfort behavior was noted: flapping, tail wagging, stretching (leg and wing, or both wings), head scratching, body shaking, bill wiping, aggressive pecking, feather pecking. Secondly the number of birds perching, thirdly, leg and wing movements were counted and fourthly of locomotory steps. The frequency of 4 types of comfort behavior varied among cage designs, and was generally more common in furnished than in C cages.

In the observation on leg and wing movement, there was a large effect of stocking density/ number of birds per bird, where there were fewer birds per cage they took many more steps. There was no difference between treatments in number of wing movement. Aggressive behavior was more frequent in furnished cages than in C cages ($P < 0.05$), but this was confounded by number of birds per cage; in furnished cages with 8 hens/ cage there were 1.6 pecks/ hen hour, with 5 hens/cage there were 0.8 and in C cages with 4 hens there were 0.4. Frequency of dust bathing did not differ between treatments, but dust bathing bouts on wire floors, which occurred in all cage designs were shorter than bouts in dust baths, so average bout length was less in C than in furnished cages F.

It was concluded that behavior was more unrestricted and varied in furnished cages compared to conventional cages, dust baths were used more for pecking and scratching than for dust bathing. Comfort behavior (tail wagging, stretching of wing and leg or both wings, body shaking) was more frequent in furnished than in conventional cages. The number of birds per cage or the space per bird greatly affected the locomotion of birds and was observed to be reduced by crowding. Most of the birds perched at night.

Behavioral observations were taken by Anderson *et al.*, (2004) during 2 consecutive day at 26,34,43,51,62,64 and at 68 week of age to examine daily behavioral patterns of birds (2 strains) maintained at 2 stocking density -361 and 482 cm²/bird. Each cage was observed for two 5-min periods at approximately 50-min intervals beginning at lights on (0500 h), midday (1200 h), and evening (1800 h). The behavioral acts independent of cage mates that were recorded was of standing (ST), crouching (CR), preening (PR), moving (MV), feeding (FD),

drinking (DR), comfort movements (CM), and pecking inedible objects (PI), within each 5- min period.

Cooper *et al.*, (2004) studied the 24 hours activity of hens in furnished cages in groups of ten with stocking density of 1219 cm² per bird in eight pairs of adjoining furnished cages with sloping floors. Over the entire 24 h, rest and eat were the most frequently observed behavior patterns at 3.7±0.22 and 3.10± 0.15 respectively (mean number of observed cases ± SE) and they were most active during the hours of light. Other recorded behaviors included preen 0.80±0.06, stand 0.760±0.06, locomotion 0.50±0.05 and drink 0.40 ±0.04. The period of rest commenced when the lights went out at approximately 19:00 h. At night, 86% of resting birds were on a perch usually next to another bird. Not all hens could fit on one perch, the maximum number observed resting on a perch was seven. Hens were observed to rest on the floor of the cage on 14% of occasions, of which 8%, were close to a perching bird and 5% at the front of the cage next to the food trough.

Similar appetitive behavior frequencies were noticed for both strains and densities and, surprisingly, there were no significant interactions between strain, density. In this study PI was the highest at 26 weeks and during the molt, and significantly higher ($P < 0.05$) than at 43, 51, 62 and 68 weeks in the first and second cycle. The behavior frequencies for ST, CR, PR, MV, and CM were similar between the strains. The hens in the low-density cage expended more ($P < 0.05$) time MV within the cage while the behavior frequencies for ST, CR, PR, and CM were similar. This may be a function of space and the ability of hens to move within the cage with less interference from other hens.

It was reported that the increased movement (pacing within the cage) was related to frustration in nest site selection in a cage, or it may also be related to foraging behavior during this same time period when nutrients are in high demand to support reproduction. FP-Feather Pecking was highest during the early periods 26 and 34 weeks and during the molt at 64 weeks, which may be the result of social facilitation during periods of PR of feathers in young hens and PR associated with feather push out during the molt period. Prior to molt at 51 and 62

weeks and after molt at 68 weeks, FP was at its lowest frequencies. The strain and density had no significant impact on FP. Fearfulness, as measured by the MHT-Modified Hansen's test scores, was not influenced by the strain and stocking density of the laying hen. In this study, age of the hen had a greater impact on the fearfulness score. As the hens aged, the level of fearfulness increased ($P < 0.001$) from a low of 1.36 at 20 week of age to a high of 2.79 at 64 weeks of age that coincided with the molt period.

Zimmerman *et al.*, (2006) aimed to study laying hen behavior under commercial stocking densities, flock sizes and management practices by giving different combinations of stocking densities that is low: 7 birds m^2 , medium: 9 birds m^2 , high: 12 birds m^2 , flock sizes small: 2450, 3150 birds, large: 4200 birds and management conditions – standard (T1, T2 and T3) and modified. Behavioral observations during this study were carried out 3, 7 and 10 months into the laying cycle, when the birds were about 32, 48 and 60 weeks of age, respectively.

There was significant overall effect of treatment on the incidence of feather pecking and aggression. On average, feather pecking and aggression were highest in the small flocks under standard management and lowest in treatments under modified management. The incidence of dust bathing was not significantly affected by stocking density (7 m^2 : 0.14 ± 0.07 , 9 m^2 : 0.23 ± 0.16 , 12 m^2 : 0.20 ± 0.09 ; $P = 0.27$), age (32 weeks: 0.14 ± 0.06 , 48 weeks: 0.23 ± 0.15 , 60 weeks: 0.20 ± 0.09 ; $P = 0.41$) or their interaction ($P = 0.72$). The incidence of preening was affected by stocking density ($F_{2,29} = 10.0$, $P < 0.01$). More preening was noticed in the low ($P < 0.05$) and high ($P < 0.001$) density pens compared to the medium stocking density pens. There was more incidence of preening ($P = 0.06$) at the high density than at the low stocking density. The incidence of allopreening was not affected by stocking density. In the small flocks, however, more feather pecking was shown in week 48 than in week 32 ($P < 0.001$) and more feather pecking was performed in week 60 compared to weeks 32 and 48 ($P < 0.001$ and $P < 0.05$, respectively).

Behavioral observations in the above study did not show that the welfare of laying hens was compromised by housing them at 12 birds m², when compared with birds housed at 9 or 7 birds m² in single-tier aviary system. However, modifications in management could decrease the feather pecking and aggression in the birds.

Albentosa *et al.*, (2007) studied the effect of cage height and stocking density on the behavior of laying hens in furnished cages and observed few behavioral differences due to cage treatment. 5 stocking densities were maintained between 609 and 870 cm²/bird by varying the number of birds per cage from 10 to 7 in standard full-width cages or housing 7 hens in a narrower cage. The recorded behavior feeding had the longest duration (265%), then preening (6%), drinking (3%) and resting (35%). There was an overall effect of stocking density on mean feeding bout length. Hens allowed at space allowance of 609cm² (10 hens in full size cages) and 762 cm² per hen (8 hens) had longer mean feeding bouts than hens housed at 870cm² per hen (7 hens). In all treatments stretching and self-maintenance behaviors were rarely observed, but yawning was more common in cages that had a minimum height of 38cm. The number of preening bouts, their mean duration and time spent preening increased with the age of the hens but the number of feeding bouts decreased with hen age. The number of steps taken was highest when hens were 33 to 36 weeks old and lowest when hens were 46 weeks old. The incidence of wing raise/flaps (H=22, N=60, P<0.001) was highest when hens were 68 weeks old. Full wing flaps were not observed. It was concluded that in both flocks there was considerable variation in mean feeding bout length, with an indication of longer bout lengths at the higher stocking densities.

Sarica *et al.*, (2008) observed that pecking related mortalities were higher in cages with stocking density of 667cm²/hen and 500cm²/hen. No pecking related mortalities were observed in cages where birds were kept in density of 2000 and 1000 cm² space allowance per hen.

The non-cage systems, especially Free-range, had a low score for freedom from pain, injury, and disease, together with other disadvantages, such as pale eggs and increased feed intake for production as reported by Shimmura *et al.*,

(2010). However, the score for freedom to express normal behavior was high and immune response was good in the non-cage systems. In the furnished cages, behavior was more diverse in small-furnished cages than in large-furnished cages, and in Small-furnished immune response was comparable with the non-cage systems. For freedom from fear and distress, the non-cage systems had high scores for some indicators such as TI duration, H/L ratio and claw length, while aggressive pecking and feather pecking was worse in the housing systems with large group sizes.

Doring (2012) for the dissertation work studied exploratory and dust bathing behavior of laying hens kept in commercial aviaries and furnished cages to identify management factors that affect the extent of exploratory behavior (pecking and scratching) as well as quantitative and qualitative aspects of dust-bathing behavior in laying hens kept in commercial furnished cages ('small group housing') and aviaries. Within the range of environmental conditions found on-farm, pecking, scratching and dust-bathing behavior was performed on average 25 %, 2 % and 7 % of the observation time. Hen numbers in the 1 sqm litter areas were positively associated with stocking density ($P=0.0016$) and group size ($P=0.0178$). More scratching was performed with increasing litter height ($P=0.0002$) as well as in humid litter areas ($P=0.0088$). In altogether 28 observation areas (eight aviaries) no litter had been provided, corresponding to a reduced proportion of dust-bathing hens ($P=0.0453$). No influencing factor on the amount of pecking behavior could be identified, although a large range from 4 to 60 % was observed.

Guo *et al.*, (2012) observed that the hens spent most of the time for eating and standing in all three housing systems. Compared to hens kept in Small furnished cages or Large furnished cages, controls that is in conventional cages with 398cm^2 as stocking density and 4 birds as flock size had a higher frequency of sitting ($P < 0.0001$, $F_{2,67} = 26.49$) and lower frequency of walking ($P < 0.0001$, $F_{2,68} = 21.91$). SFC hens showed a lower frequency of sitting ($P < 0.0001$, $F_{2,67} = 26.49$) and perching ($P < 0.01$, $F_{1,38} = 10.99$) behaviors compared to birds in LFC. In contrast, the SFC hens showed a higher frequency of nesting compared to the

LFC treatment (20.4 vs 2.2%, $P < 0.0001$, $F_{1, 45} = 112.7$). No significant ($P > 0.05$) differences were found in pecking, feeding, drinking and standing between different treatments. Moreover, the proportion of hens displaying other behaviors (preening, flapping wing, lying and severe aggression) was small in all of the three housing systems. The frequency of panting was lower ($P < 0.001$, $F_{2, 67} = 9.61$) in hens housed in SFC than those housed in control or LFC. Birds kept in small furnished cages showed more nesting and less perching behaviors than hens in large furnished cages.

Campbell *et al.*, (2016) on the use of perches by laying hens at different aviaries showed that there was significant variation in hens roosted and for diurnal observation time ('dark', 'light'; $P < 0.001$). At both observation times ('dark' and 'light') within each age point, hens significantly preferred perches (excluding ledges) in the upper tier and occupied those perches more than in the lower tier (where no ledges are present). The United Egg Producers guidelines recommends a 15 cm/hen, a minimum 20% of perch space ≥ 40 cm above the house floor, and perch diameter and materials that allow hens to wrap their toes around the perch and balance while resting. However, it did not appear that all perches were equally preferable by the hens. This study showed hens sometimes chose to crowd (over 100% capacity) the middle and upper level perches while leaving the 3 perches on the lower level relatively empty.

Behavioral observation was recorded by Li *et al.*, (2017) by keeping birds in different types of furnished cages of small-SFC and medium size-MFC-I, II, III with varied facility for perch, nest and sandbox and Conventional Cages-CC. Behavioral observation was carried out by spraying dyes of different colors (black, white or blue) on the body of three hens from each cage that were randomly selected.

There was no effect of conventional and furnished cages on the feeding and lying behaviors of all hens ($P > 0.05$). It was concluded that hens in the furnished cages had significantly lower standing than hens in conventional cages ($P < 0.05$), while their walking was significantly higher than CC hens ($P < 0.05$) with the highest walking found for Small Furnished Cages (SFC) hens. Higher

dust bathing was found for the hens in Medium Furnished cages (MFC)-I than in SFC ($P < 0.05$), and the lowest perching was for the hens in SFC, which was significantly lower than the hens in MFC-I, -II, and -III with the highest perching found for the hens in MFC-III. No difference in nesting behavior was found between the furnished cages ($P > 0.05$). The birds in MFC-III had significant higher preening than other cages ($P < 0.05$). Also the hens in MFC-I and -II tended to show higher preening than SFC and CC, but not significant. Hens in MFC-III had a significant higher scratching and feather-pecking behavior than the other cages ($P < 0.05$).

In this study the design of furnished cage was found to affect perching, dust bathing and nesting, and hens in larger furnished cages (MFC-I, -II, and -III) showed more perching than in SFC. This may be attributed to different perch allowance per bird in each type of cage where each SFC hen had only 16 cm perch allowance compared with 22.3 cm/hen for MFC-I, 21.3 cm/hen for MFC-II and 26.7 cm/hen for MFC-III. Hens in furnished cages performed more walking and less standing than in Conventional cages. This may be due to the provision of a perch that promoted activity so that standing was less and walking was more. Also, the stocking density may be another important factor that restrains hens' activity. The results of this study showed that stocking density affected socializing and preening behavior of hens. And it suggested that low stocking density (MFC-I, -II, and -III) seemed to result in more socializing and preening behavior in hens than high stocking density as in SFC and CC. It also may indicate hens in low stocking density cages feel more comfortable than in high stocking density cages.

MATERIALS AND METHODS

The present research study was carried out at Poultry Research and Training Centre, Department of Poultry Science, Nagpur Veterinary College, M.A.F.S.U, Nagpur. The objective was to observe the effect of stocking density, flock size in conventional California and furnished cages on well-being and the performance of commercial layers. The birds reared in conventional and cages furnished with perch, nest box and scratch pad was evaluated in terms of egg production performance, egg quality, immune response, stress level, behavioural and welfare response of the birds. The detail account of the materials used as well as the various techniques and methodologies adopted during the course of the research study has been presented in this chapter.

3.1 Experimental birds and feed

The 240 available commercial White Leghorn layer (strain BV300) birds of age 28 weeks, at Poultry Research and Training Centre, Department of Poultry Science were employed to conduct the experiments. Before the initiation of an experiment the birds were dewormed and vaccinated against New Castle Disease. As per experimental design the birds were shifted three weeks before into the assigned cages so that the birds get acclimatize with change in housing environment. All the biological experiments were conducted during academic year 2018-19 and 2019-20.

The birds were maintained on standard commercial layer feed. A commercial layer feed was procured from renowned poultry feed company M/s Megataj Agrovvet Pvt Ltd, MIDC, Nagpur. As per recommendation (BV 300 manual) for commercial laying hen, the birds were offered with 110 gm feed/bird/day. At the end of each week the feed refusals were collected to measure the feed intake of the experimental birds. The feed was offered twice a day in the proportion of 40g (Morning): 60g (Evening) for optimum production performance.

3.2 Housing and Management

The White Leghorn layer birds were reared in high rise California (conventional) cage housing arranged in 3-tier system. Before the experiment initiated the entire shed and drinking water pipe line were thoroughly cleaned. The experimental birds were randomly distributed in the four different type of treatment groups as per the experimental design. The experimental birds were distributed in different replicates in such way to nullify the row and tier effect of cage housing system. Throughout the experimental period 16 hours of light was provided with ideal management conditions. The birds were given free access to fresh, clean and wholesome drinking water. The specifications of BV-300 manual were followed for all management practices including for medication and vaccination.

3.3 Closed Circuit Television (CCTV) recording

Four CCTV cameras were installed in the experimental shed to cover all angles of the birds rearing. The basic aim for CCTV cameras was to record the welfare and behaviour activities of the laying birds. The camera were mounted on different corners of the poultry shed (Plate 3.1) and the angle of the camera were so adjusted that the specific birds painted with colours were in better focus so that all the welfare and behavioural activities captured. The recording was stored in the digital video recorder (DVR) and retrieved time to time to study the different behavioural activities of the birds.

3.4 Experimental Designs

In this research study total three experiments were conducted on BV-300 layers (28th week age) for the duration of 20 weeks each. First experiment was conducted to observe effect of different stocking density in commercial laying hens on the basis of production, immune response, egg quality, economics, welfare and behaviour of the birds. A well being scorecard was developed by Delphi technique to choose the best stocking density from experiment first. Two best suited stocking densities from treatment groups were selected and further

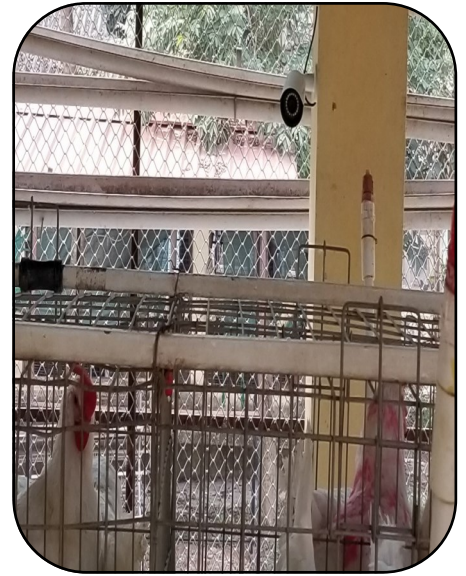


Plate 3.1: CCTV Recording of birds for behavioral and welfare parameters



used in second experiment to record interaction effect of stocking density and flock size of laying hens. On the basis of performance record of second experiment best suited stocking density and flock size treatment were selected to conduct the third experiment. The best suited stocking density with flock size treatment groups were compared (conventional California cage) with furnished (facility of perch, scratch pad and nesting area) cages for the egg production, egg quality, immune response, economics, welfare and behavioural changes of the laying hens. The experimental birds were distributed in replicates and each cage box represented one replicate consisting of birds as per assigned stocking density and flock size of that treatment group. All the birds were reared in California and furnished cages as per trial design under standard and uniform managerial condition.

3.4.1 Experiment I: Effect of different stocking density on production, welfare and behaviour of laying hens.

The first experiment was conducted for 20 weeks period and initiated during October 2018 at 28th week age of birds till 47th week. A total 240 birds were divided into four different stocking densities having 16 replicates in each treatment to determine the effects on production, welfare and behaviour of laying birds. Each cage box was considered as one replicate and the number of birds per cage varied as per the stocking density.

The four different stocking density groups consisted of

Treatment A- 387cm²/bird or 60 sq. inch/bird (five birds in each cage or replicate) with high stocking density;

Treatment B- 484cm²/bird or 75sq. inch/bird or (four birds in each cage or replicate) with a moderate stocking density;

Treatment C- 548cm²/bird or 85 sq. inch/bird (three birds in each cage or replicate) with a medium stocking density and

Treatment D-645 cm²/bird or 100 sq. inch/bird (three birds in each cage or replicate) with the lowest stocking density.

Available cage dimension measured 20” X 15” X16” (width X depth X height) were used for treatment A, B and D while treatment C birds were caged in restructured (Plate 3.2) by placing a wire-mesh across the width so as to have a dimension of 17” X 15” X16”.

Table 3.1. Experiment I-Rearing of laying hens at different stocking density in California cage system

Treatment groups	One cage box is equal to one 1 replicate		Number of replicates	Number of birds
A	387cm ² or 60 sq. inch per bird (5 birds per cage box)	High density	16	80
B	484 cm ² or 75 sq. inch per bird (4 birds per cage box)	Moderate density	16	64
C	548 cm ² or 85sq. inch per bird (3 birds per cage box)*	Medium density	16	48
D	645 cm ² or 100 sq. inch per bird (3 birds per cage box)	Lowest density	16	48

*Cage box restructured to achieve the mentioned space

3.4.2 Cage Reared Commercial laying hen Well Being Scorecard:

During the first experiment the laying birds were reared at different stocking density to evaluate the performance. Well being scorecard was developed on the basis of production, egg quality, immunity, stress parameters, economics, welfare and behaviour of the birds to select the best performance treatment groups. On the basis of Delphi technique (Balamurugan *et al.*, 2019) Well being scorecard, weightage and guidelines was developed comprising of panel of national experts from academic, scientist and poultry industry. The opinions from different panels (statutory student advisory, College Advisory Board, Board of Studies of subject Poultry Science :Appendix I) were invited, collated, discussed and summarized for the preliminary Well Being scorecard. During round one, the experts were asked to comment on the parameter to be



Stocking Density: 60 sq. inch or 387cm²/bird



Stocking Density: 75 sq. inch or 484 cm²/bird



Stocking Density: 85 sq. inch or 548 cm²/bird



Stocking Density: 100 sq. inch or 645 cm²/bird



Restructured Cage in Stocking Density 85 sq. inch or 548 cm²/bird

Plate 3.2: Rearing of laying hens at different stocking densities

undertaken for evaluation, scoring pattern, and weights to be assigned. During the next round, the refined score card was circulated to the experts through email and opinions were received. The performance scorecard was discussed in Animal ethical committee of college too (Appendix-II). On the basis of consensus specific mark weightage was given to each parameter to be tested during the experiment I. (Table 3.2)

Table 3.2. Development of cage reared commercial laying hens well-being Scorecard on the basis of stocking density, production and welfare of the birds

Parameters	Weightage (%)	Sub-division of Weightage (%)		Sub-division of Parameters	Marks Distribution
1. Production and Economics	48	24	16	a) Percent Egg Production	Treatment achieving the highest egg production will be given maximum 16 marks and subsequent treatment as 12, 08 and 04.
			08	b) Feed Conversion Ratio per egg	Treatment achieving the best FCR will be given the highest 08 marks, followed by 06, 04 and 02 for the other treatments.
		24	16	a) Cost of production per Egg In relation to Recurring expenses.	Treatment group with the minimum cost of production would be assigned score 16, followed by 12, 08 and 04.
			08	b) Cost of production per Egg In relation to Space Utilization.	Treatment group with the maximum space utilization will score 08, followed by 06, 04 and 02.
2. Egg Quality, Immunity and Stress Parameters	24	12	04	a) Egg Weight	Treatment with highest egg weight will receive the maximum 04 marks followed by 03,02,01 for the subsequent treatments.
			04	b) Percentage of Broken Egg	Treatment conforming to the less broken eggs will have the maximum 4 marks followed by other group as 03, 02,01.
			04	c) Percentage of Clean Eggs	Treatment group producing cleanest eggs among the treatments will have the highest 04 marks followed by subsequent groups as 03, 02,01.
		12	04	a) HI Titer	Depending on the best titre, the treatment groups would be assigned score from 04 to 01
			04	b) Heterophil/ Lymphocyte Ratio	Depending on the H/L, the treatment groups would be assigned score from 04 to 01.
			04	c) Corticosterone level	Depending on the corticosterone, the treatment groups would be assigned score from 04 to 01
3. Welfare and behaviour (Abrahamsson <i>et al.</i> , 1996 Kestin <i>et al.</i> ,1992)		16	08	Physical condition of bird- Feather Scoring	Depending on the feather scoring suggested by Abrahamsson <i>et al.</i> , 1996 at the end of the trial marks will be assigned from 8,6,4 and 2 for full feathers cover, worn feathers, detectable small and large bare patches to no feather most of the body.
			08	Gait Scoring	Gait performance as described by Kestin <i>et al.</i> ,1992 at the end of the trial

Behaviour data will be collected with the help of CCTV	28				observed marks would be assigned from 8,6,4 and 2 to treatment groups having sound birds with no detectable impairment, bird moving fast with flight, significant or serious deficiency and birds that barely able to move or total inability to walk.
		12	06	a) State behaviour (standing, walking, sleeping etc)	The activity number for state behaviours would be calculated for each treatment and group with the highest activity were offered highest score of 06, followed by others 04,02 and 00.
			06	b) Event Behaviour (feather pecking, drinking , feeding, preening etc)	The activity number for event behaviours would be calculated for each treatment and group with the highest activity were offered highest score of 06, followed by others 04,02 and 00.
Total	100				

Table 3.3. Well Being Scorecard of commercial laying hens at stocking density 60 sq. inch per bird

Stocking densities (Sq. Inch)		Weightage %	Sub division of weightage %		Parameters
60	1. Production performance and economics	12/48	6/24	4/16	Percent Egg Production
				2/08	Feed Conversion Ratio per egg
			6/24	4/16	Cost of Production per egg in relation to recurring
				2/08	Cost of production per egg in relation to space utilization
	2.Egg Quality, Immunity and Stress Parameters	10/24	4/12	1/4	Egg weight
				1/4	Percentage of Broken egg
				2/4	Cleanliness
			6/12	4/4	HI Titre
				1/4	Heterophil/ Lymphocyte Ratio
				1/4	Corticosterone level
	3. Welfare and Behaviour	10/28	4/16	2/8	Feather scoring
				2/8	Gait scoring
			6/12	4/6	State Behaviour
				2/6	Event Behaviour
Total	32/100				

Table 3.4. Well Being Scorecard of commercial laying hens at stocking density 75 sq. inch per bird

Stocking densities (Sq. Inch)		Weightage %	Sub division of weightage %		Parameters
75	1. Production performance and economics	38/48	18/24	12/16	Percent Egg Production
				6/08	Feed Conversion Ratio per egg
			20/24	16/16	Cost of Production per egg in relation to recurring
				4/08	Cost of production per egg in relation to space utilization
	2.Egg Quality, Immunity and Stress Parameters	17/24	7/12	3/4	Egg weight
				3/4	Percentage of Broken egg
				1/4	Cleanliness
			10/12	3/4	HI Titre
				4/4	Heterophil/ Lymphocyte Ratio
	3. Welfare and Behaviour	18/28	12/16	3/4	Corticosterone level
				4/8	Feather score
			6/12	8/8	Gait score
				2/6	State Behaviour
				4/6	Event Behaviour
	Total	73 /100			

Table 3.5. Well Being Scorecard of commercial laying hens at stocking density 85 sq. inch per bird

Stocking densities (Sq. Inch)		Weightage %	Sub division of weightage %		Parameters
85	1. Production performance and economics	48/48	24/24	16/16	Percent Egg Production
				8/08	Feed Conversion Ratio per egg
			24/24	16/16	Cost of Production per egg in relation to recurring
				8/08	Cost of production per egg in relation to space utilization
	2.Egg Quality, Immunity and Stress Parameters	18/24	9/12	4/4	Egg weight
				4/4	Percentage of Broken egg
				1/4	Cleanliness
			9/12	3/4	HI Titre
				2/4	Heterophil/ Lymphocyte Ratio
				4/4	Corticosterone level
	3. Welfare and Behaviour	18/28	12/16	4/8	Feather score
				8/8	Gait score
			6/12	0/6	State Behaviour
				6/6	Event Behaviour
Total	84/100				

Table 3.6 - Well Being Scorecard of commercial laying hens at stocking density 100 sq. inch per bird

Stocking densities (Sq. Inch)		Weightage %	Sub division of weightage %		Parameters
100	1.Production performance and economics	44/48	24/24	16/16	Percent Egg Production
				8/08	Feed Conversion Ratio per egg
			20/24	16/16	Cost of Production per egg in relation to recurring
				4/08	Cost of production per egg in relation to space utilization
	2.Egg Quality, Immunity and Stress Parameters	16/24	11/12	3/4	Egg weight
				4/4	Percentage of Broken egg
				4/4	Cleanliness
			5/12	1/4	HI Titre
				1/4	Heterophil/ Lymphocyte Ratio
				3/4	Corticosterone level
	3. Welfare and Behaviour	22/28	16/16	8/8	Feather score
				8/8	Gait score
			6/12	6/6	State Behaviour
				0/6	Event Behaviour
	Total	82/100			

After completion of the first experiment of rearing the laying hens at different stocking density, data were compiled and analysed for production, welfare and behaviour. On the basis of well being score card (Table 3.2) it was noted that the birds reared at stocking density 548 cm² or 85 sq. inch per bird and 645cm² or 100 sq. inch per bird scored highest marks 84 % and 82 % respectively (Table 3.5 & 3.6). Mentioned two highest scoring treatment groups were further tested for flock size and its effect on production performance, antibody titre, welfare, behaviour and their economics in the experiment II.

3.4.3 Experiment II: Interaction effect of stocking densities (on the basis of I experiment) and flock size on production, welfare and behaviour of laying hens

In consecutive year October 2019, second experiment was carried out for 20 week period on the laying birds (BV-300) of age, 28– 47th weeks to note the effect of two different flock sizes at best suited stocking density of 548 cm² or 85 sq. inch per bird and 645cm² or 100 sq. inch per bird (on the basis of Experiment I) to test production, welfare and behaviour of laying hens at two flock size of 6 and 9 birds. A total of 180 birds were divided into four treatment groups A1, A2, B1 and B2(Plate 3.3). Similar to Experiment I, each cage box represented one replicate and total six replicate were used to conduct the interaction effect of stocking density and flock size experiment to determine the best stocking density and flock size.



Stocking Density 85 sq. inch/bird & Flock size of 6 birds



Stocking Density 85 sq. inch/bird & Flock size of 9 birds



Stocking Density 100 sq. inch/bird & Flock size of 6 birds



Stocking Density 100 sq. inch/bird & Flock size of 9 birds

Plate 3.3: Rearing of laying hens at two different stocking densities and flock sizes



Stocking Density 85 sq. inch/bird & Flock size of 6 birds in furnished cage



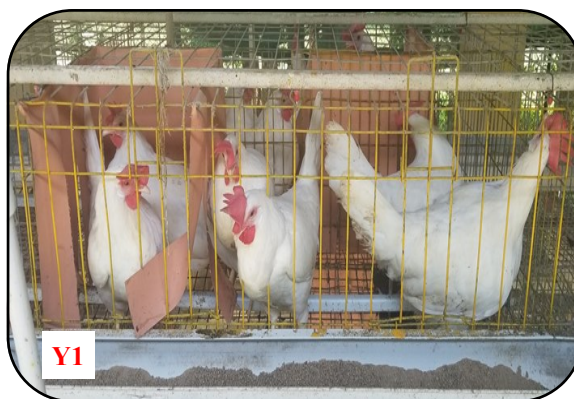
Stocking Density 85 sq. inch/bird & Flock size of 9 birds in furnished cage



Furnished cage



Non-Furnished cage



Stocking Density 100 sq. inch/bird & Flock size of 6 birds in furnished cage



Stocking Density 100 sq. inch/bird & Flock size of 9 birds in furnished cage

Plate 3.4: Rearing of laying hens at two different stocking densities and flock sizes in furnished cage

Table 3.7: Experiment II Rearing of laying hens at two different stocking densities and flock sizes in California cages

Treatments		Space per bird	Flock Size	Bird X cage Box	Flock Size each box	Replicate	No. of birds
A	A1	85 sq. inch/bird (548cm ² /bird)	Double	3x2	06 birds	6	36
	A2		Triple	3x3	09 birds	6	54
B	B1	100 Sq. inch / bird (645 cm ² /bird)	Double	3x2	06 birds	6	36
	B2		Triple	3x3	09 birds	6	54

*Restructured cage box specification of group A1 34''X15''X16 '' and A2 51''X15''X16 '' (width x depth x height) Cage box specification of group B1 40''X15''X16''and B2 60''X15''X16'' (width x depth x height)

3.4.4 Experiment III : Interrelationship between best stocking densities (548cm² and 645cm²/b) and flock size (6 and 9 birds) of conventional cage and furnished cage rearing of commercial laying hens on production, welfare and behavior.

In parallel with second experiment (October 2019), the third experiment was conducted with the aim of rearing the commercial White Leghorn (BV300) layer birds in furnished cages to meet welfare and behaviour needs of the laying birds. Similar to second experiment (same stocking density and flock size) the third experiment was conducted for 20 weeks period on total 180 birds of age 28 weeks. Each cage box represented one replicate to form six replicate per treatment to achieve specific flock size and stocking density. The major difference in Experiment-II and Experiment-III was furnished cages.

In this Experiment –III the existing conventional California cages were furnished with additional facility of perch, scratch pad and nesting area for laying birds (Plate 3.4) At one corner of the furnished California cage, nesting area was provided with the help of hanging curtains flaps so as to provide privacy for the birds during the oviposition/ laying time. The nesting area was covered from three sides

and the front was kept open for the access of feed. The nesting area of 265 cm² per hen was provided in each cage as experimented and suggested by Guo and Song, 2012. A perch was provided across the width of the cage at middle portion. The provided perch space was 14.39 cm and 16.93 cm per bird for 548cm²/b or 85 and 645cm²/b or 100 sq. inch/bird stocking densities. A scratch pad of astro-turf (5x15”) was tied to floor wire of furnished cages so that layer birds had the facility of scratch.

The furnished cage treatment were divided into four treatment groups X1, X2, Y1 and Y2. All the furnitures were provided in allotted (548 and 645cm²/b) area only. After conducting Experiment-III the best suited stocking density and flock size of Experiment-II (conventional cage 548 and 645cm²/b X 6 Bird) were compared with Experiment-III best findings of furnished cage 548 and 645cm²/b X 6 Bird, for production, performance, egg quality, economics and welfare of the laying hens, to arrive on final conclusion. The basic purpose of the Experiment-III was to compare the best suited conventional California cages (548 and 645cm²/b X 6 Bird) with furnished California cages of same stocking density and flock size (548 and 645cm²/b X 6 Bird).

Table 3.8: Experiment III- Rearing of laying hens at two different stocking densities and flock sizes with Modified/Furnished California cage

Treatment		Space per Bird	Flock size	Bird X Cage box	Flock Size	Nesting Area /Bird	Perch space/ Bird	Scratch-pad space/cage box	No. of Replicates	No of Birds
X	X1	548 cm ² or 85 sq. inch per bird	Double	3 X 2	06 birds	265cm ²	14.39 cm	483.87cm ²	6	36
	X2		Triple	3 X 3	09 birds					54
Y	Y1	645cm ² or 100 sq. inch or per bird	Double	3 X 2	06 birds	265cm ²	16.93 cm	483.87cm ²	6	36
	Y2		Triple	3 X 3	09 birds					54
<p>Restructured cage box specification of group X1 is 34''x 15''x 16 ''and X2 is 51''x 15''x16 '' (width x depth x height)with furnished facility of perch, nest and scratch pad.</p> <p>Cage box specification of group Y1 is 40''x 15''x 16''and Y2 is 60''x 15''x 16'' (width x depth x height) with furnished facility of perch, nest and scratch pad.</p>										

3.5 Parameters studied

The following observations were recorded during all the experiments.

3.5.1 Production Parameters

3.5.1.1 Egg Production and Egg weight:

The eggs were collected twice a day and record of each replicate egg production were maintained on daily basis. The egg mass/egg weights of the each replicate production were noted once in a week.

Egg mass=Number of egg produced in a period (15 days) X Average egg weight (gm)

3.5.1.2 Feed Consumption:

The experiments were conducted on birds (BV 300) of age 28 – 47th week therefore; as per standard a precisely weighed quantity (110 g/b/d) of feed was offered to all the experimental birds. The residual feed from feeding trough of each replicate was collected at the end of each week which was negligible. Due care was taken to avoid feed wastage to note precise quantity of feeding.

3.5.1.3 Feed Conversion Ratio:

Based on the data of egg production and feed intake the feed conversion ratio (FCR) were calculated on weekly basis during the experimental period.

$$\text{Feed Conversion ratio (FCR)} = \frac{\text{Total feed intake in a period (kg)}}{\text{Total egg mass (kg) produced in that period.}}$$

(Per Kg egg mass)

$$\text{Feed Conversion ratio (FCR)} = \frac{\text{Total feed intake in a period (kg)}}{\text{Total egg produced in that period}} \times \frac{12}{12} \quad \text{X}$$

(Per dozen egg)

3.5.1.4 Live Body Weight:

Live body weight (LBW) changes of 10 % of the experimental flock were recorded by individual weighing of the birds to note the changes in LBW at the start and end of the experiment.

3.5.1.5 Body Weight Gain:

Body weight gain (BWG) changes were calculated at 10% of the experimental flock by individual weighing of the birds to note the changes in BWG.

3.5.2 Egg quality traits

The egg quality comprises of external egg quality and internal egg quality traits. At each fifteen days twelve eggs per treatment were used to assess the egg quality of the experimental birds which were reared at different stocking density, flock size (Plate 3.5).

3.5.2.1 Measurement of External egg quality traits

3.5.2.1.1 Shape Index:

It is the ratio of width to length of the egg. The length and width of the eggs were measured with Vernier calliper and the shape index was calculated as per Schultz (1953)

$$\text{Shape index} = \frac{\text{Maximum width of egg (cm)}}{\text{Maximum length of egg (cm)}} \times 100$$

3.5.2.1.2 Cracked and Dirty Eggs (%):

At each fifteen days, all the treatment eggs were visually graded to check for the cracks and dirtiness of the egg. During laying process the eggs use to get contaminated with faecal material and blood smears on the shell, those eggs were noted to calculate the effect of stocking density flock size and cage effects.

3.5.2.2 Measurement of internal egg quality traits

3.5.2.2.1 Shell weight:

In order to determine the egg shell weight, the content of the eggs were emptied, the shell was thoroughly washed in running water, dried for two hours at room temperature with the shell membrane intact, and weighed on an analytical scale. Eggshell weight was determined after drying.

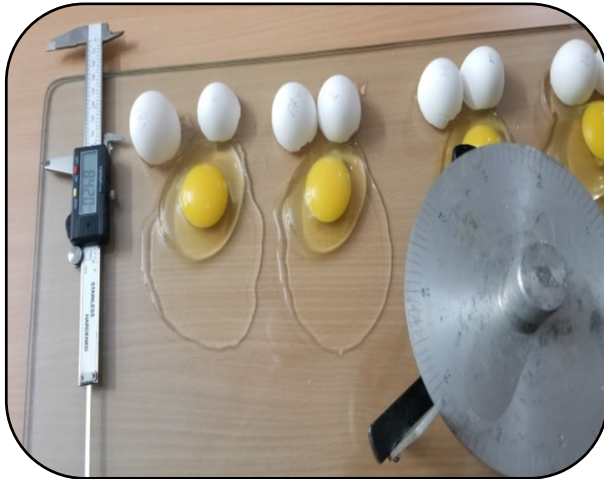
3.5.2.2.2 Shell thickness:

Egg shell thickness was measured with Screw gauge at three different identical area (mid/broad/narrow) of egg after removal of shell membranes.

3.5.2.2.3 Albumen Index:

Albumen index a measure of the quality or freshness of an egg. It is the height: width ratio of the albumen when the egg is broken on a flat surface, height (mm) of thick albumen was measured at two different points with the help of Spherometer and their average value was worked out. Similarly, the width of thick albumen was measured by Vernier calipers. The albumen index was calculated according to Heiman and Carver (1936) as shown hereunder:

$$\text{Albumen index} = \frac{\text{Average thick albumen height (mm)}}{\text{Average thick albumen width (mm)}}$$



Internal Egg Quality Assessment



Yolk Color Assessment By Roche Yolk Color Fan



Clean Eggs



Dirty Eggs

Plate 3.5 : Egg Quality Parameters

3.5.2.2.4 Yolk Index:

Yolk index is the ratio of yolk height over yolk diameter. The height of yolk was measured at the highest yolk point, the diameter was measured at two different points and the average was considered for calculation.

$$\text{Yolk index} = \frac{\text{Height of yolk (mm)}}{\text{Average width of yolk (mm)}}$$

3.5.2.2.5 Haugh Unit:

It is an equation combining the weight of the egg with the height of the central portion of the thick albumen determines the haugh unit (HU), as per formula suggested by Haugh (1937).

$$\text{H.U.} = 100 \log (H + 7.57 - 1.7W^{0.37})$$

Where: H= Albumen height in mm; W= Egg weight in g

3.5.2.2.6 Yolk colour:

The yolk fan provides a simple means of measuring and evaluating yolk colour. A Roche colour fan strip with numerals was used to determine the egg Yolk colour. The blades of the yolk fan was held immediately above the egg yolk and viewed vertically from above, with the blade numbers facing down and the yolk positioned between the tips of the blade. The reader was always faced the side of the blade without numbers and showed the number to the assistant for recording. The fan was closed from one egg to the next to ensure. The independence of every measurement the evaluation of yolk colour was carried out by the same person on a white, non-reflective surface, in order to eliminate the influence of adjacent colors. Roche Yolk Color Fan is 15 scales colour index to distinguish the yolk color density and is widely used in the poultry industry.

3.5.3 Immunological Status:

The immune responsiveness of layer birds maintained in different stocking densities, flock sizes and in furnished cages were analyzed by humoral immune response. Humoral immune response was tested by estimating haemagglutination inhibition titer against New Castle Disease virus (NCDV) during start, mid and end of the 20 week experimental period. All birds were vaccinated two weeks prior to blood collection with New Castle Disease Virus (lyophilized La Sota strain of NDV) through drinking water which was used as a test antigen to quantitatively analyze specific antibody responses, as a measure of humoral immunocompetence. Six (experiment II & III) to eight (experiment I) birds from each treatment were randomly selected and marked for the blood collection. Blood was collected fourteen days post immunization from the brachial vein of the birds on 28 (start of experiment), 37 (Mid) and 47th (end) weeks of age. The serum was separated by centrifugation at 3000 rpm for 20 minutes and decanted into clean, sterile plastic vials and stored under deep freeze at -18°C to -20°C . Haemagglutination inhibition (HI) test was performed as per procedure of O.I.E (1992). Two fold serial dilutions of antigen and serum was made for HA and HI, respectively. The HI titer was expressed as \log_2 value of the highest dilution of serum causing complete inhibition of 8 HA unit of antigen.

3.5.3.1 Preparation of Reagents:

Alsever's solution and phosphate buffer saline (PBS) were prepared as per the composition given below.

Alsever's solution

Dextrose	= 20.5 g
Sodium citrate	= 8.0 g
NaCl	= 0.55 g
Citric acid	= 0.055 g
Distilled water	= 1000 ml

Stored in a refrigerator at 4°C.

PBS (phosphate buffer saline)

Sodium chloride (NaCl) = 8.0 g

Potassium chloride (KCl) = 0.29 g

Potassium dihydrogen phosphate (KH₂ PO₄) = 0.24 g

Disodium hydrogen phosphate (Na₂ HPO₄.2H₂O) = 1.44 g

Distilled water = 1000 ml

The pH of the solution was adjusted to 7.2

3.5.3.2 New Castle Disease Virus (NCDV) Titer

HA test was performed in 'U' bottom micro-titer plate as per O.I.E. Procedure.

1. 40µl of PBS (0.1% and 7.2 pH) was added in first well of U bottom micro titer plate.
2. 25µl of PBS was distributed in subsequent well (11 well).
3. 10µl of antigen was added in first well.
4. Two-fold serial dilutions were made up to 11th well and last well was kept as control.
5. 25µl chicken RBC (1% v/v) added to each well.
6. After gentle mixing, the plates were covered and incubated at 37°C for 45 minutes.
7. The micro titer plates were read under bright light.
8. The reciprocal of highest dilution of the antigen showing 50% haemagglutination was considered as HA titer.
9. The virus was diluted to contain 8 HA units and was used as HA antigen in the HI test.

3.5.4 Welfare of cage laying birds:

Assessing the hen's welfare should consider freedom from hunger, malnutrition and thirst; freedom from fear and distress; freedom from physical and thermal discomfort; freedom from pain, injury and disease; and freedom to

express normal patterns of behaviour (FAWC, 1992). The welfare of the laying birds were judged by physical, physiological and behavioural indicators at different stages (Plate 3.7) of the experimental period {28 (start), 37 (mid) and 47th (end) week age of the bird}.

3.5.4.1 Physical Indicators

3.5.4.1.1. Feather score:

Feather scores were recorded at different time period, considering the same bird for every time to measure the difference and effect of stocking density, flock size and rearing of laying birds in furnished cages. Plumage condition was scored using a modified eight point scoring system (Abrahamsson *et al.*, 1996) for different areas of the body parts that is head, neck, back wings, tail, abdomen and breast.

Feather score description

Marks	Description
2	No feathers over most of the areas of head, neck, back, wings, tail, and abdomen or breast or large bare patches of > 30 mm diameters with no feathers
4	Body showed marked deterioration with few small bare patches <30 mm diameter.
6	Worn feathers detectable but complete feather coverage was observed.
8	Full feather cover and indicated very good feathering.



Plate 3.7 : Blood Collection and Processing



3.5.4.1.2. Gait score:

Similar to feather score method, gait score was recorded in the same birds used for feather score at different phases of the experimental period with modified 4-point scoring system (Kestin *et al.*, 1992). The gait scoring was performed by two persons, one gently driving and herding each bird with a light cane and the other observing from a crouched position. An understanding between the two assessors/examiners for each bird was maintained before the score was recorded.

Gaits score description

Marks	Description
2	The bird is incapable of sustained walking on its own feet. Although it may be able to stand, locomotion could be achieved with the assistance of the wings or by crawling on the shanks.
4	The bird has a severe gait defect, it is still capable of walking, but only with difficulty and when driven or strongly motivated. Otherwise it squatted down at the first available opportunity. Its acceleration, speed and, manoeuvrability is all severely affected.
6	The bird had a definite and identifiable defect in its gait but the lesion did not hinder it from moving or competing for its resources.
8	The bird walked normally with no detectable abnormality; it was dexterous and agile. Typically the foot was picked up and put down smoothly and each foot was brought under the bird's centre of gravity as it walked, rather than the bird swaying.

3.5.4.2 Physiological Indicators

3.5.4.2.1 Heterophil : Lymphocyte ratio:

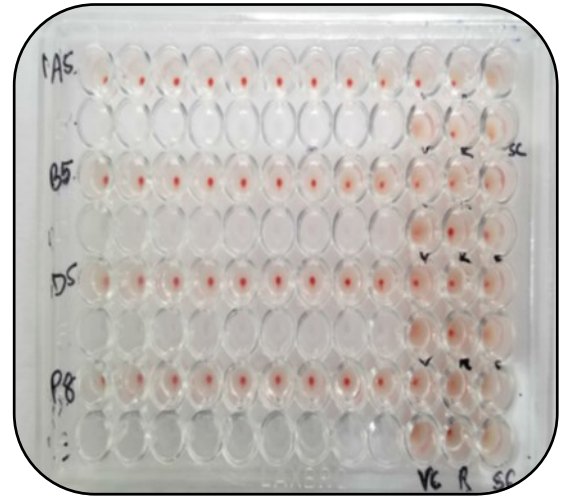
The heterophil:lymphocyte ratio (H:L) is an haematological measure that has been documented as an index of stress in chickens (Gross and Siegel, 1983). Although the influence of cage rearing demonstrated scarcely effect, other stressors that negatively impact poultry live performance often cause the H:L ratio to increase. To obtain heterophil to lymphocyte ratio, six (experiment II & III) to eight (experiment I) birds from each treatment were randomly selected for the blood collection (Plate 3.6) at 28 (start of experiment), 37 (mid of experiment) and 47th (end of experiment) weeks of age. The blood drop smear was stained using Wright's stains and fixed approximately 1 hour later by application of methyl alcohol (Shen and Patterson, 1983). One hundred leukocytes, including granular (heterophils) and non-granular (lymphocytes), were counted on every slide (duplicate each for four birds) under the microscope at 100X. Heterophil to lymphocyte ratio was calculated as the method described by Gonzales *et al.*, 2003.

3.5.4.2.2. Corticosterone:

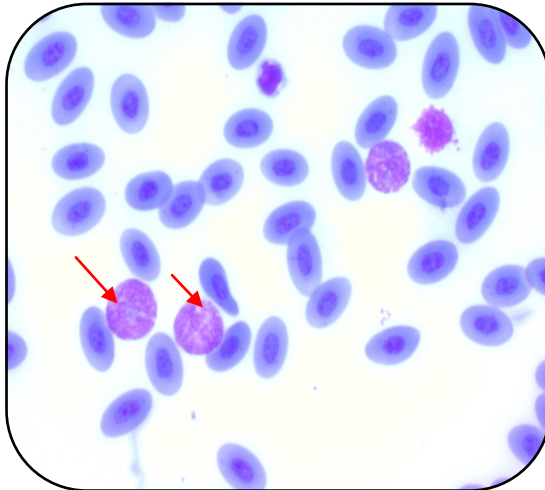
Corticosterone, also known as the stress hormone, is the major glucocorticoid in birds. In commercial poultry, stressors such as heat, food and water deprivation (Beuving, 1980) and transportation (Broom and Knowles, 1989) increase glucocorticoids production. This is mediated by the release of adrenocorticotrophic hormone from the pituitary gland. The stress response is largely characterized by activation of two systems, the hypothalamic-pituitary-adrenal axis and the sympathoadrenal axis. The common measures of stress that result from the activation of these systems are glucocorticoids, predominantly corticosterone in poultry and the hormones epinephrine and norepinephrine. Researchers measure changes in these hormones when assessing the animal's response to potential stressors and gain information about how the individual perceives the stressor. In this study corticosterone has been analyzed by ELISA kit (Eiahcor 96-Invitrogen-Thermo



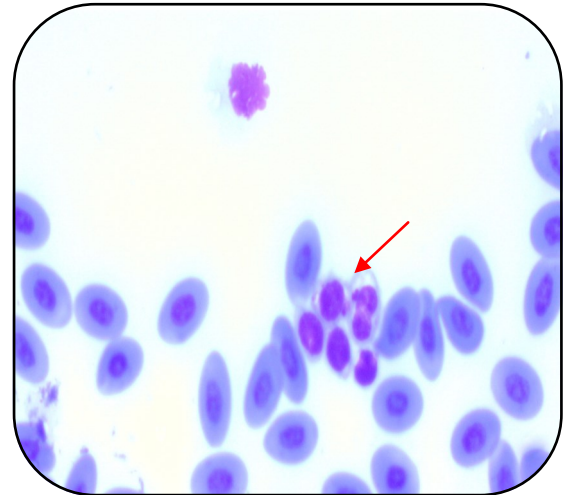
Corticosterone tested by ELISA Kit



Estimation of HI titer against ND Virus



Heterophils



Lymphocytes



Feather Score



Gait Score

Plate 3.6 : Physiological and Physical Parameters of Welfare

Fisher Scientific). Serum samples for corticosterone estimation were obtained from six birds randomly selected from each treatment on 28 (start of experiment), 37 (mid of experiment) and 47th (end of experiment) weeks of age. The bleeding procedure was less than one minute to minimize the influence of handling stress, which was common for all birds. All blood samples were allowed to clot for 1 hour and serum was decanted in centrifuge tubes and stored at -20°C for further analysis.

The detail procedure of ELISA test as below:

1. Added 50 µl of standard or sample to the appropriate wells of the plate.
2. Added 75 µl of 1X Assay buffer into the wells for detecting non-specific bindings (NSB).
3. Added 25 µl of Cortisol conjugate to each well.
4. Then added 25 µl Cortisol Antibody to each well except NSB wells.
5. Tapped the side of the plate to mix and then kept the plate covered with plate sealer and incubated for 1 hour at room temperature with shaking.
6. The solution from the well is thoroughly aspirated out and washed four times with 300 µl of 1X wash buffer.
7. Next the chromogen (TMB substrate) 100 µl is added to each well, the substrate solution will begin to turn blue and let it incubate for 30 minutes.
8. Added 50 µl of stop solution to each well and tapped on the side of the plate, the solutions in the well change from blue to yellow.
9. Next the absorbance is being read at 450 nm within 10 minutes of adding stop solution to the plate. Corticosterone standard solution is being provided to generate a standard curve for the assay and all samples are read off from the standard curve.

3.5.4.3 Behavioural Studies of cage laying birds

Behavioural observations were assayed to quantify animal biological responses. Poultry behaviour is a reflex of their welfare status at a particular moment and is related to internal (physiological) and external (environmental) factors. The correct interpretation of the behaviours expressed by poultry, including their frequency, duration and sequence was used to estimate their welfare. Animal ethology has divided animal behavioural repertoires into two components, events which are relatively short in duration and states which are relatively long in duration.

State behaviour: States are behaviour of longer duration, such as prolonged activities, body postures or proximity measures. The activities which were taken under state behaviour are standing, sitting, sleeping, walking, perching and investigation.

Event Behaviour: Events are behavioural patterns of relatively shorter duration, such as discrete body movements or vocalization, which can be approximated as points in time. The activities undertaken are preening, feather pecking, feeding, drinking, scratching, wing flapping and fighting. All the state and event behavioural activity of the birds were video recorded in CCTV camera for 24 hours during the entire experiment. Periodically the recording was extracted from hard disc of DVR on 28 (start of experiment), 37 (mid of experiment) and 47th (end of experiment) weeks of age of the bird to study the behaviour pattern of the laying birds at different stocking density, flock size and in furnished cages. During the experiment, continuous sampling method Martin and Bateson (2007) was used to observe the cage layer bird's behaviour. When using this method, the observer simply records all of the activity that occurs while the bird/animals are being watched. This sampling method was very helpful in recording social interaction between two or more animals in a group. Continuous samplings for three birds per treatment were individually compared for 24-hours. The specific birds (03) from each treatment were marked with different colours (waterproofed, non-toxic dyes-black, red, blue, yellow and pink etc). From the specific 24 hours recording, an activity chart or ethogram was

prepared, an example shown for half an hour time period in Table 3.9. The time devoted (01 min) to each activity was expressed. The time budgets for feeding(1), drinking(2), standing(3), sleeping(4), sitting(6), walking(8), investigation(5), perching(7), feather pecking(11), wing flapping(13), scratching(10), fighting(12) and preening(9) on the perches were calculated as the mean proportion of birds performing each behavior over the 24 hours on 28 (start of experiment), 37 (mid of experiment) and 47th (end of experiment) weeks of age of the bird. As suggested by Li *et al.*, (2016) the description for the various state and event behaviors is given in Table 3.10.

Table 3.9 : Activity chart format for half-hour time (12:00 to 12:30)

12-12:30 pm Birds	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1																														
2																														
3																														
4																														
5																														
6																														

F= Feeding(1), D=Drinking(2), Sl= Sleeping(4), Si= Sitting(6), S=Standing(3), W= Walking(8), I=Investigating(5), F= Fighting, Ph=Perching(7), FP=Feather pecking(11), Sc=Scratching(10), P= Preening(9), WF-Wing Flapping(12).

Table 3.10: Behavioural categories and Definitions

Behavioural categories	Definition
Feeding	Hen directs its beak to feed trough and carries out pecking or eating,
Drinking	Hen directs its beak to nipple drinker and raises its head when getting water.
Standing	Both legs are straightened on the floor.
Sleeping	Hens' abdomen contacts with the floor and both legs are twisted under the body with eyes closed.
Investigation	Visual exploration and head movements while body is stationary.
Sitting	Hens' abdomen contacts with the floor and both legs are twisted under the body with eyes open.
Perching	Behaviors exhibited when the hens are on perches.
Walking	Hen raises one of its legs with the other leg standing on floor and moves forward.
Preening	Hen directs its beak to its own plumage of several body parts and carries on pecking, nibbling, combing or rotating movements, once or repeatedly.
Scratching	Hen directs its claw to the scratch-pad provided and rubbing it repeatedly.
Feather-Pecking	Hen pecking or pulling others' feather.
Fighting	Behaviors happened between two or more hens including pecking.
Wing Flapping	Bilateral movement of the wings including wing raising while standing.

3.5.5 Cost of Production:

During this study all attempts were made to calculate the cost of production incurred for rearing the birds at different stocking densities and flock size as well cost incurred for furnished cages. The cost of pullets, feed, medication, vaccination and other overheads for restructuring cages were considered while calculating cost of production. The cost of production was estimated by two methods:

3.5.5.1 Cost of Production per egg (CPPE) in relation to recurring expenditure: The cost of production per egg was calculated considering the recurring expenses incurred during the experiment. Recurring expenditure includes cost of pullets per egg, feed, medicine, vaccination and miscellaneous.

Cost of cages (non-recurring): The existing California cages were restructured to furnished cages with extra amenities (nesting area, scratch-pad and perches) for laying birds in Experiment III. Hence considering stocking density, flock size and restructuring of cage cost per bird was also calculated considering the approx. market prevailing rates to find the cost of production per egg.

3.5.5.2 Cost of Production per egg in relation to space utilization:

The number of birds were vary as per the stocking density and flock size of the laying birds, therefore the space utilized to produce per egg was differed in each treatment group. Hence cost of production of egg in relation to space utilization was calculated considering the mentioned formula.

$$\begin{array}{ccccccc} \text{CPPE} & & \text{CPPE on basis of} & & \text{Space Provided} & & \text{No. of birds} \\ \text{(Space utilization)} & = & \text{Recurring Expenditure} & \times & \text{(sq. inch Per bird)} & \times & \text{(Flock size)} \end{array}$$

3.6. Mortality:

The birds that were dead during the trial period was replaced by layers of same age that was reared (extra birds) in similar condition so as to maintain the stocking density and flock size of the experiments as documented for the different experiments. For experiment I a total of 3 birds were dead for the entire 20 week experimental period during the 30th week, 31st week and 45th week of bird's age. In experiment II a total of 4 birds were found dead at 29th, 38th, 39th and 40th week of bird's age and in experiment III 3 birds were found dead at 38th, 42nd and 43rd week of bird's age for the entire 20 weeks of experimental period. Post-mortem examination revealed no any gross lesion indicating of any kind of disease. All mortality was well within the limit.

3.7. Statistical Analysis:

Data emanated from different treatments were analyzed for statistical significance using completely randomized factorial designs (Snedecor and Cochran, 1989). All data were statistically analyzed using SPSS software package version 20.0. Variables having unequal observations were analyzed following least square design method and the Duncan's multiple range test (Duncan, 1955). Each replicate served as an experimental unit for statistical analysis of growth, production and economics while each representative bird from respective replicate served as an experimental unit for statistical analysis of immunity, welfare and behavior.

RESULTS AND DISCUSSION

Three experiments were conducted to test the production, welfare and behavior of commercial White Leghorn hens (BV 300) at different stocking densities and flock size in California and furnished cages. The results and observations of the study are presented in this chapter.

4.1 Experiment 1: Study the effect of different stocking density on production, welfare and behavior of commercial laying hens.

The results of the best suited stocking density in terms of egg production, egg quality, feed efficiency, immune response, economics, welfare and behavioral changes in conventional California cages were observed in commercial BV 300 layer (White Leghorn) birds. The total 240 experimental birds were maintained in four different stocking density for a period of 20 weeks, starting from 28 to 47th week of age of the birds. The results of the experiment obtained are presented and discussed as below.

4.1.1 Hen Day Egg Production (HDEP)

The data pertaining to HDEP % obtained from the birds reared in different stocking density groups is presented in Table 4.1 and same is depicted in Figure 4.1 for the overall 20 week period.

The laying birds from treatment 645cm²/bird provided with highest space allowance showed the non-significant difference in HDEP % compared to the birds reared with space 548 and 484 cm²/bird during 28-31, 32-35, 40-43 and 44-47 week of age. While, compared during overall production period i.e 28 to 47 week significant difference ($P < 0.01$) in HDEP % were noted among the laying birds reared in 645 and 484 cm²/bird. During the same duration non-significant difference were observed among the laying birds reared in 645 and 548 cm²/bird. Treatment 387cm²/bird having the highest stocking density with 05 laying birds had the significantly ($P < 0.01$) lowest HDEP % during entire experimental period when compared to all other space allowance provided to birds except during 44-47 week of age. If compared the overall HDEP % during 28-47 week age i.e 20

week period significantly ($P < 0.01$) highest egg production was recorded in the commercial laying hens reared in $645 \text{ cm}^2/\text{bird}$ compared to 387 and $484 \text{ cm}^2/\text{bird}$. Whereas, the HDEP % was non-significant difference among laying hens reared in 645 and $548 \text{ cm}^2/\text{bird}$. Similar observations were recorded among laying hens reared in 548 and $484 \text{ cm}^2/\text{bird}$. It was observed that as the space allowance increased from 387 to $645 \text{ cm}^2/\text{bird}$, the performance of the laying birds improved from 83.54 to 90.72 % HDEP. All the while the birds provided with $387 \text{ cm}^2/\text{bird}$ showed poor HDEP % compared to its counterpart treatment with higher space allowances. On the other side the birds received highest space allowance i.e $645 \text{ cm}^2/\text{bird}$ recorded highest HDEP % during the entire experimental period.

Craig and Milliken (1989) recorded that stocking density affected the rate of lay, the recorded rate of lay were 64 , 71.8 and 72.6% when the birds were maintained at 348 (high), 464 (medium) and 580 (low) cm^2/bird . Hens in medium and low density had significantly ($P < 0.005$) better rate of production than in high density cages, corroborating the present experiment. Lee and Moss (1995) also observed that treatment with highest density level had the lowest percentage hen-day egg production than hens in lowest density level. Kang *et al.*, (2016) observed hen-day egg production was lower ($P < 0.01$) for $10 \text{ birds}/\text{m}^2$ stocking density than stocking densities of 7 , 6 and $5 \text{ birds}/\text{m}^2$. They concluded laying performance was shown to decline in response to increased stocking density which was attributable to a reduction in the amount of feeding area per hen.

Anderson *et al.*, (2004) inferred that cage density effected the percentage HDP which was significantly greater ($P < 0.01$) for hens provided a density of $482 \text{ cm}^2/\text{bird}$ (82.3%) than for hens provided a density of $361 \text{ cm}^2/\text{bird}$ (77.4%). Similar results of negative effect on egg production percentage with increasing stocking density was reported by On basilar and Aksoy (2005). Increased stocking density had a negative effect on egg production percentage 94.1 , 89.3 and 78.5% for 1968 , 656 and 393.8 cm^2 per hen space respectively. Benyi *et al.*, (2006) reported significant better 85 % HDEP at $1100 \text{ cm}^2/\text{hen}$ and a higher egg

Fig. 4.1. Effect of Stocking Density on Hen Day Egg Production %

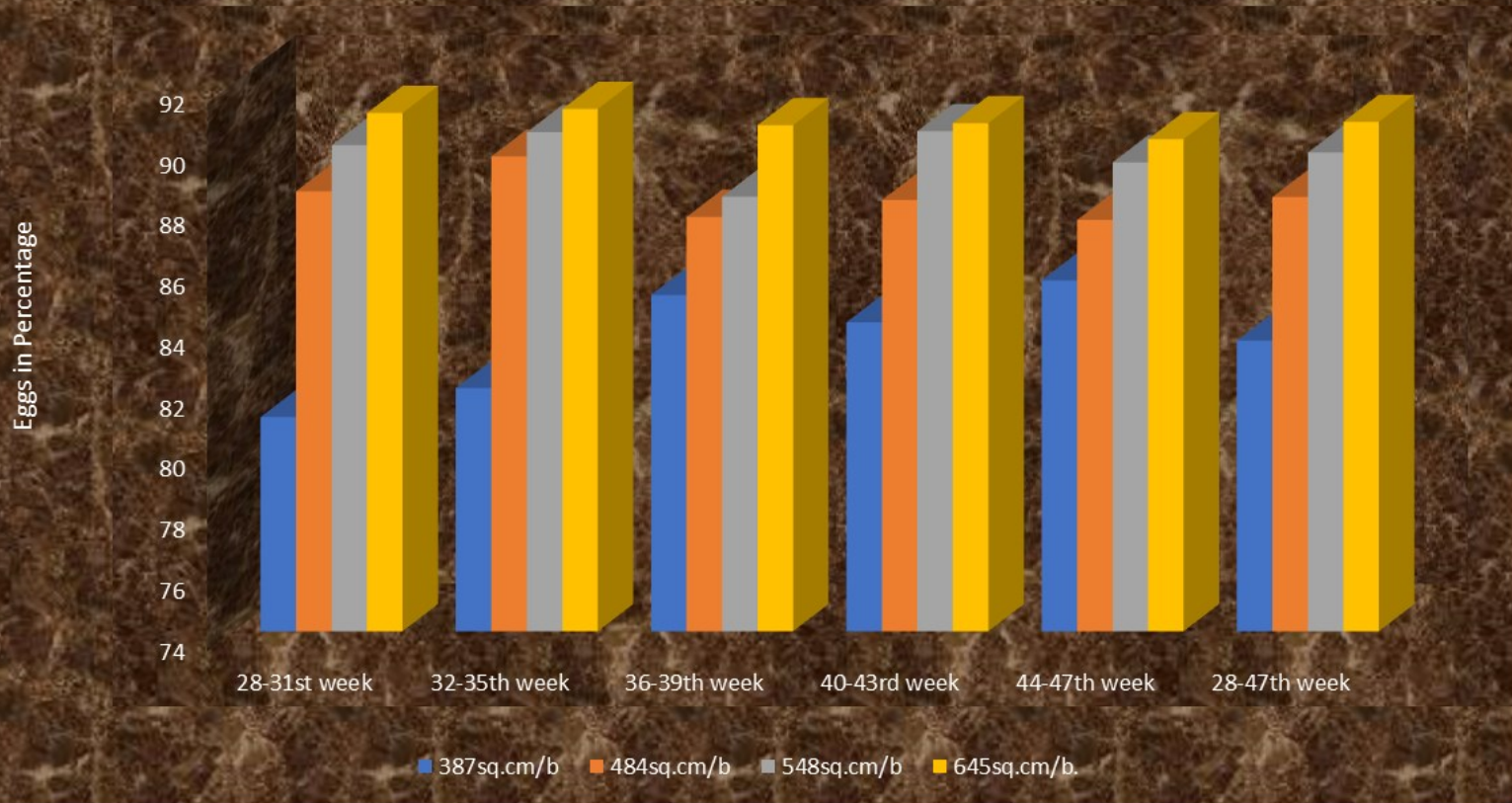


Table 4.1. Effect of stocking density on hen day egg production%

Treatment	Experimental duration/ birds age					
	28- 31wk	32- 35wk	36-39wk	40- 43wk	44- 47wk	28- 47wk
387cm ² /bird	81.03 ^c ±1.38	81.98 ^c ±0.76	85.04 ^c ±0.95	84.14 ^c ±0.88	85.52 ^{bc} ±0.65	83.54 ^c ±0.73
484cm ² /bird	88.44 ^{ab} ±1.31	89.58 ^b ±1.08	87.60 ^b ±0.91	88.15 ^{ab} ±0.80	87.50 ^{ab} ±0.95	88.25 ^b ±0.82
548cm ² /bird	89.95 ^{ab} ±1.15	90.38 ^{ab} ±0.88	88.26 ^b ±0.71	90.41 ^a ±0.86	89.39 ^a ±0.65	89.71 ^{ab} ±0.59
645cm ² /bird	91.01 ^a ±1.65	91.14 ^{ab} ±1.70	90.61 ^a ±0.98	90.68 ^a ±1.17	90.15 ^a ±1.07	90.72 ^a ±1.09
SEM	0.689	0.616	0.494	0.457	0.480	0.422
P value	**	**	**	**	**	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P<0.05, **P< 0.01.

output as compared to birds maintained at stocking density of 733 and 550 cm² per bird i.e 73 and 70 % HDEP respectively. Jalal *et al.*, (2006) confirmed that egg production declined as much as 10.1% (86.19 Vs 76%) as the number of hens per cage was increased from 3 to 6 with space allowance of 690, 516, 413 and 342 cm²/hen. Sarica *et al.*, (2008) concluded that increased space allowance or decreased cage stocking density (2000, 1000, 667 and 500cm² per hen) significantly enhanced egg production. Hens kept either at 667 or 1000 cm² cage densities produced the same amount of eggs (212 and 211 numbers) while those kept at 500 cm² space allowance decreased egg production. In Indian conditions Rajendran *et al.*, (2013) concluded that hen day egg production in WLH birds was significantly higher at space 270 sq. inch per bird as compared to 135, 90, 67.5 and 54 sq. inch per bird. HDEP for other spaces were non-significant among themselves. The results of the present study are also in contrary to reports by Craig (1986) who did not observe any changes of housing condition and stocking density in egg production (pen Vs cages with different stocking density of 2900 Vs 929 and 464cm²/hen) and Balogun *et al.*, (1997) that stocking densities have no effects on egg production.

It could be concluded from the above various observations that as the stocking density improved the HDEP % improved which is in agreement of the

present trial. High stocking density results in more competition for space, food and water and might result in physiological stress and reduced egg production.

4.1.2 Feed Intake and Feed Conversion Ratio:

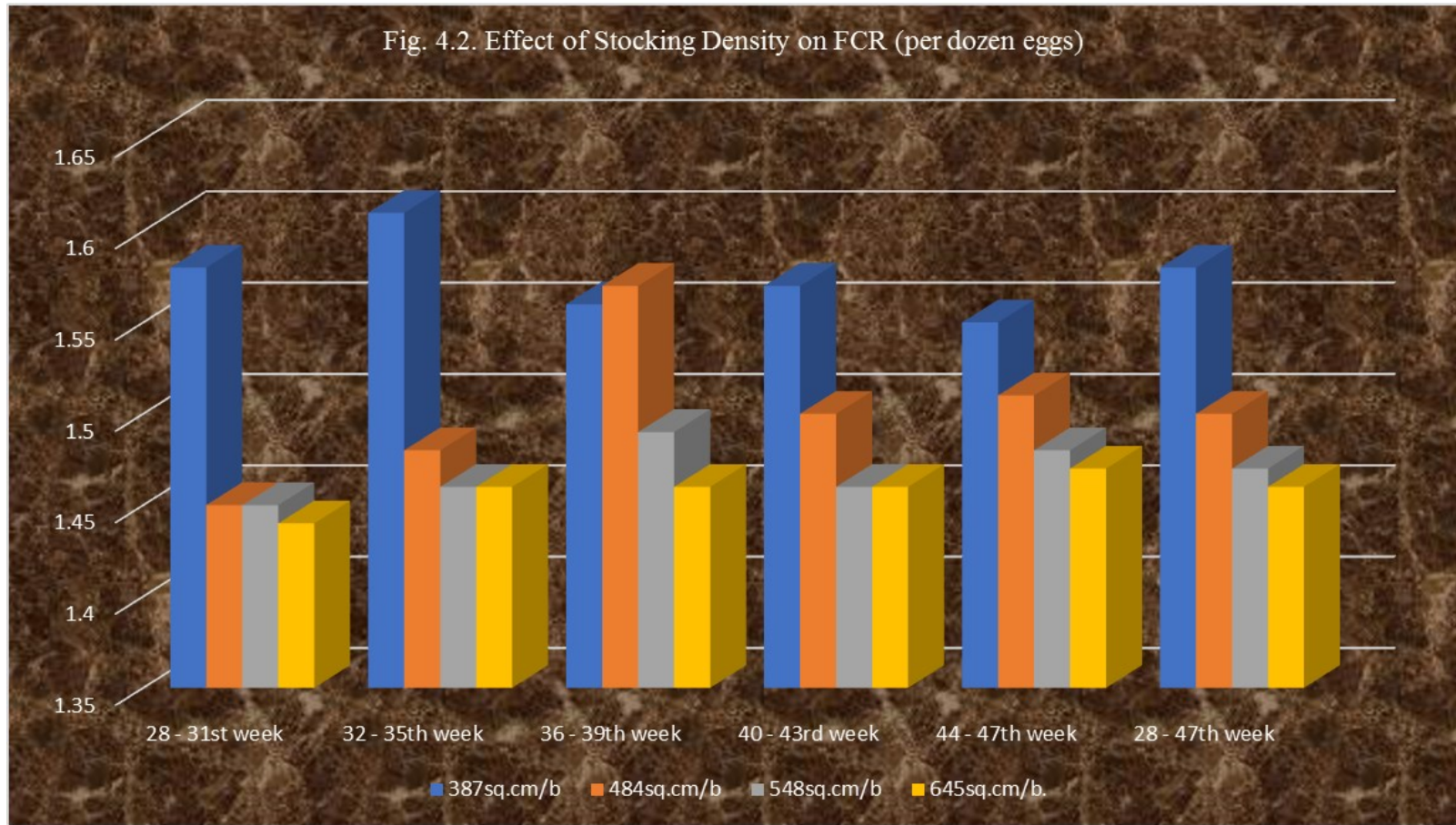
As per standard specification of BV 300 commercial WLH birds a weighed quantity of feed 110 g/bird was offered to all the birds during the entire 20 weeks of experimental period. At the end of the each week the residual feed from the feeding trough of each cage box was collected quantitatively and weighed and divided by the number of birds in that cage which was merely 0.5 gm/bird. Therefore, the actual feed intake of birds was considered to be 110g/bird for all the experimental birds.

The data pertaining to Feed Conversion ratio (per dozen egg and per kg egg mass) obtained from the birds reared at different stocking density group is presented in Table 4.2 and 4.3 and FCR (per dozen eggs) is depicted in Figure 4.2.

The aggregate of the FCR (per dozen eggs and per kg egg mass) for the 20 weeks of rearing showed a significant difference ($P < 0.01$) in FCR due to difference in stocking density treatment. The laying birds reared with highest stocking density with lowest space allowance i.e 387 cm²/bird recorded the significantly poorest FCR among all the treatment groups during entire experimental period except for 36-39 week age. The continuous non significant difference of FCR among treatment 645, 548 and 484 cm²/bird were changed to significant difference among treatment 645 and 484 cm²/bird at 44-47 week period. This change could be attributed due to higher space allowance. While comparing the overall, 20 week period, the FCR of birds reared with 484, 548 and 645 cm²/bird were non-significant among themselves with the best FCR noted 1.46 per dozen eggs in 645 cm²/bird. During same period, the birds reared at highest stocking density i.e 387 cm²/bird recorded significantly poor FCR.

Davami *et al.*, (1987) observed decrease in feed consumption (g/hen/day) 135 Vs. 117 g as the stocking density changed from 420 to 300 cm²/hen by increasing the number of birds from five to seven. Feed conversion ratio

Fig. 4.2. Effect of Stocking Density on FCR (per dozen eggs)



(kg/dozen egg) was significantly better ($P < 0.05$) at lower stocking density $420 \text{ cm}^2/\text{hen}$ than at higher stocking density $300 \text{ cm}^2/\text{hen}$ which is in agreement with our study that as stocking density improved from 387 to $645 \text{ cm}^2/\text{bird}$, there is improvement in FCR which may be attributed to the fact that hens in lower density cages faced less competition for feed intake and stress free movements resulted into better feed conversion to form the eggs. Similarly, Lee and Moss (1995) found feed efficiency was poorer ($P < 0.05$) for White Leghorn birds kept with 4 bird per cage as compared to 1 bird per cage which was because of reduced rate of egg production in 4 bird per cage group. Correlation coefficient analysis of study showed that egg production was negatively correlated with population density and feed required to produce a dozen egg was positively correlated ($P < 0.01$) with population density.

Contrasting to present study, non-significant interaction between density (361 and $482 \text{ cm}^2/\text{bird}$) and age for feed consumption and feed efficiency was reported by Anderson *et al.*, (2004). Hens raised at $690 \text{ cm}^2/\text{hen}$ had greater feed intake than those hens maintained at 342 and $413 \text{ cm}^2/\text{hen}$, but was not observed any difference with stocking density $516 \text{ cm}^2/\text{hen}$. The feed efficiency followed the trend that as stocking density was better and more space provided group, the feed efficiency declined in contrast to present experiment. Non-significant results for feed consumption and feed efficiency was reported by Karkulin (2006) when maintained birds at different stocking density in conventional (550 cm^2 per hen) and enriched cages (as per EU directives).

The effect of stocking density on FCR followed the same trend as HDEP% and was found to better in groups that had low stocking density. The treatment group having space of 484 , 548 and $645 \text{ cm}^2/\text{bird}$ had superior/better FCR than $387 \text{ cm}^2/\text{bird}$ space and this is more or less related with increased number of eggs produced (83.54 HDEP% for $387 \text{ cm}^2/\text{bird}$ space Vs 88.25 , 89.71 and 90.72 HDEP % for the rest) as the feed intake was constant. The birds reared at $645 \text{ cm}^2/\text{bird}$ exhibited better feed conversion ratio than 484 , $548 \text{ cm}^2/\text{bird}$ and best than $387 \text{ cm}^2/\text{bird}$, which indicates that feed was efficiently utilized for egg production rather than body tissue formation within the cage because of higher space.

Table 4.2. Effect of stocking density on feed conversion ratio (per dozen of eggs)

Treatment	Experimental duration/birds age					
	28- 31wk	32-35wk	36-39wk	40- 43wk	44-47 wk	28-47 wks
387cm ² /bird	1.58 ^a ±0.02	1.61 ^a ±0.01	1.56±0.02	1.57 ^a ±0.02	1.55 ^a ±0.01	1.58 ^a ±0.01
484cm ² /bird	1.45 ^b ±0.01	1.48 ^b ±0.02	1.57±0.26	1.50 ^b ±0.01	1.51 ^{ab} ±0.02	1.50 ^b ±0.01
548cm ² /bird	1.45 ^b ±0.01	1.46 ^b ±0.01	1.49±0.02	1.46 ^b ±0.01	1.48 ^{bc} ±0.01	1.47 ^b ±0.01
645cm ² /bird	1.44 ^b ±0.01	1.46 ^b ±0.03	1.46±0.02	1.46 ^b ±0.02	1.47 ^c ±0.02	1.46 ^b ±0.02
SEM	0.01	0.01	0.07	0.01	0.01	0.01
P value	**	**	NS	**	P=0.001	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P<0.05, **P< 0.01.

Table 4.3. Effect of stocking density on feed conversion ratio (per kg egg mass)

Treatment	Experimental duration/birds age					
	28- 31wk	32-35wk	36-39wk	40- 43wk	44-47 wk	28-47 wks
387cm ² /bird	2.60 ^a ±0.05	2.44 ^a ±0.03	2.31±0.03	2.31 ^a ±0.02	2.29±0.03	2.38 ^a ±0.02
484cm ² /bird	2.34 ^b ±0.06	2.31 ^b ±0.05	2.25±0.03	2.19 ^b ±0.02	2.22±0.03	2.26 ^b ±0.03
548cm ² /bird	2.32 ^b ±0.03	2.22 ^b ±0.02	2.30±0.05	2.18 ^b ±0.06	2.24±0.06	2.21 ^b ±0.02
645cm ² /bird	2.29 ^b ±0.05	2.22 ^b ±0.04	2.21±0.03	2.13 ^b ±0.03	2.21±0.05	2.21 ^b ±0.04
SEM	0.028	0.021	0.017	0.020	0.022	0.015
P Value	0.000	0.000	0.191	0.010	0.641	0.000
	**	**	NS	**	NS	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

4.1.3 Live Body Weight (LBW) and Body Weight Gain (BWG):

The result of the effect of stocking density on the live body weight changes in the layers are presented in Table 4.4 The results revealed that body weight change in layers did not differ significantly when maintained at different stocking density.

Table 4.4. Effect of stocking density on Live Body Weight (LBW) and Body Weight Gain (BWG)

Treatment	Initial LBW (28 th week of birds age)	Final LBW (47 th week of birds age)	BWG
387cm ² /bird	1298.22 ± 23.19	1375.06 ± 20.03	76.83±6.49
484cm ² /bird	1319.44 ± 24.41	1374.44 ± 21.40	55±5.63
548cm ² /bird	1294.33 ± 26.31	1354.89 ± 23.48	60.61±6.57
645cm ² /bird	1315.78 ± 27.66	1389.61 ± 23.96	73.83±8.90
SEM	12.520	10.865	3.591
P Value	0.867	0.646	0.093
	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

In contrast to present experiment Benyi *et al.*, (2006) observed significant changes in the body weight of layers maintained at stocking density of 1100, 733 or 550 cm²/bird, the hens housed at 1100 and 733 cm²/bird had a higher final body weight than 550cm²/bird space. These two groups were noticed to have consumed more feed and had higher final body weight as well as body weight change, feed intake was a factor affecting the observed lower body-weight change and lower final body weight of hens housed at density of 550cm²/bird. The effect of stocking density was thought to be confounded with the effect of the group size. Davami *et al.*, (1987) observed significant lower body weight at 300cm²/bird stocking density when compared to 420 cm²/bird stocking density. Onbasilar and Aksoy (2005) indicated differences in body weight, when maintained at stocking density of 1968, 656 and 393.8 cm²/bird by keeping one, three and five birds per cage. Increasingly cage density, from one and three to five hens/cage, resulted in a lower body weight.

These reports are contradictory to our present experiment, similar to present result non-significance in body weight was reported by Sarica *et al.*, (2008) at stocking density of 500, 667 and 1000 cm²/bird, however at higher stocking density 2000 cm²/bird had resulted in significant difference. As feed

consumption was same in all the treatment groups of the present experiment, as well the experiment was conducted for short period of 20 weeks which did not reflect any significant change in live body weight probably this could be reasons for not able to bring in any significant difference in the body weight gain of experimental birds in present study.

4.1.4 External and Internal Egg Quality Parameters :

The results of the effect of stocking density on egg quality traits were divided into two categories: external and internal. External egg quality which comprises of egg weight, shape index and the percentage of dirty and broken eggs. Internal egg quality includes albumin index, yolk index, Haugh unit, shell weight, yolk color, shell thickness. At each fifteen days of the experimental period twelve eggs per treatment groups were used to assess the egg quality of the experimental birds, hence total 480 eggs were used to assess the egg quality of the experimental birds. The average details of the external and internal egg quality parameters are presented in Table 4.5 and 4.6 and Figure 4.3 and 4.4.

The egg laid by the experimental birds reared at highest stocking density ($387\text{cm}^2/\text{bird}$) were recorded significantly ($P < 0.01$) lowest egg weight (52.44 ± 0.34) among all the birds reared at different treatment stocking densities. As the stocking density improved (387 to $548\text{ cm}^2/\text{bird}$) the egg weight was increased (52.44 to 55.88 g), whereas, there was not much difference in egg weight of the birds reared at stocking density 484 to $645\text{ cm}^2/\text{bird}$.

In agreement to the present experiment Onbasilar and Aksoy (2005) reported a difference in egg weight in birds that were reared in different stocking density of 1968 , 656 and 393.8 cm^2 floor area per hen, respectively for hens kept single, three and five hens per cage. Rajendran *et al.*, (2013) also reported significant difference in egg weight by stocking density of 270 sq. inches per bird as compared to the other stocking density of 135 , 90 , 67.5 and 54 sq. inch space per bird and concluded stocking density had high influence on egg weight. Davami *et al.*, (1987) concluded that hens in lower density cages were allowed

more movement, resulting in less stress and when birds are in stressful condition most of the food is utilized to cope with unpleasant conditions. Food is partitioned between body functions, including maintenance, growth, reproduction, and health. In healthy animals, 10 % of food ingredients consumed are used to maintain health, while the remaining portion is divided into three equal parts for reproduction, maintenance, and health. So, in the present experiment, this condition may explain the reduction in egg weight in groups with 5 hens in cages.

In contrast to our finding Craig and Milliken (1989), Lee and Moss (1995) reported non-significant effect of different stocking density on egg weight, it may be due to the adverse effects of crowding of hens in cages and more evident with prolonged exposure to such densities and which have missed entirely in relatively short term experiments. Similarly, Sarica *et al.*, (2008) observed no effect of stocking densities (2000, 1000, 667 and 500 cm² per hen) on egg weight and remarked that reports regarding egg weight and egg quality is expectable as the study was conducted in a traditional cage system providing optimum condition for animal welfare.

No significant difference were found in shape index, broken and dirty egg percentage among any of the present treatment stocking density reared birds. For dirty and cracked eggs percentage, after 40 weeks of age, Sarica *et al.*, (2008) indicated the total cracked and broken eggs amounted to 2.86, 3.04, 3.40, 3.44% at 2000, 1000, 667 and 500 cm² per bird of stocking density with the highest proportion at 500 cm²/bird, though the differences were not significant similar to present experiment. Moinard *et al.*, (1998) also observed no changes in cracked egg percentage at stocking density of 450, 600 or 800 cm² per hen. Windowski *et al.*, (2017) observed no changes in cracked and dirty egg percentage at space allowance of 520 and 748 cm² per hen in furnished cages but the percentage of dirty eggs was higher at week 69 compared to week 50; also more cracked eggs at week 69 observed showing interaction of age and space allowance.

Fig. 4.3. Effect of Stocking Density on Egg Weight, Shape Index and Haugh Unit

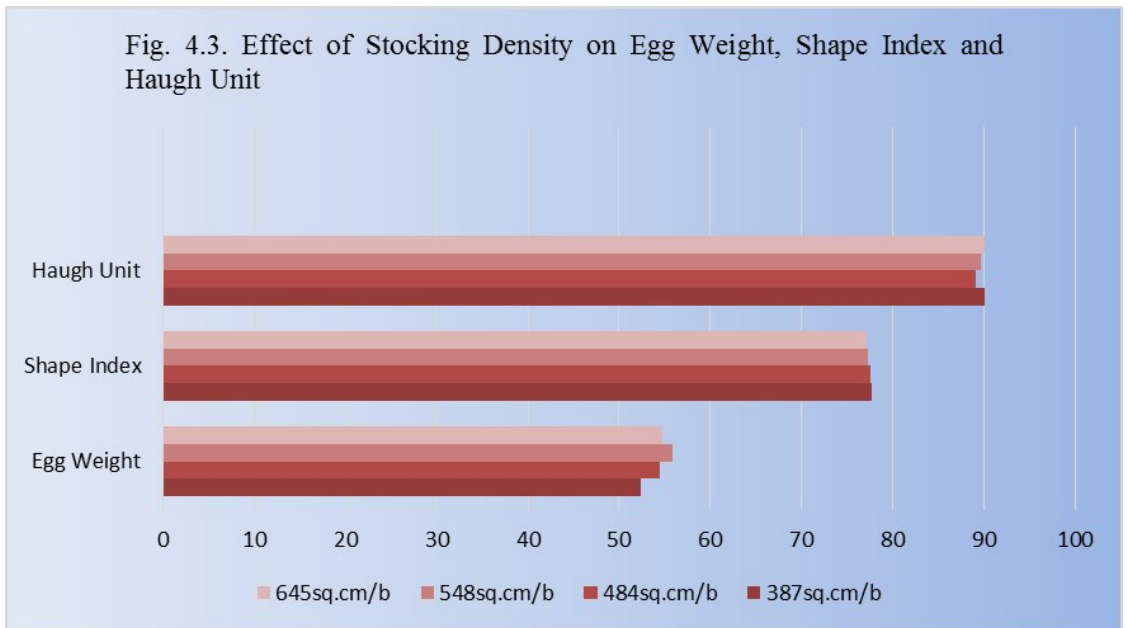


Fig.4.4 Effect of Stocking Density on Egg Quality parameters

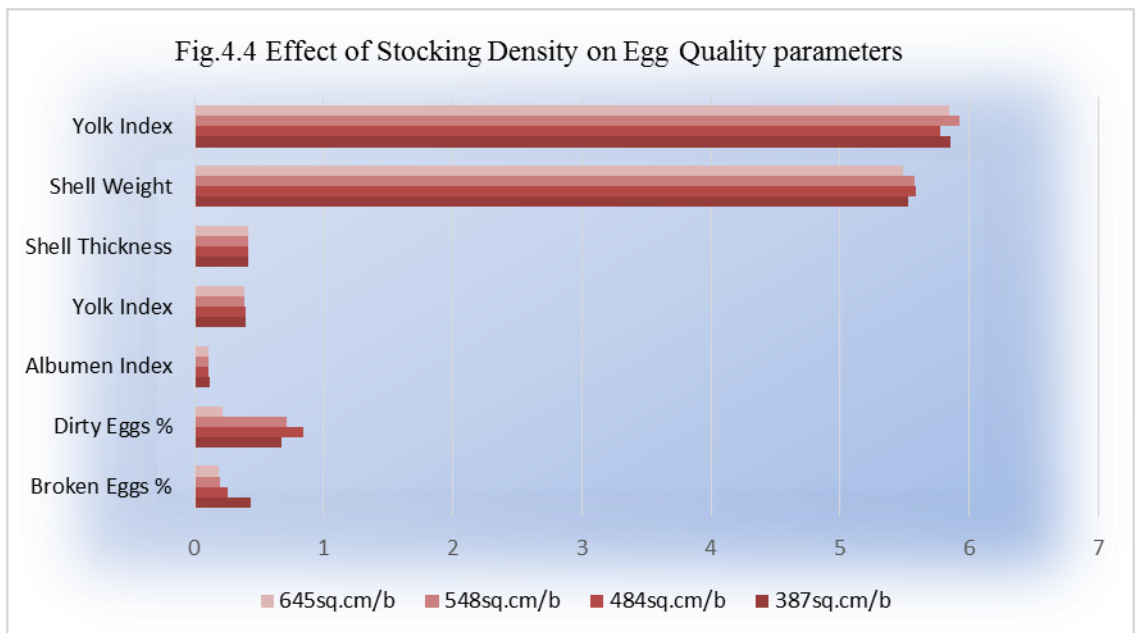


Table 4.5. Effect of stocking density on external egg quality parameters

Parameters	Experimental birds reared with different stocking density						
	387cm ² /b	484 cm ² / b	548 cm ² / b	645 cm ² /b	SEM	P-value	
Egg weight (gm)	52.44 ^b ± 0.34	54.48 ^{ab} ± 0.49	55.88 ^a ± 0.98	54.67 ^a ±0.46	0.32	P<0.01	**
Shape Index	77.77±0.19	77.62±0.26	77.25±0.27	77.19±0.21	0.10	P>0.05	NS
Broken Eggs %	0.44±0.14	0.26±0.13	0.20±0.08	0.19±0.09	0.057	P>0.05	NS
Dirty Eggs %	0.67±0.00	0.84±0.34	0.71±0.29	0.22±0.08	0.125	P>0.05	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.6. Effect of stocking density on internal egg quality parameters

Parameters	Experimental birds reared with different stocking density						
	387cm ² /b	484 cm ² /b	548 cm ² /b	645 cm ² /b	SEM	P-value	
Albumen Index	0.118 ^a ±0.00	0.113 ^b ±0.00	0.112 ^b ±0.00	0.113 ^b ±0.00	0.001	P< 0.05	*
Yolk Index	0.394 ^a ±0.00	0.396 ^a ±0.00	0.383 ^b ±0.00	0.386 ^b ±0.00	0.00	P<0.05	*
Haugh Unit	90.11±0.85	89.17±0.32	89.73±0.55	90.04±0.58	0.27	P>0.05	NS
Shell Thickness	0.414±0.01	0.413±0.00	0.418±0.01	0.416±0.01	0.00	P>0.05	NS
Shell weight	5.53±0.01	5.59±0.03	5.58±0.04	5.49±0.05	0.02	P> 0.05	NS
Yolk colour	5.86±0.05	5.78±0.08	5.93±0.06	5.85±0.04	0.03	P>0.05	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Significant ($P < 0.05$) differences were found in albumen and yolk index values, whereas egg Haugh unit, shell thickness, shell weight and yolk colour found non significant at any stage of the experiment. Albumen index of egg obtained from birds reared at highest stocking density i.e $387\text{cm}^2/\text{b}$ was significantly better compared to the eggs received from lower stocking density treatment groups. While egg albumen index was found non-significant among the birds reared at 484, 548 and $645\text{ cm}^2/\text{b}$. Onbasiler and Aksoy (2005) recorded the higher values of albumen index in 5 birds per cage or $393.8\text{ cm}^2/\text{b}$ as compared to 3 birds or $656\text{ cm}^2/\text{b}$, similar to present experiment of 5 birds or $387\text{cm}^2/\text{bird}$ space. Yolk index was observed significantly better in birds reared at higher stocking density i.e 387 and $484\text{ cm}^2/\text{b}$ than lower density reared birds 548 and $645\text{ cm}^2/\text{b}$. The other internal egg quality parameters Haugh unit, shell thickness, shell weight and yolk colour showed non-significant difference in all the birds reared at different stocking densities. Onbasiler and Aksoy (2005) reported non-significant effect of cage density (1968, 656 and 393.8 cm^2 floor area per hen) on egg shape index, egg-shell breaking strength, shell thickness or yolk index, similar to present experiment except for yolk index parameter. Lee and Moss (1995) found that albumen height and egg shell thickness was affected in only one of the four experiments conducted on two commercial strains of WLH- Hyline and Shaver. In most study it was observed that decreasing the space allowance tend to decrease the yolk and albumen quality without statistical difference, while the opposite trend was seen in present experiment with yolk and albumen index with statistical difference.

Kang *et al.*, (2016) also reported non-significant differences in egg quality among stocking density treatments (5, 6, 7 and 10 birds/ m^2) for eggshell thickness, egg yolk color and Haugh unit. In the present study stocking density did not affect any external or internal quality traits except for egg weight, albumen and yolk index, possible because the study was conducted in traditional cages with optimum condition for animal welfare, stands true as indicated by Sarica *et al.*, (2008).

4.1.5 Physical and physiological welfare study

Table No. 4.7 & 4.8 indicate the physical indicators of welfare parameter like feather score and gait score and Physiological indicators of welfare parameter heterophil-lymphocyte ratio, corticosterone level in the blood and the immune status of experimental birds reared at different stocking density. The H/L ratio and corticosterone values are depicted in Figure 4.5. Feather score and gait score of 12 birds from each treatment stocking density were measured on 28, 37 and 47th week of age that is at the start, mid and end of the experiment. The scores were averaged for the different period and presented in the Table 4.7 Similarly, Physiological indicators like H/L ratio, corticosterone value (ng/ml) and immune status were also recorded. The immune response was judged by employing HI test to detect the antibody titer against New Castle Disease (ND) and the results are represented as log₂ values.

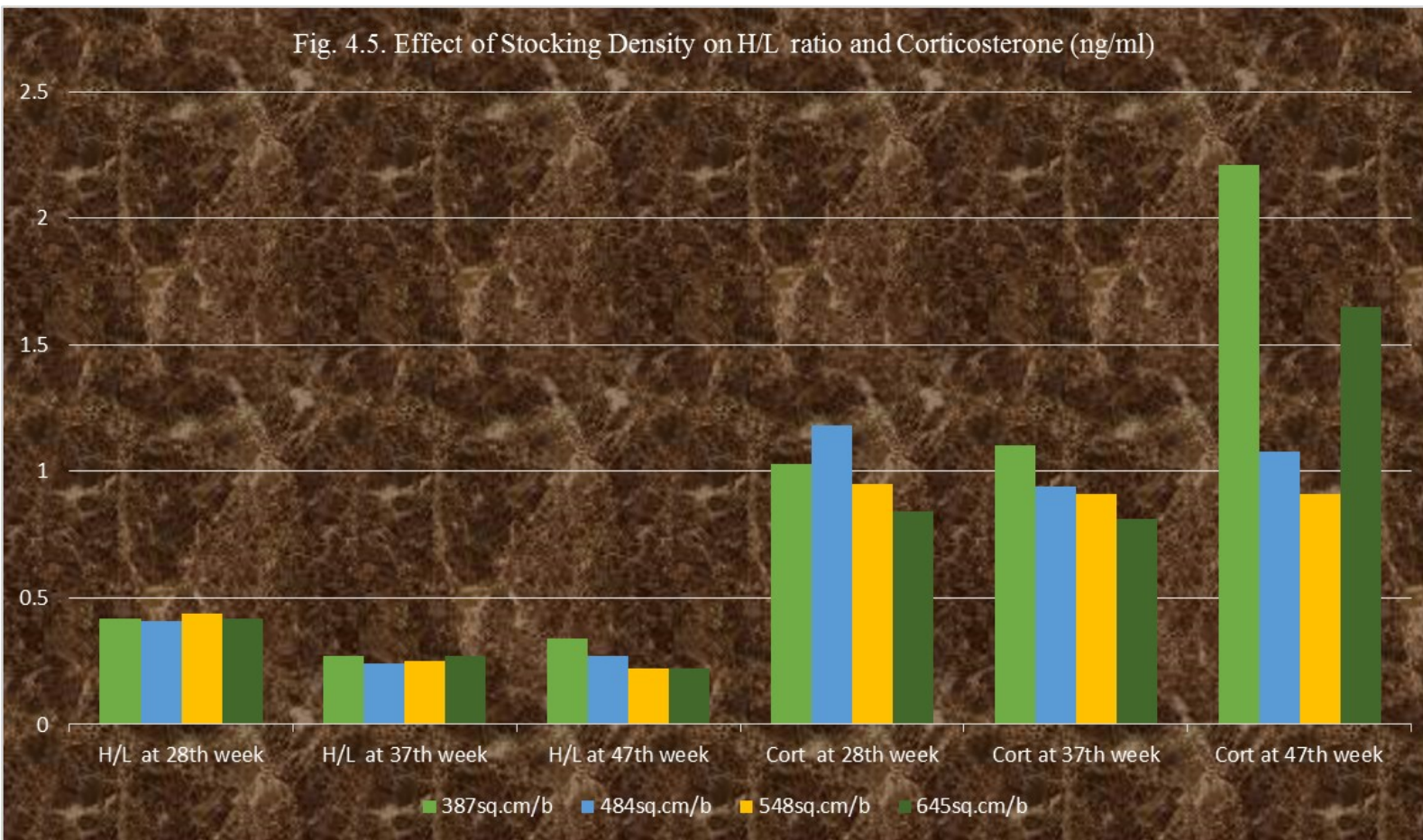
The average value for the gait score and the antibody titer against New Castle Disease (ND) measured at different period interval were found non-significant for the birds reared at different treatments stocking density during the entire period of the experiment. Effect of stocking density on immuno competence was reported to be non-significant by Mench *et al.*, (1986) when compared the micro-hemagglutination log₂ values to SRBC for stocking densities of 1394 and 697cm²/hen. It was concluded social stress associated with competition and changing group composition is known to cause decreases in circulating antibody levels in both chickens (Siegel and Latimer, 1975), which was not seen by Mench *et al.*,(1986). This is in corroboration with the present study for different stocking densities where the immune status was checked against New Castle Disease (ND). Non-significant effect of cage density on hemagglutination titer against SRBC for different cage density 97.8, 116.1, 142.9 and 185.8 cm²/ bird, respectively, from Day 1 to 6 week of age was reported by Paterson and Siegel (1988). On contrary Onbasiler and Akshoy (2005) reported significant difference (P<0.01) in antibody titers at different stocking density with

Table 4.7. Effect of stocking density on immune status, feather and gait score

Treatment	Feather Score	Gait Score	Antibody titer against ND (log ₂ values)		
			28 th week	37 th week	47 th week
387cm ² /b	6.67 ^b ± 0.30	7.89 ± 0.11	3.17 ± 0.04	3.05 ± 0.07	3.01 ± 0.10
484cm ² /b	7.44 ^{ab} ± 0.44	8.00 ± 0.00	3.13±0.10	2.97 ± 0.14	3.05 ± 0.09
548cm ² /b	7.45 ^{ab} ± 0.27	8.00 ± 0.00	3.21±0.10	2.84 ± 0.06	3.17 ± 0.13
645cm ² /b	8.00 ^a ± 0.00	8.00 ± 0.00	2.97±0.06	2.98 ± 0.11	3.02 ± 0.16
SEM	0.170	0.028	0.040	0.049	0.059
P-value	0.037	0.413	0.144	0.494	0.793
	*	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Fig. 4.5. Effect of Stocking Density on H/L ratio and Corticosterone (ng/ml)



highest titer at lower stocking density. When the cage density was increased from 3 to 5 hens/cage it resulted in a significant decrease ($P < 0.01$) in the antibody titers to SRBC. Though feather score, showed significant change across the various stocking density and a linear regression as the stocking density increased. Experimental bird reared with highest space allowance $645 \text{ cm}^2/\text{b}$ had significantly ($P < 0.05$) better feather score than the birds provided with lowest space $387 \text{ cm}^2/\text{b}$, space, but could not elicit a significant difference with moderate ($548 \text{ cm}^2/\text{b}$) and low ($484 \text{ cm}^2/\text{b}$) stocking density treatment group. Similar to present experiment result of feather score deterioration with increasing stocking density Craig *et al.*, (1986) reported feather damage and loss increased step-wise and significantly with stocking density of 929, 464 and $310 \text{ cm}^2/\text{hen}$. Feather loss in stocking density of $929 \text{ cm}^2/\text{hen}$ was only in the tail and wing feathers that were frayed at the ends because of rubbing on cage wires, but feather coverage of the skin was intact while birds in stocking density $464 \text{ cm}^2/\text{hen}$ had a moderate loss of about 10%. Significant differences in plumage score between various stocking densities of 1968, 656 and 393.8 cm^2 floor area per hen was reported by Onbasiler and Akshoy (2005) with significant poorer plumage score at highest stocking density. The poorer plumage score of densely populated cages was caused by abrasion against cage wire or other hens and also higher stocking density appears to increase the level of nervousness and feather-pecking activity. Significant ($P < 0.05$) poorer plumage score at stocking density of $667 \text{ cm}^2/\text{hen}$ and $500 \text{ cm}^2/\text{hen}$ was reported by Sarica *et al.*, (2008) in comparisons to birds at stocking density of 1000 and $2000 \text{ cm}^2/\text{bird}$. While in contrast Moinard *et al.*, (1998) observed no changes in feather scores for stocking density of 450, 600 and $800 \text{ cm}^2/\text{hen}$.

At the end of the experimental period significant difference ($P < 0.05$) was observed in heterophil-lymphocyte ratio and corticosterone (ng/ml) level among the different stocking density treatment groups as given in Table.4.8. The birds reared at 548 and $645 \text{ cm}^2/\text{bird}$ had significantly better heterophil-lymphocyte ratio and corticosterone level as compared the birds reared in $387 \text{ cm}^2/\text{bird}$, while compared with the treatment group $484 \text{ cm}^2/\text{b}$ showed non-significant changes at 47th week of bird's age. The birds reared at space $548 \text{ cm}^2/\text{b}$ had recorded the

lowest corticosterone level as compared to others, indicating less stress upon birds, and highest stress was observed in birds reared with space allotment 387cm²/bird. With increased corticosterone level the antibody titer tends to decrease and heterophil counts was increased as observed by Post *et al.*,(2003).

Similar to our results of H/L ratio at 47th week of age, Onbasiler and Akshoy (2005) reported the ratio of heterophils to lymphocytes was significantly higher (P<0.01) in the group having highest stocking density than those of groups having low and moderate stocking density. This condition was explained by the elevated concentration of corticosterone in blood circulation, which causes an increase in heterophil count and a decline in lymphocyte count. Kang *et al.*, (2016) reported significant difference in H/L ratio for layers in pens at stocking density of 5, 6, 7 and 10 birds/m² with H/L ratio were greater (P < 0.01) for 10 birds/m² than stock density 5, 6, and 7 birds/m². Mashaly *et al.*, (1984) observed significant differences (P < 0.05) in serum corticosterone concentrations of laying hens as a response to increased population density of 516, 387 and 310 cm² per bird similar to present experiment when tested at 47th week of age. Increase in circulating corticosterone observed in the study was indicative of an active adrenal gland. Regardless of the treatment, elevation in circulating corticosterone did not significantly differ until first 10 week and seemed to reach a plateau thereafter, which suggest that the longer the duration of exposure for new environment and social stressors could be a factor in higher production of corticosterone which shows more physiological changes.

In parallel with present experiment, Cheng and Muir (2004) reported that laying hens showed significantly lower plasma corticosterone levels in single-bird cages (525 cm²/bird) than in the 10-bird cages (419 cm²/bird), indicating that social stressors could be a factor in higher production of corticosterone in hens. Similarly, Kang *et al.*,(2016) also reported significant serum corticosterone to be greater (P < 0.01) at 10 birds/m² stock density than other stock densities that is at

Table 4.8. Effect of stocking density on corticosterone and heterophil: lymphocyte ratio

Treatment	H/L Ratio at 28 th week	H/L Ratio at 37 th week	H/L Ratio at 47 th week	Corticosterone (ng/ml) 28 th week	Corticosterone (ng/ml) 37 th week	Corticosterone (ng/ml) 47 th week
387cm ² /b	0.42±0.04	0.27 ± 0.04	0.34 ^a ± 0.05	1.03 ± 0.18	1.10 ± 0.23	2.21 ^a ±0.28
484cm ² /b	0.41±0.03	0.24 ± 0.01	0.27 ^{ab} ± 0.03	1.18 ± 0.10	0.94 ± 0.14	1.08 ^{ab} ±0.16
548cm ² /b	0.44 ± 0.02	0.25 ± 0.03	0.22 ^b ± 0.01	0.95 ± 0.11	0.91 ± 0.10	0.91 ^c ±0.10
645cm ² /b	0.42±0.03	0.27 ± 0.01	0.22 ^b ± 0.01	0.84 ± 0.02	0.81 ± 0.04	1.65 ^b ±0.07
SEM	0.015	0.013	0.011	0.062	0.071	0.128
P-value	0.841	0.717	0.041	0.265	0.562	0.000
	NS	NS	*	NS	NS	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

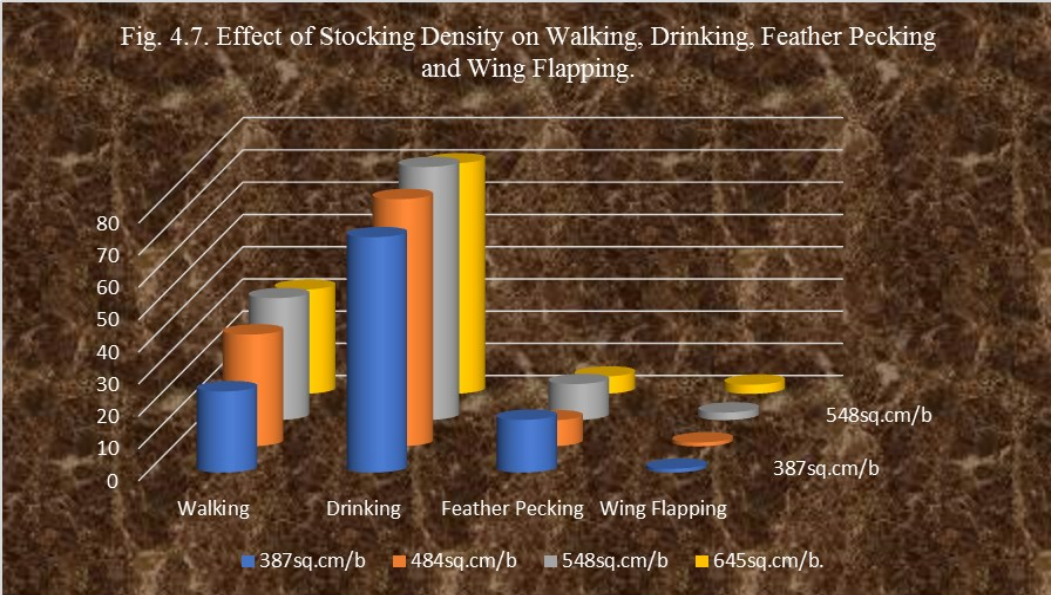
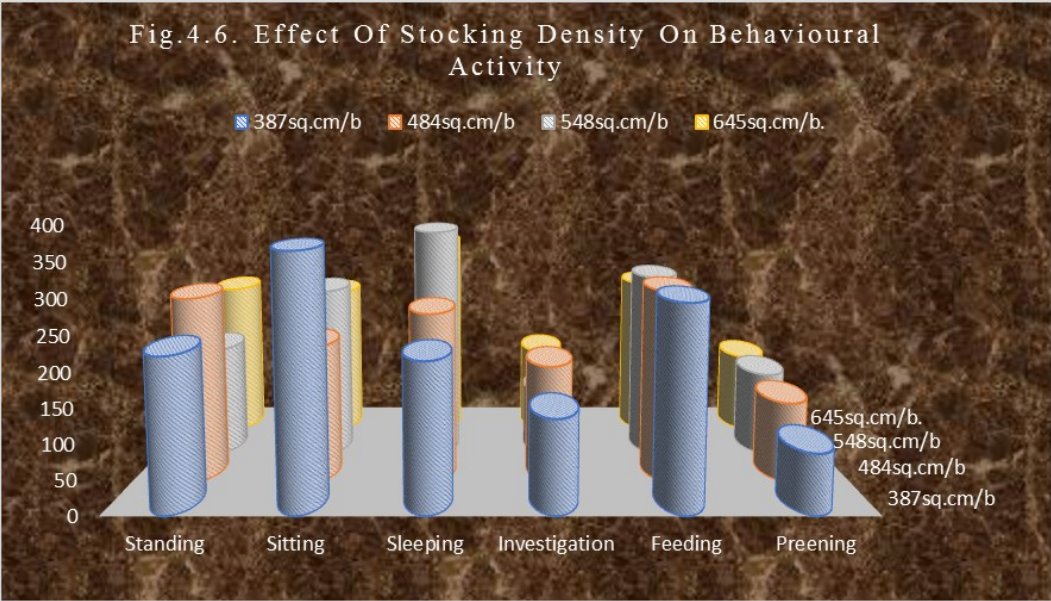
5, 6, and 7 birds/m² stock density. Contrasting reports on corticosterone was observed by Craig *et al.*, (1986), who found non-significant differences in plasma corticosterone (ng/ml) between different stocking densities when measured at 45 weeks of housing. Hens at stocking density of 310cm²/hen had 15% higher level than those in 464 and 929 cm²/hen but could not elicit a significant change. It was observed that plasma corticosteroid concentrations present during the first 2 weeks after housing was higher as compared to later after adaptation and also, non-significant difference in plasma corticosterone for hens at different stocking density by Mench *et al.*, (1986).

4.1.6 Behavioral Study:

The various observations of behaviors (standing, sitting, sleeping, investigating drinking, feeding, preening, feather pecking, scratching, wing flapping, fighting etc.) of the experimental birds were recorded at start (28th week), mid (37th week) and at the end (47th week) of the experimental period are presented in Table 4.9, 4.10 and 4.11 respectively. The various activities of behavior of the birds were recorded in CCTV camera for 24-hour period and an activity chart or ethogram from the video recordings were noted. The time spent on each behavior was observed and expressed in minute. The specific 24 hour (1440 minute) behavioral observations collected at three different period (28, 37 and 47 week) of experiment were averaged and presented in Table 4.12 and Figure 4.6 and 4.7. The average of three different stages/weeks of each behavioral activity is discussed as below.

Standing and walking:

It was observed experimental layers reared with 548 cm²/b had spent the lowest time on standing behavior with significant differences compared to the other stocking density treatment groups which indicated that the hens were engaged in other activities. Although increased movement (pacing within the cage) may also be related to foraging behavior during this same time period when nutrients are in high demand to support reproduction. In support of this the birds



reared at 548 cm²/b recorded highest walking behavior i.e 37.83 minutes (3%) and less standing (12%) when compared to other stocking density groups during 24 hour period. Similar to this findings Mench *et al.*, (1986) recorded locomotion range from 1 to 3% and standing as 5 to 15%. Though various factors like age, nest selection site, near molting age could be a factor for more movements, but in this study it would be related more of space and ability to move. The birds maintained on 387, 484 and 645 cm²/b showed non-significant difference among themselves in standing behavior activity. Standing and locomotion are interrelated and as stated by Hughes and Black (1974) and similar observation by Mench *et al.*, (1986), the decrease in locomotion in high density cages was accompanied by more standing. Similarly, Moinard *et al.*, (1998) reported birds housed in the biggest cages showed higher frequencies of walking behaviour in the treatment group which had highest space allowance, increased surface area of cage might have induced increased activity. In relation to present study Anderson *et al.*, (2004) also indicated more movement acts ($P < 0.05$) when birds were kept in low density (482cm²/bird) than in high density (361 cm²/bird) while the behaviour frequencies for standing, crouching and comfort movements were similar. Further it was stated that these behaviours are a function of space and the ability of hens to move within the cage with less interference. The frequency of movement observed by Anderson *et al.*, (2004) was highest during the early stages of the production cycle (26 to 34 week) when egg production is at high. The birds maintained on 484, 548 and 645 cm²/b showed non-significant difference among themselves in walking behavior activity.

Investigation:

Another important behavioral activity on scanning/ investigation (visual exploration by head movements while body is stationary) reported by Mench *et al.*, (1986) reported that caged birds showed less locomotor activity and more scanning even in the relatively spacious low-density environment. On the basis of its frequency, it appears that such stationary visual exploration may substitute for locomotors exploration of the environment. In this study, the birds reared at 548

cm²/b had the most locomotion (walking) and resulted in lowest for the average of the investigation (or scanning) activity of 7% (Table 4.12) compared to 9, 10 and 12% for other birds maintained at different stocking density (387 cm²/b; 645 cm²/b; 484 cm²/b respectively) indicating visual exploration had substituted for walking or locomotion.

Sleeping:

Sleeping or resting that is the birds lying down with eyes close was mostly noticed after the lights went off at 9 pm (timer facility to maintain 16 hr light) and it was observed that the birds reared at 548 cm²/b had the significantly better time of resting throughout the weeks as recorded at different periods and presented in Table (4.9, 4.10, 4.11 and 4.12). On an average time spent on resting for the birds reared at 548 cm²/b was observed to 25%, followed by 23% for the birds reared at 645 cm²/b, 18% for the birds reared at 484 cm²/b and 15% for the birds reared at 387 cm²/b which were highly significantly ($P < 0.001$) different from each other. It was observed that in high density cages, the sudden movement of one bird displaces the resting state of other birds causing it to stand or investigate. In contrast to present experiment Mench *et al.*, (1986) observed the resting activity for the various stocking density varied from 16 to 20 % with no significant differences between high density and low density two-caged birds.

Sitting:

The act of lying down with eyes open was mostly observed for the birds reared at 387 cm²/b which was highly dense compared to other treatment groups and the birds were observed to sit at the back of the cages as there was less space to stand in the front with other birds in the same cage to perform activities like preening and investigation after the basic essential like feeding and drinking is completed. The average time spent on sitting by the various groups are 25% for the birds reared at 387 cm²/b, 14% (484 cm²/b), 18% (548 cm²/b) and 17% (645 cm²/b). The birds maintained on 387 cm²/b exhibited significant time for sitting as compared to any other treatment birds of the experiment.

Grooming and Preening:

The incidence of preening is observed to be consistently significantly higher in low density cages for all the periods recorded (28th, 37th and 47th week) and also the averaged figure mentioned in Table 4.12 indicate that the birds maintained on stocking density 484, 548 and 645 cm²/b showed significantly better preening (8-9 %) than the birds reared at 387 cm²/b (6%). Mench *et al.*, (1986) reported grooming activity to be more in two-caged birds than in single birds but did not display the density-related decrease as reported by Huges and Black (1974) and present study findings. They further indicated that the reason for this discrepancy is not clear, but preening can increase because of feather damage, ectoparasite infestation, or frustration but in this study, the highest stocking density or insufficient space could be the reason for less preening.

Feeding and drinking:

There was no significant differences for the averaged behavioral values for feeding and drinking records (Table 4.12). Anderson *et al.*, (2004) reported non-significant appetite activity and fairly constant drinking at stocking densities of 361 and 482 cm²/bird. Probably the case of dominant birds to monopolize the limited resources because of less feeder space which did not arise in our study. At certain periods that is 28th week (Table 4.9), feeding and drinking were highly significant with highest minutes spent on feeding and drinking was found in the birds reared at 548 cm²/b and at 47th week drinking activity only showed significant differences (Table 4.11), in the birds reared at 484cm²/b. Similar higher drinking for birds reared in 1016 cm²/hen of low stocking density was reported by Anderson *et al.*, (2004) when compared to high stocking density of 762cm² and 609cm²/bird. Mench *et al.*, (1986) observed feeding was less frequent in high density cages than in low density cages which they reasoned for limited feeder space which was seen in present study.

Table 4.9. Effect of stocking density on state and event behaviour of bird at 28th week age

Parameters	387 cm ² /b	484 cm ² /b	548cm ² /b	645 cm ² /b	SEM	P value
Standing	232.89 ^b ± 7.66	293.89 ^a ± 8.88	165.11 ^c ± 6.50	218.67 ^b ± 23.65	10.114	0.000**
Sitting	273.00 ^a ± 23.78	142.67 ^c ± 9.36	155.56 ^{bc} ± 11.28	209.11 ^b ± 26.41	12.649	0.000**
Sleeping	215.33 ^b ± 4.63	306.78 ^a ± 14.08	311.89 ^a ± 11.67	336.00 ^a ± 13.95	9.573	0.000**
Investigation	128.00 ^b ± 5.59	155.33 ^a ± 10.56	115.56 ^b ± 3.11	112.56 ^b ± 4.41	4.238	0.000**
Walking	22.89 ^b ± 1.58	33.11 ^a ± 3.87	30.00 ^a ± 0.53	28.11 ^{ab} ± 0.72	1.198	0.015**
Total state behaviour	872.11 ^a ± 24.18	931.78 ^a ± 6.28	778.11 ^b ± 21.45	904.44 ^a ± 44.22	16.423	0.003**
Drinking	76.33 ^b ± 4.31	54.89 ^c ± 5.52	105.78 ^a ± 10.25	67.78 ^{bc} ± 2.27	4.370	0.000**
Feeding	387.67 ^{ab} ± 25.01	320.78 ^b ± 11.26	421.67 ^a ± 17.52	318.89 ^b ± 36.83	13.914	0.012**
Grooming & Preening	86.56 ^b ± 0.84	119.33 ^a ± 12.81	115.33 ^{ab} ± 7.26	131.11 ^a ± 15.30	5.784	0.038**
Feather pecking	15.11 ^a ± 0.75	7.33 ^c ± 0.17	11.00 ^b ± 0.58	9.78 ^{bc} ± 1.41	0.627	0.000**
Wing Flapping	0.67 ± 0.21	1.00 ± 0.00	2.17 ± 0.60	1.71 ± 0.71	0.313	0.310-NS
Fighting	4.00 ± 2.31	1.83 ± 0.31	0	0	-	0.279-NS
Total event behaviour	567.89 ^b ± 24.18	503.78 ^b ± 5.32	655.22 ^a ± 19.95	528.89 ^b ± 42.53	15.980	0.002**

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.10. Effect of stocking density on state and event behaviour of bird at 37th week age

Parameters	387 cm ² /b	484 cm ² /b	548cm ² /b	645 cm ² /b	SEM	P Value
Standing	261.00±19.28	281.50±24.08	213.83±15.49	281.33±32.75	12.498	0.181-NS
Sitting	393.17 ^a ±45.19	222.17 ^b ±31.82	303.50 ^{ab} ±32.35	229.00 ^b ±28.44	21.78	0.008-**
Sleeping	216.50 ^c ±6.72	178.33 ^d ±12.09	381.67 ^a ±4.27	337.00 ^b ±16.35	18.156	0.000 - **
Investigating	127.67 ^b ±9.14	203.67 ^a ±12.78	79.50 ^c ±6.10	142.67 ^b ±7.93	10.214	0.000- **
Walking	26.67±0.56	32.33±2.16	42.83±8.26	29.00±0.37	2.377	0.068 - NS
Total state behaviour	1025.00 ^a ±28.77	918.00 ^b ±12.54	1021.33 ^a ±20.42	1019.00 ^a ±37.63	15.528	0.026 - *
Drinking	78.17±6.50	88.50±5.84	66.17±5.78	72.67±6.30	3.322	0.099- NS
Feeding	239.83±20.98	265.00±15.86	202.67±8.89	224.67±27.78	5.036	0.182- NS
Grooming & Preening	73.83 ^c ±5.04	146.50 ^a ±12.28	134.50 ^{ab} ±7.21	113.50 ^b ±6.80	6.934	0.000- **
Feather pecking	21.67 ^a ±2.33	11.17 ^b ±1.05	12.67 ^b ±1.33	4.50 ^c ±0.34	1.445	0.000- **
Wing Flapping	1.50 ^b ±0.34	1.00 ^b ±0.26	0.67 ^b ±0.21	5.83 ^a ±0.31	0.455	0.000 - **
Fighting	0.00 ^b ±0.00	9.17 ^a ±2.18	2.00 ^b ±0.93	0.00 ^b ±0.00	0.961	0.000- **
Total event behaviour	415.00 ^b ±28.77	542.50 ^a ±26.16	418.67 ^b ±20.42	421.17 ^b ±37.50	17.529	0.013- **

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.11. Effect of stocking density on state and event behaviour of bird at 47th week age

Parameters	387 cm ² /b	484 cm ² /b	548cm ² /b	645 cm ² /b	SEM	P value
Standing	173.50 ^{ab} ±13.09	253.67 ^a ±33.05	128.50 ^b ±27.06	239.33 ^a ±42.96	17.889	0.03-*
Sitting	434.33 ^a ±39.64	245.17 ^b ±37.21	333.17 ^{ab} ±49.37	279.67 ^b ±43.03	24.816	0.027-*
Sleeping	217.00 ^c ±8.37	277.00 ^b ±15.03	382.33 ^a ±5.61	301.00 ^b ±17.35	13.677	0.000- **
Investigating	153.33 ^a ±16.35	175.33 ^a ±3.68	109.67 ^b ±10.91	154.17 ^a ±5.34	6.935	0.002- **
Walking	25.67 ^b ±1.36	25.83 ^b ±1.64	42.00 ^a ±7.74	40.33 ^a ±1.41	2.492	0.011-**
Total state behaviour	1003.83±13.28	977.00±14.27	995.67±31.13	1010.50±37.81	12.565	0.823-NS
Drinking	63.00 ^b ±1.90	85.00 ^a ±5.88	63.67 ^b ±4.51	71.33 ^b ±3.90	2.724	0.006-**
Feeding	255.50±12.46	262.83±9.47	240.50±16.16	221.50±28.02	9.003	0.399-NS
Grooming & Preening	103.00 ^b ±3.63	110.17 ^b ±3.49	129.67 ^a ±8.54	129.67 ^a ±7.21	3.773	0.010-**
Feather pecking	12.67 ^a ±1.48	5.33 ^b ±0.55	10.00 ^a ±2.39	3.67 ^b ±0.56	1.012	0.001-**
Wing flapping	In some groups no observations were recorded, so couldn't compare.					
Total event behaviour	436.17±13.28	463.33±14.19	441.00±30.76	429.50±37.81	12.517	0.816-NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.12. Effect of stocking density on state and event behaviours of bird on 28, 37 and 47 week (average) of age

Parameters	387 cm ² /b		484 cm ² /b		548cm ² /b		645 cm ² /b		SEM	P value
	Minutes	%	Minutes	%	Minutes	%	Minutes	%		
Standing	222.50 ^a ±9.74	15	277.00 ^a ±17.92	19	169.50 ^b ±13.46	12	245.17 ^a ±27.52	17	11.851	0.004**
Sitting	365.50 ^a ±29.67	25	204.17 ^b ±21.54	14	264.50 ^b ±20.40	18	239.17 ^b ±17.29	17	16.393	0.000 **
Sleeping	216.17 ^d ±5.42	15	254.50 ^c ±10.02	18	359.00 ^a ±8.58	25	325.00 ^b ±13.354	23	12.597	0.000**
Investigating	136.33 ^b ±8.18	9	175.67 ^a ±8.59	12	101.17 ^c ±4.99	7	137.17 ^b ±2.94	10	6.298	0.000**
Walking	25.17 ^b ±0.60	2	34.67 ^a ±2.78	2	37.83 ^a ±3.81	3	32.33 ^a ±0.56	2	1.479	0.009**
Total state behaviour	965.67±15.17	67	946.17±8.91	66	931.83±13.58	65	978.83±33.36	68	10.070	0.382-NS
Drinking	73.00±3.27	5	76.67±4.24	5	78.50±3.19	5	71.50±3.21	5	1.735	0.484-NS
Feeding	295.67±14.97	21	287.83±8.80	20	286.50±9.25	20	254.67±26.71	18	8.397	0.337-NS
Grooming & Preening	87.83 ^b ±2.17	6	120.17 ^a ±6.13	8	129.50 ^a ±4.72	9	126.50 ^a ±8.33	9	4.389	0.000**
Feather pecking	16.33 ^a ±1.17	1.1	11.17 ^b ±1.01	0.6	8.00 ^c ±0.58	0.8	5.33 ^c ±0.76	0.4	0.954	0.000**
Wing flapping	1.17 ^b ±0.31	0.1	1.17 ^b ±0.17	0.1	2.33 ^a ±0.42	0.2	2.83 ^a ±0.31	0.2	0.211	0.002**
Total event behaviour	474.17±15.30	33	493.67±8.82	34	508.17±13.58	35	461.33±33.37	32	10.074	0.386

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Feather Pecking:

Feather pecking shows one bird pecks or pulls at the feathers of another. There was significant difference in aggressive behavior like feather pecking with most feather pecking was seen in the treatment (387 cm²/b) birds reared at higher density compared to the birds maintained at 484, 548 and 645 cm²/b (Table 4.12). Although some agonistic behavior like head-pecks, threats and fights are more popular encounter in pens, here in cages the most noticeable was some pushing leading to displacement of birds from feeder and drinker but could not be recorded well as the observation technique did not provide an accurate measurement of very short duration behavior. On an average 1.1% of the time was engaged on feather pecking activity by the birds reared at 387 cm²/b which was significantly (P<0.01) higher than the rest of the treatment groups. The birds reared at 548 and 645cm²/b showed significantly less feather pecking compare to the birds reared at 387 and 484cm²/b. Mench *et al.*, (1986) reported significant differences (P<0.05) between single bird at stocking density of 1394cm²/bird and two-caged bird at 1394 (Low Density) and 697cm²/bird (High density), and the total percentage of time for feather pecking 3.2, 1.4 and 0.2%; aggressive activity (head-pecks) was 1.9, 1.7 and 0 % for the birds in High density birds, Low Density and single bird in cage (S) respectively.

Anderson *et al.*, (2004) had observed no significant differences in feather pecking for stocking density at 361 and 482 cm²/bird, however there was difference during the different age period recorded at 26, 34, 43, 51, 62 and 64 weeks of age. Feather pecking was highest during the early periods 26, 34 week and during the molt at 64 week, which may be the result of social facilitation during periods of preening of feathers in young hens and preening associated with feather push out during the molt period. Feather pecking, lacking of any response from the hen being pecked, appears to be gentle in nature and a component of social recognition or grooming in chickens.

Wing Flapping:

The birds spent an approximate of 0.1 to 0.2 % of time was spent on wing flapping activity on a 24-hour activity recorded. The average value for wing flapping observed for the entire period showed a linear increase (Table 4.12) as the stocking density improved from 387 cm²/bird or 60 sq. inch to 645cm²/bird or 100 sq. inch. The birds reared at 548 and 645 cm²/b showed significantly (P<0.01) more wing flapping compare to the birds reared at 387 and 484 cm²/b.

Nicol (1987) recorded an increase in the rate of body shaking, head scratching and feather raising with increasing cage area, and that the increase from 807 to 1045 cm² per bird had more effect on the rate of head scratching than the difference between 570 and 807 cm². Spatial restriction appears to affect behaviour by altering the rate at which certain activities are performed rather than by preventing their outright occurrence. In present experiment as the stocking density decreased the wing flapping behaviour got more opportunity to express among the birds reared at 548 and 645 cm²/bird (Table 4.12).

4.1.7. Cost of production per egg (CPPE) on the basis of recurring expenses and space utilization:

The interrelation between stocking density and space utilization for the cost of production per egg is presented in Table 4.13. The CPPE was estimated on the basis of egg production and recurring expenses i.e feed, pullet, medicine, vaccine and miscellaneous incurred during experimental period (20 weeks). CPPE in relation to space utilization was calculated considering CPPE(on basis of recurring expenses) x Space allowance (sq. inch) x number of birds.

It was observed that, because of egg production difference the birds reared at 387cm²/bird had significantly (P < 0.01) higher cost of production per egg (on basis of recurring expenses) than the rest of the treatment groups. The hen day egg production was 83% for birds maintained in stocking density of 60 sq. inch or 387cm²/bird while for other treatment groups production percentage ranged from 88 to 90%. It was noticed that, as stocking density decreased the egg production percentage as well CPPE on the basis of recurring expenses improved, with better

welfare points as cited by Magnale (2018) who reported the profit per egg for birds maintained in California cages at stocking density 750 cm²/bird was significantly better than at stocking density of 450 and 465cm²/bird. The CPPE on the basis of recurring expenditure was found non-significant among the birds reared with space in between 484, 548 to 645 cm²/bird.

Table 4.13. Effect of stocking density on Cost of Production Per Egg (CPPE) and inter-relation between (CPPE) and Space Utilization (SU)

Treatment	Space allowance & Number of birds per cage box	CPPE on the basis of recurring expenses (₹)	CPPE on the basis of SU= (CPPE on the basis of recurring expenses x space allowance provided per bird in inches x number of birds)
387cm ² /b	387 cm ² /b or 60 sq. inch/b (5 birds)	2.74 ^a ±0.02	822.49 ^a ±6.85
484cm ² /b	484 cm ² /b or 75 sq. inch/b (4 birds)	2.62 ^b ±0.02	786.19 ^b ±7.34
548cm ² /b	548 cm ² /b or 85 sq. inch/b (3 birds)	2.62 ^b ±0.02	667.20 ^c ±4.39
645cm ² /b	645 cm ² /b or 100 sq. inch/b (3 birds)	2.59 ^b ±0.03	777.91 ^b ±10.18
	SEM	0.014	7.799
	P-Value	**	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

The CPPE on the basis of space utilization was found to be higher with higher stocking density group, as number of birds are more in limited space with less production performance. Hence the birds reared at highest stocking density (387 cm²/b) recorded significantly highest value (822.49) and on the contrary the birds reared at 548 cm²/b or 85 sq. inch observed significantly lowest value (667.20).

Thus in present experiment space allowance higher than 484 cm²/ bird, observed lower cost of production per egg on the basis of recurring expenses and better value of CPPE considering space utilization. While considering the CPPE on the basis of recurring expenses and space utilization it was confirmed that the birds reared at 548 cm²/b emerged as best treatment group to rear the laying birds in California cages.

4.1.8. Development of Scorecard for Cage reared commercial laying hens well being:

Before the start of the experiment, the outline of the project work was discussed at various scientific platform. The developed commercial laying hen well-being score card was well discussed at students advisory committee, college advisory council, animal ethical committee at college level and finally thoroughly discussed in the university statutory board of studies. The board of studies consist of renowned poultry industry personal and national institute subject experts who all had discussed, suggested and approved the developed commercial laying hens well being scorecard. The score card was developed on the basis of Delphi techniques (Balamurugan *et al.*, 2019). After first experiment the birds/treatments for different stocking density were assessed on parameters like production, economics, behaviour and welfare of the birds on the basis of developed score card. The top two groups (548 and 645 cm²/b) that attained highest score (84 and 82) were selected for further investigation. The data concerning the performance and well being of the layers with score allotment to various stocking density groups as per marks obtained are presented in Table 4.14.

Table 4.14. Development of cage reared commercial laying hens well-being scorecard on the basis of stocking density, production and welfare of the birds

Parameters	Sub-Group of Parameters	Total Score	Range	Score Allotment	Rank of treatments	Scores of treatment			
						387 cm ² /b	484 cm ² /b	548 cm ² /b	645 cm ² /b
Production and Economics	1.Egg Production (HDEP) %	16	1) 83.54 to 85.34	04	Treatment 387 cm ² /b	04			
			2) 85.3 to 87.14	08					
			3) 87.15 to 88.94	12	Treatment 484 cm ² /b		12		
			4) 88.95 to 90.74	16	Treatment 548 cm ² /b and 645 cm ² /b D			16	16
	2. FCR (per dozen eggs)	08	1) 1.46 to 1.49	08	Treatment 548 cm ² /b and 645 cm ² /b			08	08
			2) 1.5 to 1.53	06	Treatment 484 cm ² /b		06		
			3) 1.54 to 1.57	04					
			4) 1.58 to 1.6	02	Treatment 387 cm ² /b	02			
	3. Cost of Production Per egg in relation to recurring expenses	16	1) 2.59 to 2.63	16	Treatment 484 cm ² /b, 548 cm ² /b and 645 cm ² /b		16	16	16
			2) 2.64 to 2.68	12					
			3) 2.69 to 2.73	08					
			4) 2.74 to 2.78	04	Treatment 387 cm ² /b	04			
	4. Cost of production per egg in relation to space utilization	08	1) 667.20 to 706.02	08	Treatment 548 cm ² /b			08	
			2) 706.03 to 744.85	06					
			3) 744.86 to 783.68	04	Treatment 484 cm ² /b and 645 cm ² /b		04		04

			4) 783.69 to 822.49	02	Treatment 387 cm ² /b	02			
	Subtotal	48				12	38	48	44
Egg Quality, Stress and Immunity	1.Egg weight	04	1) 52.44 to 53.29	01	Treatment 387 cm ² /b	01			
			2) 53.3 to 54.15	02					
			3) 54.16 to 55.01	03	Treatment 484 cm ² /b and 645 cm ² /b		03		03
			4) 55.02 to 55.87	04	Treatment 548 cm ² /b			04	
	2.Broken egg %	04	1) 0.19 to 0.25	04	Treatment 548 cm ² /b and 645 cm ² /b			04	04
			2) 0.26 to 0.32	03	Treatment 484 cm ² /b		03		
			3) 0.33 to 0.39	02					
			4) 0.4 to 0.46	01	Treatment 387 cm ² /b	01			
	3. Clean egg %	04	1) 99.16 to 99.32	01	Treatment 484 cm ² /b and 548 cm ² /b		01	01	
			2) 99.33 to 99.49	02	Treatment 387 cm ² /b	02			
			3) 99.50 to 99.66	03					
			4) 99.67 to 99.83	04	Treatment 645 cm ² /b				04
	4. H/ L ratio	04	1) 0.26 to 0.293	04	Treatment 484 cm ² /b		04		
			2) 0.294 to 0.327	03					
			3) 0.328 to 0.361	02	Treatment 548 cm ² /b			02	

			4) 0.362 to 0.390	01	Treatment 387 cm ² /b and 645 cm ² /b	01			01
	5. Corticosterone level	04	1) 0.920 to 1.053	04	Treatment 548 cm ² /b			04	
			2) 1.054 to 1.187	03	Treatment 645 cm ² /b and 484 cm ² /b		03		03
			3) 1.188 to 1.321	02					
			4) 1.322 to 1.450	01	Treatment 387 cm ² /b	01			
	6. Immunity (HI Titre)	04	1) 2.96 to 2.993	01	Treatment 645 cm ² /b				01
			2) 2.994 to 3.027	02					
			3) 3.028 to 3.061	03	Treatment 484 cm ² /b and 548 cm ² /b		03	03	
			4) 3.062 to 3.09	04	Treatment 387 cm ² /b	04			
	Subtotal	24				10	17	18	16
Welfare and behaviour	1. Feather score	08	1) 6.89 to 7.17	02	Treatment 387 cm ² /b	02			
			2) 7.18 to 7.46	04	Treatment 387 cm ² /b and 548 cm ² /b		04	04	
			3) 7.47 to 7.75	06					
			4) 7.76 to 8.04	08	Treatment 645 cm ² /b				08
	2. Gait score	08	1) 7.89 to 7.91	02	Treatment 387 cm ² /b	02			
			2) 7.92 to 7.94	04					
			3) 7.95 to 7.97	06					

			4) 7.98 to 8.00	08	Treatment 484 cm ² /b , 548 cm ² /b and 645 cm ² /b		08	08	08	
	3. State behaviour	06	1) 931.00 to 942.75	00	Treatment 548 cm ² /b			00		
				2) 942.76 to 954.51	02	Treatment 484 cm ² /b		02		
				3) 954.52 to 966.27	04	Treatment 387 cm ² /b	04			
				4) 966.28 to 978.00	06	Treatment 645 cm ² /b				06
	4. Event behaviour	06	1) 461.33 to 473.04	00	Treatment 645 cm ² /b				00	
				2) 473.05 to 484.76	02	Treatment 387 cm ² /b	02			
				3) 484.77 to 496.48	04	Treatment 484 cm ² /b		04		
				4) 496.49 to 508.17	06	Treatment 548 cm ² /b			06	
	Subtotal	28				10	18	18	22	
TOTAL						32	73	84	82	

Experiment 2: Relationship between best stocking densities (548 and 645 cm²/b) and flock Size (06 and 09 birds) on production, welfare and behaviour of commercial laying hens maintained in California cage system

On the basis of well being scorecard it was confirmed that the rearing of commercial laying hens in California cage system at 548 and 645 cm²/b gave best results in term of production, welfare, behaviour and economics. Hence, second experiment was planned to test the interaction effect of best two space allowances (548 and 645 cm²/b) and flock size (06 and 09 birds) to know the production, welfare and behaviour of commercial laying hens.

During the first experiment in each cage box 3 birds (3B) were maintained for best space allowance of 548 and 645 cm²/b. In this trial two different flock sizes with 06 (6B) and 09 birds (9B) in each cage with stocking density 548 and 645 cm²/b were evaluated further for the production, welfare and behaviour of commercial laying hens.

Hence there were four different treatments 548 cm²/b with 6B and 9B; 645 cm²/b with 6B and 9B to observe the interaction effect of stocking density and flock size. The existing California cages were restructured to achieve the required specific flock size with appropriate space allowance. The performance of all the treatment groups were recorded with respect to the different parameters considered for this study. A total of 180 commercial laying hens were maintained for 20 weeks in four different treatment groups. The results obtained for the different parameters are presented and discussed as below.

4.2.1 Hen Day Egg Production (HDEP):

The data concerning to HDEP % during the entire experimental (20 week period) from all the treatment groups are presented in Table 4.15 and graphically depicted in Figure 4.8. The entire trial period was divided as 28 to 31st, 32 to 35th, 36 to 39th, 40 to 43rd and 44 to 47th week periods.

The interaction effect of stocking density and flock size on HDEP % for the overall 20 week of experimental period were statistically non-significant

among the various interaction treatment groups. Whereas, during the 44 to 47 week of age the birds reared at 645 cm²/b with flock size 6B and 9B showed significant difference with better performance in 645 cm²/b at 6B flock size. In this period, the mean of egg production of 548cm²/b with flock size 6B and 645cm²/b with 6B treatment groups was statistically higher 93.22 ± 1.40 and 92.19 ± 1.82 , respectively than 645cm²/b with flock size 9B for production 87.55 ± 0.98 . There was no statistical difference for the entire 28-47 week and individual 4 weekly period because of the main effect of stocking density, however main effect of flock size on HDEP% changes was observed. At 28 to 31st week, 44-47th week and entire period of 28-47th week of bird's age 6B flock size had statistically higher ($P < 0.05$) egg production than flock size with 9B.

In overall, birds maintained at 548 and 645cm²/b with flock size 6B was observed to provide a trend of better production due to interaction effect of stocking density and flock size, but not enough to elicit a statistical significant among the various treatment groups. In corroboration with the present study Craig and Milliken (1989) reported that group size (four and eight birds) had no detectable effect on rate of production at low (580 cm²/bird) and medium (464 cm²/bird) but cages holding four hens were superior in rate of lay, that is with smaller flock size. Craig *et al.*, (1986) indicated that hens in large group size (4 and 6 hens with space allowance of 464 and 310cm²/bird) and in high density cages are consistently performing inferior as compared to birds in single hen cages with space allowance of 929cm²/bird. By continuing the study for number of generations in the same place they further indicated that increasingly inferior performance over long periods is associated with apparent stress. Statistical difference for the main effect of flock size in present experiments is in alignment with findings of Benyi *et al.*, (2006) who reported significant differences on egg production where a stocking density of 1100, 733 and 550 cm²/bird for three treatments by housing the birds in groups of 12, 18 and 24 birds at densities 2, 3 and 4 birds per cage, respectively. Hens housed in pairs or in group size of 12 produced significantly more eggs in comparison to 18 and 24 birds group size, that is in smaller flock size more production.

Guo *et al.*,(2012) maintained two group sizes of 21 birds and 48 hens in furnished cages provided with stocking density of 586 cm² and 543 cm² per bird and another controlled group with 4 birds in conventional cage. Laying rate was not affected by the group size, the means were 86.85, 84.78 and 89.85% for 21 birds and 48 hens group size in furnished cages and 4 birds in conventional cage respectively, though it was observed that smaller group size had numerically higher egg means. Wall (2011) also observed non-significant egg laying % (hen day) among the different designed furnished cage group size of 86.3, 84, 85 and 84.9 % for T8, T10, T20 and T40 birds, with numerically higher egg percentage in the smallest flock size of eight birds.

It could be concluded that though interaction of flock size with different stocking density could not evoke significant differences, but with smaller flock size produced numerically higher eggs as resulted in present experiment along with the reports from various authors.

4.2.2 Feed Intake and Feed Conversion Ratio:

As per standard specification of BV 300 commercial WLH birds, a weighed quantity of feed 110 g/bird were offered to all the birds during the entire 20 weeks of experimental period. At the end of the each week the residual feed from the feeding trough of each cage box was collected quantitatively and weighed and divided by the number of birds in that cage which was merely 0.5gm/b. Therefore, the actual feed intake of birds was considered to be 110g/bird for all the experimental birds.

The data pertaining to Feed Conversion ratio (per dozen egg) obtained from the interaction effect of stocking density group and flock size is presented in Table 4.16 and same is depicted in Figure 4.9. Feed Conversion ratio was calculated on the basis of per dozen egg for the different periods of trial and in total for the 20 weeks period.

Fig. 4.8. Interaction effect of Stocking Density and Flock Size on HDEP%

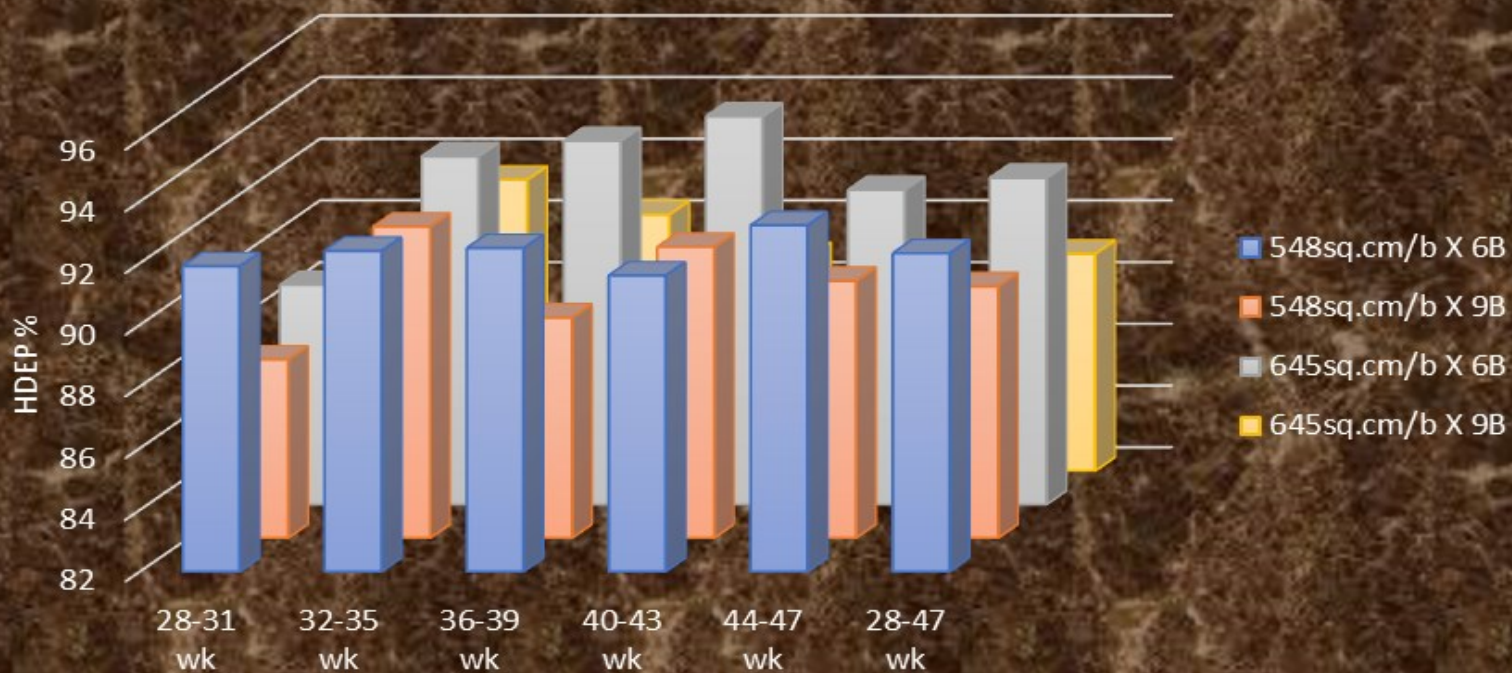


Table 4.15 Interaction effect of stocking density and flock size on hen day egg production%

Treatment	Experimental duration/ birds age					
	28- 31 wk	32-35 wk	36-39 wk	40- 43 wk	44-47wk	28-47wk
548cm ² /b X 6B	91.89±1.80	92.39±1.30	92.48±1.24	91.59±1.76	93.22 ^a ±1.40	92.31±0.82
548cm ² /b X 9B	87.78±1.42	92.09±2.22	89.14±2.78	91.43±1.55	90.33 ^{ab} ±1.05	90.15±1.42
645cm ² /b X 6B	89.1 ±1.46	93.28±1.71	93.78±1.76	94.57±1.73	92.19 ^a ±1.82	92.58±1.50
645cm ² /b X 9B	86.93±0.96	91.43±1.71	90.30±1.66	88.95±1.72	87.55 ^b ±0.98	89.03±1.04
Effect of stocking density						
548cm ² /b	89.83±1.02	92.24±1.25	90.81±1.38	91.51±1.20	91.77± 0.96	91.23±0.87
645cm ² /b	88.02±1.02	92.35±1.25	92.04±1.38	91.76±1.20	89.87 ± 0.96	90.81±0.87
Effect of flock Size						
6 Birds	90.50 ^a ±1.02	92.83±1.25	93.13±1.38	93.08±1.20	92.70 ^a ± 0.96	92.45 ^a ±0.87
9 Birds	87.35 ^b ±1.02	91.76±1.25	89.72±1.38	90.19±1.20	88.94 ^b ±0.96	89.59 ^b ±0.87
Pooled SEM	0.721	0.882	0.973	0.848	0.678	0.614
Statistical significance						
Treatment	NS	NS	NS	NS	*	NS
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	*	NS	NS	NS	**	*

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

The interaction effect of stocking density and flock size on FCR for the overall 20 week experimental period were statistically non-significant among the various interaction treatment groups. Whereas, during the 44 to 47 week of age the birds reared at 645 cm²/b with flock size 6B and 9B showed significant difference with better FCR in 645 cm²/b at 6B flock size.

There was no statistical difference for the entire 28-47 week and individual 4 weekly period because of the main effect of stocking density, however because of flock size FCR changes were observed. During 28-31st, 44-47th week and entire period of 28-47th week of bird's age 6B flock size had statistically better (P < 0.05) FCR than flock size with 9B.

In overall, birds maintained at 548 and 645cm²/b with flock size 6B was observed and provided a trend of better FCR due to interaction effect of stocking density and flock size, but not enough to elicit a statistical significant among the various treatment groups. The main effects for stocking density on FCR did not lead to any significant differences but flock size significantly (P < 0.05) improved FCR in 6B reared groups.

Table 4.16. Interaction effect of stocking density and flock size on feed conversion ratio (per dozen eggs)

Treatment	Experimental duration/ birds age					
	28- 31wk	32-35 wk	36-39 wk	40-43wk	44-47wk	28- 47wk
548cm ² /b X 6B	1.38±0.01	1.43±0.02	1.43±0.02	1.45±0.03	1.42 ^b ±0.02	1.42±0.01
548cm ² /b X 9B	1.43±0.02	1.44±0.04	1.48±0.04	1.45±0.02	1.46 ^{ab} ±0.02	1.45±0.02
645cm ² /b X 6B	1.41±0.01	1.42±0.03	1.41±0.03	1.40±0.03	1.43 ^b ±0.03	1.42±0.02
645cm ² /b X 9B	1.42±0.01	1.45±0.03	1.47±0.03	1.49±0.03	1.51 ^a ±0.02	1.47±0.02
Effect of stocking density						
548cm ² /b	1.41±0.01	1.44±0.02	1.46±0.02	1.45±0.02	1.44± 0.02	1.43±0.01
645cm ² /b	1.42±0.01	1.43±0.02	1.44± 0.02	1.45±0.02	1.47 ± 0.02	1.44± 0.01
Effect of flock size						
6 Birds	1.40±0.01	1.42±0.02	1.42±0.02	1.42±0.02	1.43 ^b ± 0.02	1.42 ^b ± 0.01
9 Birds	1.43±0.01	1.44± 0.02	1.47± 0.02	1.47±0.02	1.49 ^a ± 0.02	1.46 ^a ± 0.01
Pooled SEM	0.008	0.014	0.015	0.014	0.011	0.009
Statistical significance						
Treatment	NS	NS	NS	NS	*	NS
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	NS	NS	NS	NS	*	*
Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.						

Fig.4.9. Interaction effect of Stocking Density and Flock Size on Feed Conversion Ratio (per dozen eggs)

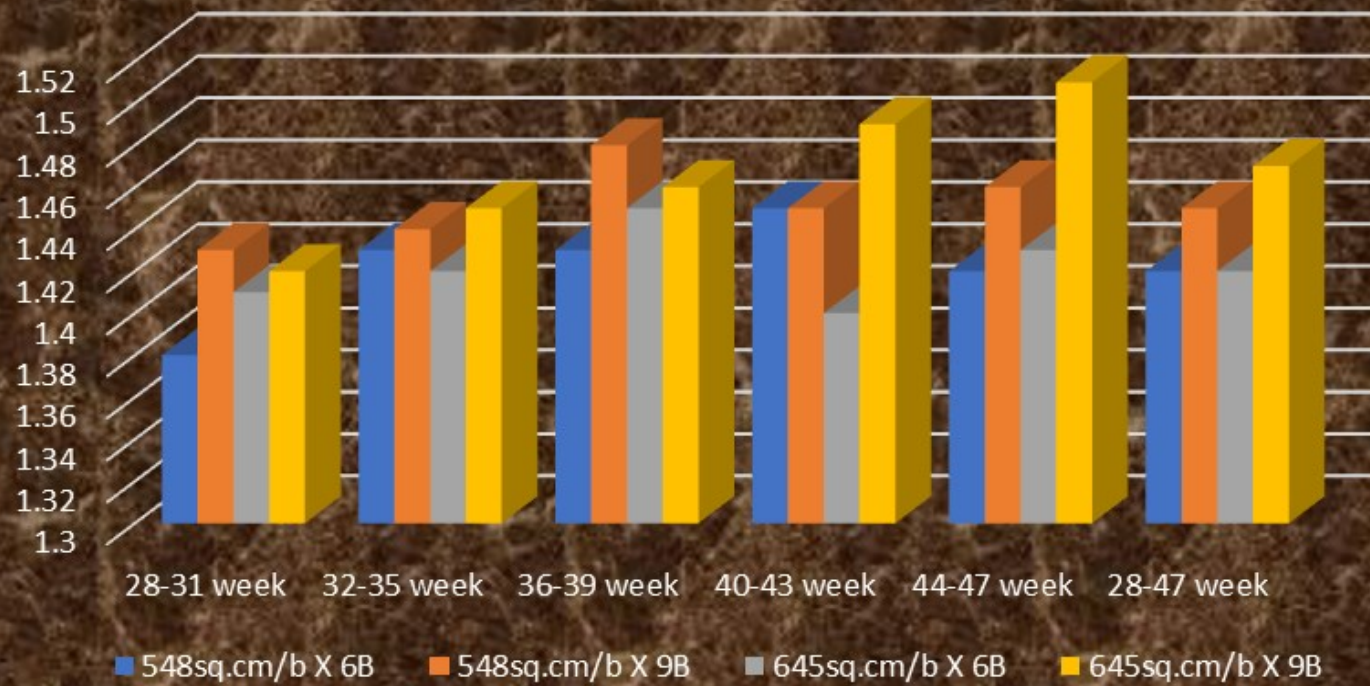


Table 4.17. Interaction effect of stocking density and flock size on feed conversion ratio (per kg egg mass)

Treatment	Experimental Birds age/period					
	28- 31 wk	32-35 wk	36-39 wk	40-43wk	44-47wk	28- 47 wk
548cm ² /b X 6B	2.33±0.03	2.28±0.03	2.28±0.03	2.19±0.05	2.17±0.05	2.25±0.02
548cm ² /b X 9B	2.31±0.04	2.30±0.06	2.30±0.07	2.20±0.04	2.25±0.03	2.27±0.04
645cm ² /b X 6B	2.27±0.04	2.24±0.05	2.18±0.04	2.12±0.04	2.18±0.05	2.20±0.03
645cm ² /b X 9B	2.32±0.02	2.26±0.05	2.24±0.06	2.26±0.06	2.29±0.03	2.28±0.03
Effect of stocking density						
548cm ² /b	2.32± 0.03	2.30±0.03	2.29±0.04	2.19±0.03	2.21±0.03	2.26±0.02
645cm ² /b	2.30±0.03	2.26±0.03	2.21±0.04	2.24±0.03	2.24±0.03	2.24±0.02
Effect of flock size						
6 Birds	2.30±0.03	2.26±0.03	2.23±0.04	2.15±0.03	2.18 ^b ±0.03	2.22 ± 0.02
9 Birds	2.32±0.03	2.28±0.03	2.27±0.04	2.23±0.03	2.27 ^a ±0.03	2.27± 0.02
Pooled SEM	0.017	0.024	0.026	0.023	0.019	0.015
Statistical significance						
Treatment	NS	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	NS	NS	NS	NS	P<0.05	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Feed conversion ratio (per dozen egg) was significantly different and better for small size group of 5 birds of 1.5 Vs 2.10 for group of 7 birds that is similar to present experiment where changes were though non-significant for interaction effect of stocking density and flock size, but the trend was similar and better in small flock size in 548 and 645 cm²/b with flock size 6B compared to 548 and 645 cm²/b with flock size 9B, as seen in main effect for flock size. Corroborating the present experiment result Anderson *et al.*, (2004) observed no difference in feed consumption and feed efficiency when birds maintained in two group sizes 24 hens and 18 hens for stocking density 361 and 482 cm² per bird only the HDEP was affected because of stress.

Guo *et al.*, (2012) observed hens housed in group size of 48 in large furnished cages and 21 in small furnished cages had a higher feed intake ($P < 0.05$) and poorer feed efficiency ($P < 0.01$) than that of controls of group size of 4 birds. The poorer efficiency was related with low production in group of 21 and 48 and significant more feed consumption in 21 and 48 as compared to group size of 4 birds. Meng *et al.*, (2014) observed that layers in large group of 40 in large furnished cages tended to have lower efficiency of feed conversion (i.e. feed: egg ratio) than group of 8 in small furnished cages or group of 12 in conventional caged hens, although 40 and 8 group size in furnished caged hens had similar daily feed intake but was different from 12 bird group, though not-significant. The possibility of lower feed intake in the large group treatment is attributed to the greater space which may increase hen's social competition for resources such as high perches or the dust-bathing box. Like if a dominant hen occupies a high perch, they are reluctant to give it away; hence, longer occupation of the high perch would mean less time to feed, which in turn, may reduce total daily feed intake. In the present investigation there was no significant difference in feed conversion ratio. Although it might have been expected that feed conversion would have been unfavorable when there is a bigger groups of 40 and 60 hens because of the larger floor space available, this was not found to be the present experiment.

4.2.3 Live Body Weight (LBW) and Body Weight Gain (BWG):

The result of the effect of flock size on the body weight changes in the layers are presented in Table 4.18. The results revealed that initial and final body weight in experimental birds did not differ significantly due to either main effect of stocking density or flock size or interaction when maintained at flock size of six and nine birds with stocking density of 85 sq. inch/548 sq.cm and 100 sq. inch or 645 sq.cm per bird.

Keeling *et al.*, (2003) recorded difference in body weight when birds were kept in group sizes of 15, 30, 60 and 120 birds for a stocking density of 5 birds /m² which is in contrast to present experiment. Birds of group size 30 and 120 were significantly lighter than 15 and 60 which was consistent for all age groups.

As the feeder and floor space were same, the changes in body weight was unlikely to be because of resource allocation but rather to be variation in social behavior of

Table 4.18. Interaction effect of stocking density and slock size on Live Body Weight (LBW) and Body Weight Gain (BWG)

Treatment	Initial LBW (28th week of birds age)	Final LBW (47th week of birds age)	BWG
548cm ² /b X 6B	1246.33 ± 34.50	1378.50 ± 30.51	132.17 ± 38.93
548cm ² /b X 9B	1279.00 ± 26.81	1399.00 ± 49.96	120.00 ± 34.06
645cm ² /b X 6B	1248.50 ± 28.05	1408.67 ± 37.60	160.17 ± 25.80
645cm ² /b X 9B	1283.50 ± 22.72	1530.67 ± 74.66	214.50 ± 85.73
Effect of stocking density			
548cm ² /b	1262.67 ± 20.04	1388.75±36.08	126.08 ±36.56
645cm ² /b	1266.00 ± 20.04	1469.67±36.08	187.33± 36.56
Effect of flock size			
6 Birds	1247.42± 20.04	1393.58 ± 36.08	146.17±36.56
9 Birds	1281.25± 20.04	1464.83 ± 36.08	167.25± 36.56
Pooled SEM	13.68	26.84	25.27
Statistical significance			
Treatment	NS	NS	NS
Stocking density	NS	NS	NS
Flock size	NS	NS	NS

birds. Davami *et al.*, (1987) also observed significant difference in body weight in group size of seven and five birds respectively.

Abrahamsson and Tauson (1997) observed no significant difference on body weight when different group size of birds 5, 6, 7 and 8 were kept in furnished cages with a floor area of 600cm²/bird excluding nest area and sand bath. Similarly, Meng *et al.*, (2014) observed non-significant differences among various group size of birds on body weight. These findings are similar to present experiment on the weight gain and final body weight due to main and interaction effect of stocking density and flock size. No any variation in body weight due to social behavior was observed at the end of the experiment and because the feed and water allocation quantity was similar in all treatment groups that could be the reason for non-significant difference in BWG.

4.2.4 External and Internal Egg Quality Parameters :

The results of the interaction effect of flock size and stocking density on egg quality traits divided into two categories: external and internal. External egg quality like egg weight, shape index and the percentage of dirty and broken eggs were observed. Internal egg quality includes albumen index, yolk index, Haugh unit, shell weight, yolk color or score, shell thickness. At each fifteen day of the experimental period twelve eggs per treatment groups, hence total 480 eggs were used to assess the egg quality of the experimental birds, the average detail of which is presented in Table 4.19 and 4.20 and Figure 4.10 and 4.11.

Table 4.19. Interaction effect of stocking density and flock size on external egg quality parameter

Treatment	Egg Weight (g)	Shape Index	Dirty eggs(%)	Broken eggs(%)
548cm ² /b X 6B	54.13 ± 0.56	77.44 ^{ab} ± 0.21	2.03 ± 1.09	0.06 ± 0.04
548cm ² /b X 9B	55.28 ± 0.55	77.19 ^b ± 0.24	1.10 ± 0.41	0.29 ± 0.12
645cm ² /b X 6B	54.57 ± 0.52	76.93 ^b ± 0.26	0.31 ± 0.07	0.18 ± 0.14
645cm ² /b X 9B	54.58 ± 0.13	78.11 ^a ± 0.28	1.07 ± 0.35	0.08 ± 0.04
Effect of stocking density				
548cm ² /b	54.70 ± 0.34	77.31 ± 0.18	1.57 ± 0.43	0.17 ± 0.07
645cm ² /b	54.57 ± 0.34	77.52 ± 0.18	0.69 ± 0.43	0.13 ± 0.07
Effect of flock size				
6 Birds	54.35 ± 0.34	77.18 ± 0.18	1.17 ± 0.43	0.12 ± 0.07
9 Birds	54.93 ± 0.34	77.65 ± 0.18	1.09 ± 0.43	0.18 ± 0.07
Pooled SEM	0.239	0.124	0.304	0.047
Statistical significance				
Treatment	NS	**	NS	NS
Stocking density	NS	NS	NS	NS
Flock size	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P<0.05, **P< 0.01.

Table 4.20. Interaction effect of stocking density and flock size on internal egg quality parameters

Treatment	Alb Index	Yolk Index	Haugh unit	Shell Thickness	Shell weight	Yolk score/colour
548cm ² /b X 6B	0.135±0.00	0.408±0.00	95.16±0.75	0.345±0.00	5.40 ±0.04	5.25±0.08
548cm ² /b X 9B	0.131±0.00	0.409±0.00	93.87±0.92	0.353±0.00	5.35±0.04	5.25±0.06
645cm ² /b X 6B	0.132±0.00	0.405±0.00	93.79±0.60	0.344±0.00	5.49± 0.08	5.28±0.05
645cm ² /b X 9B	0.142±0.00	0.408±0.00	95.69±0.71	0.352±0.00	5.48±0.02	5.42±0.09
Effect of stocking density						
548cm ² /b	0.133±0.00	0.408±0.00	94.52±0.53	0.349±0.00	5.37 ^b ±0.04	5.25±0.05
645cm ² /b	0.137±0.00	0.407±0.00	94.74±0.53	0.348±0.00	5.48 ^a ±0.04	5.35±0.05
Effect of flock size						
6 Birds	0.133±0.00	0.406±0.00	94.48±0.53	0.344 ^b ±0.00	5.44±0.04	5.27±0.05
9 Birds	0.137±0.00	0.409±0.00	94.78±0.53	0.352 ^a ±0.00	5.41±0.04	5.33±0.05
Pooled SEM	0.002	0.002	0.377	0.002	0.026	0.034
Statistical significance						
Treatment	NS	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	*	NS
Flock size	NS	NS	NS	*	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

The results revealed that the external egg quality parameters observed for the layers for the entire period (20-47 weeks) were non-significant for the interaction effects, main effect of stocking density and flock size, except for Shape Index at interaction.

Egg Shape Index was found to be best for birds in 645cm²/b with flock size 9B for the interaction effect with highly significant differences (P = 0.01) with 548cm²/b with flock size 9B and 645cm²/b with flock size 6B, but non-significant with 548cm²/b with flock size 6B. The main effect of stocking density and flock size were non-significant for all the internal egg quality parameters except for shell weight for stocking density and shell thickness with flock size. The shell weight was significantly better at lower stocking density (100 sq. inch /

645 cm² per hen) as compared to higher stocking density (85 sq. inch/ 548 cm² per hen). Shell thickness was significantly better in flock size of nine birds as compared six birds.

Craig *et al.*, (1986) observed the interaction of flock size (1, 4 and 6 birds) and stocking density (929, 464 and 310 cm²/bird) and indicated that hens in large group size 6 hens had significantly low egg mass (% HDEP X average egg weight) produced per hen over the entire 40week period (P<0.05) as compared to birds maintained at flock size of 4 and 1 bird per cage. Probably HDEP has a significant role as compared to egg weight for significant difference in egg mass. Mench *et al.*, (1986) did not notice any change in egg weight for group size (1, 2, and 25) and stocking density (1394, 1394, 697 and 1394 cm²/bird) similar to present experiment. Davami *et al.*, (1987) in their experiment of group size (5 and 7 birds) and stocking density (420 and 300 cm²/bird) reported no significant difference in shell thickness, specific gravity, and Haugh units of eggs. They conclude that bird density had little effect on egg quality traits except for egg weight, where egg weight was significantly(P<0.05) lower in small group size of 5 birds as compared to 7 bird group. Though higher feed consumption in the small group resulted in the expectation of larger egg size in the small group, but it may be possible that extra feed was needed to maintain the larger body size of the small group.

In corroboration with the present experiment, non-significant effect of stocking density of 348 (high), 464 (medium) and 580 (low) cm²/bird and in flock size of four and eight hens for each of the stocking densities on egg weight was observed by Craig and Milliken (1989). In contrast Keeling *et al.*, (2003) reported difference in egg weight by keeping at different group size of 15, 30, 60 and 120 from 0 day to 40th week with same stocking density of 5 bird per sq. meter space in litter. The treatment group with flock size of 30 birds laid smaller eggs (P<0.05) as compared to flock size of 15, 60 and 120. Further they concluded that birds in intermediate group 30 experiencing social disruption because the groups were too large for a stable hierarchy to develop but too small for a tolerant social system to occur.

Fig. 4.10. Interaction effect of Stocking Density and Flock Size on Egg Quality Parameters

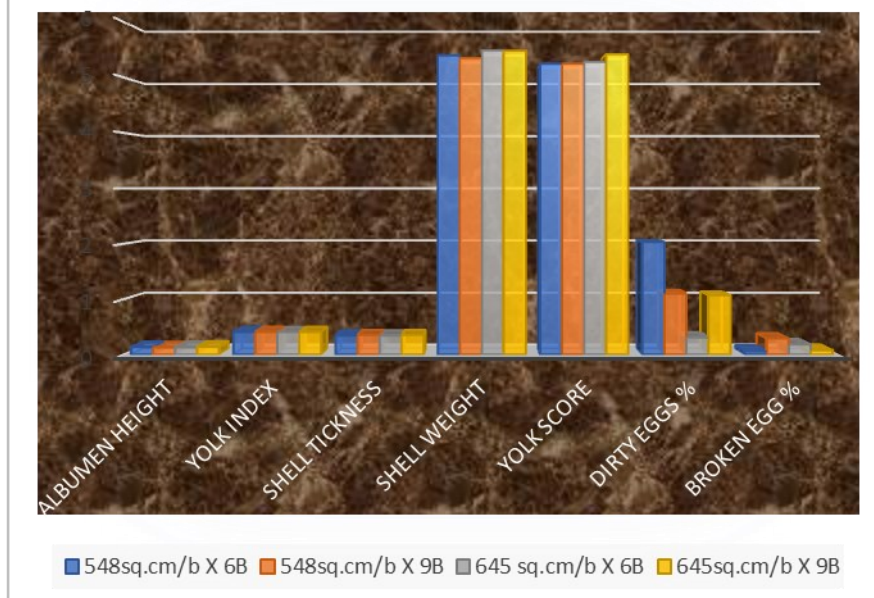
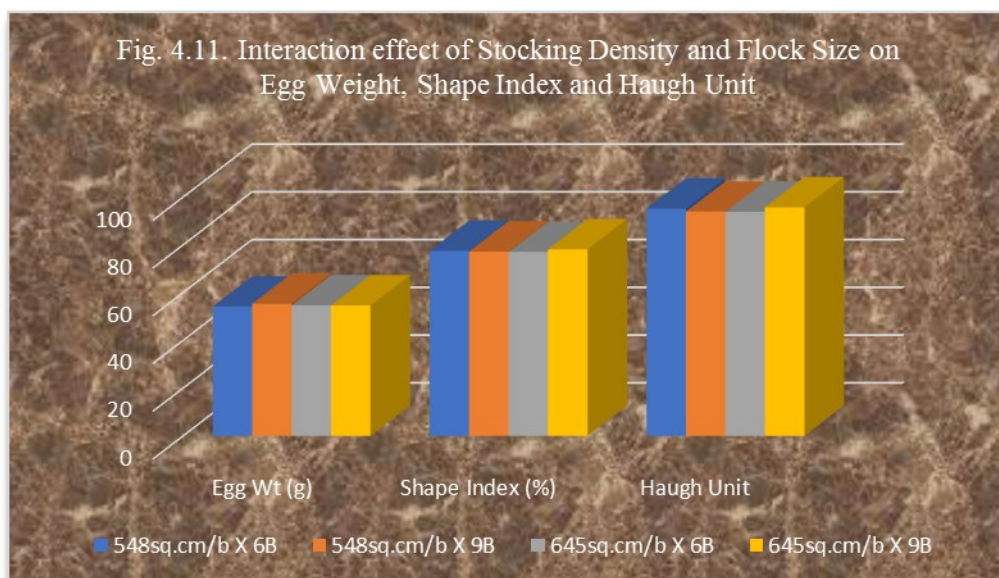


Fig. 4.11. Interaction effect of Stocking Density and Flock Size on Egg Weight, Shape Index and Haugh Unit



Guo *et al.*, (2012) did not observe any effect on egg weight, shell thickness and shape index when birds were maintained at different flock sizes in conventional and furnished cages. The results indicate group size has no role on egg quality, but only on laying rate and egg breakage.

Significant differences in percentage of cracked eggs for flock size was observed by Carey (1987) when commercial layer strain was maintained at stocking density of 364.1cm²/bird with flock size of 6, 8, 12 and 24 birds which is in contrast to the present experiment. Higher incidence of cracked egg percentage in cages with 12 and 24 birds per cage could be the result of increased bird activity, while the highest flock size in present experiment was only 9 birds due to which non-significant effect of cracked egg percentage observed.

It was concluded that neither the interaction effect of flock size of six and nine birds at the two stocking density of 548 and 645cm² nor the main effects of stocking density and flock size showed any significant and consistent effect in external and internal egg quality traits probably the flock size were too small to produce a difference and the stocking density were optimum.

4.2.5 Physical and physiological welfare study :

The results of the interaction effect of flock size and stocking density on the physical indicators of welfare parameter like feather score, gait score and physiological indicators like heterophil-lymphocyte ratio, serum corticosterone and immune status is presented in Table 4.21 and 4.22.

There was no interaction effect of flock size and stocking density observed for the various physical and physiological indicators like feather score, gait score, antibody titre, heterophil-lymphocyte ratio and corticosterone level. The mean feather score, gait score, antibody titre, heterophil-lymphocyte ratio and corticosterone level did not differ significantly due to main effect of stocking density and various levels of flock size. Significant improvement in plumage condition ($P < 0.05$) was observed by Davami *et al.*, (1987) due to interaction effect of flock size and stocking density. Birds at flock size of 7 birds and

stocking density 300 cm²/hen performed poorer than 5 birds with stocking density of 420cm²/bird as the poorer plumage of densely populated cages results because of abrasion against cage wire and other hens. The plumage condition of hens has been reported to be related to feed conversion and in this instance FCR was better. This is in contrast to present experiment where both the feather score or feed conversion were non-significant among the various treatment groups probably because the best stocking density of 548 and 645 cm² space was provided, may be with higher stocking density and flock size on the feather and gait score issue arises. Craig and Milliken (1989) observed step wise adverse influence on feather score due to increase in stocking density, but there was no effect of flock size- (four and eight birds) on the feather score and no interaction effect of stocking density and flock size.

Effect of stocking density and flock size on immunocompetence was reported to be non-significant by Mench *et al.*, (1986) when compared the micro-hemagglutination log₂ values to SRBC for stocking densities of 1394 and 697cm²/hen with flock size of either one bird per cage or two bird cage and as well when compared with birds raised in pens with flock size of 25 birds. Social stress associated with competition and changing group composition is known to cause decrease in circulating antibody levels was not observed here. Conversely, the findings indicate that the immune response of hens in systems with improved welfare might be higher. Scanty information is available for the relationships between physiological stress and immunity in housing systems and group sizes for laying hens.

Non-significant difference in H/L ratio was observed by Mench *et al.*, (1986) when two group sizes of 1 and 2 birds were maintained in cages at same stocking density of 1394cm²/bird also in comparison to 25 bird group size in pen at the same stocking density. Non-significant differences in H/L ratio for different flock sizes 2450 vs 4200 was also observed by Nicol *et al.*, (2001). In parallel present experiment also had non-significant differences in H/L ratio observed at different periods for the different flock sizes. Stress decreases the number of

Table 4.21. Interaction effect of stocking density and flock size on immune status, feather and gait score					
Treatment	Feather Score	Gait score	Antibody titer against ND (log₂ values)		
			28th week	37th week	47th week
548cm ² /b X 6B	6.89 ± 0.22	8.00 ± 0.00	2.64 ± 0.12	2.99 ± 0.09	2.95 ± 0.11
548cm ² /b X 9B	6.67 ± 0.00	8.00 ± 0.00	2.59 ± 0.13	2.90 ± 0.08	2.85 ± 0.12
645cm ² /b X 6B	7.00 ± 0.62	8.00 ± 0.00	2.82 ± 0.19	3.02 ± 0.09	2.92 ± 0.11
645cm ² /b X 9B	6.67 ± 0.52	8.00 ± 0.00	2.92 ± 0.10	2.91 ± 0.04	2.94 ± 0.04
Effect of stocking density					
548cm ² /b	6.78± 0.29	8.00± 0.00	2.61± 0.10	2.94± 0.06	2.90±0.07
645cm ² /b	6.83± 0.29	8.00± 0.00	2.87 ± 0.10	2.90± 0.06	2.93± 0.07
Effect of flock size					
6 Birds	6.94 ± 0.29	8.00 ± 0.00	2.73 ± 0.10	3.00 ± 0.06	2.93 ± 0.07
9 Birds	6.67 ± 0.29	8.00 ± 0.00	2.76 ± 0.10	2.90 ± 0.06	2.89 ± 0.07
Pooled SEM	0.208	0.00	0.068	0.040	0.050
Statistical significance					
Treatment	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS
Flock size	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.22. Interaction effect of stocking density and flock Size on corticosterone and heterophil: lymphocyte ratio

Treatment	H/L Ratio at 28 th week	H/L Ratio at 37 th week	H/L Ratio at 47 th week	Corticosterone (ng/ml) at 28 th week	Corticosterone (ng/ml) at 37 th week	Corticosterone (ng/ml) at 47 th week
548cm ² /b X 6B	0.26 ± 0.02	0.31 ± 0.03	0.23 ± 0.01	1.202±0.01	0.933±0.09	1.022±0.06
548cm ² /b X 9B	0.30 ± 0.04	0.34 ± 0.02	0.23 ± 0.01	1.158±0.07	0.915±0.09	0.857±0.08
645cm ² /b X 6B	0.31 ± 0.04	0.29 ± 0.02	0.25 ± 0.02	1.078±0.07	1.010±0.04	1.005±0.06
645cm ² /b X 9B	0.27 ± 0.05	0.29 ± 0.04	0.23 ± 0.02	1.233±0.06	0.870±0.09	0.893±0.16
Effect of stocking density						
548cm ² /b	0.28 ± 0.03	0.33± 0.02	0.23± 0.01	1.180±0.04	0.924±0.06	0.939±0.07
645cm ² /b	0.29 ± 0.03	0.29 ± 0.02	0.24± 0.01	1.156±0.04	0.940±0.06	0.949±0.07
Effect of flock size						
6 Birds	0.28 ± 0.03	0.30 ± 0.02	0.24 ± 0.01	1.140±0.04	0.972±0.06	1.013±0.07
9 Birds	0.28 ± 0.03	0.32 ± 0.02	0.23 ± 0.01	1.196±0.04	0.893±0.06	0.875±0.07
Pooled SEM	0.019	0.014	0.007	0.029	0.041	0.049
Statistical significance						
Treatment	NS	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	NS	NS	NS	NS	NS	NS
Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.						

lymphocytes and consequently a rise in the H/L ratio, the non-significant difference is an indicative of no effect of interaction of stocking density and flock size on stress. Hens at same flock size of two birds per cage at different stocking density (1394 and 697cm²/bird) had significant difference (P<0.05) in corticosterone level, however different flock size -single and two at same stocking density (1394cm²/bird) did not bringt any change in the corticosterone level as reported by Mench *et al.*, (1986).

Craig *et al.*, (1986) reported non-significant difference in plasma corticosterone level for different group size of one, four and six bird per cage at stocking density of 929, 464 and 310 cm²/bird measured at 45 weeks after housing. Although hens in 6-bird cages had corticosterone levels approximately 15% higher than those of hens in single- and 4-hen cages at this age, which is consistent with the magnitude of the difference found between hens in 6-bird cages and cages holding 1 and 4 hens in the first 22weeks of the study, this difference was not significant. Corticosteroid concentration in plasma appears to be so variable among hens treated alike that significance may be detectable only when a relatively large number of difference in the treatment and number of samples were collected. Nicol *et al.*, (2001) did not observe any significant differences in faecal corticosterone level at large different flock size of 2450 birds and 4200 birds at same stocking density of 12 bird/m². In contrast Cheng and Muir (2004) reported that laying hens showed significantly (P<0.05) lower plasma corticosterone levels in single-bird cages (525 cm²/bird) than in the 10-bird cages (419 cm²/bird), indicating that social stressors could be a factor in higher production of corticosterone in hens.

In the present experiment non-significant results in corticosterone level due to interaction effect, main effect of flock size and stocking density was similar to the finding of few authors and was probably due to less stress at stocking density of 548 and 645 cm²/bird in combination with flock size of six and nine birds. Significance may be detectable only when a relatively large number of difference in the flock size.

4.2.6 Behavioral Study:

The interaction effect of stocking density (548 and 645cm²per bird) and flock size (6 and 9 birds) on various behaviors (standing, sitting, sleeping, investigating, walking, drinking, feeding, preening, feather pecking, scratching, wing flapping etc) of the experimental birds are presented phase wise (Start-28, Mid-37 and End-47 week) in Table 4.23, 4.24 and 4.25. The various activities of the birds behavior were recorded by a CCTV camera for 24-hour period and an activity chart or ethogram from the video recordings were noted for behavior analysis. The time spent on each behavior was observed and expressed in minute for the specific 24 -hour (1440 minutes) of the day. The specific 24 hour (1440 minute) behavioral observations collected at three different period (28, 37 and 47 week) of experiment were averaged and presented in Table 4.26 and Figure 4.12 and 4.13. The average of three different stages/weeks of each behavioral activity for the interaction effect and main effect of stocking density and flock size is discussed as below.

Interaction Effect of Stocking Density and Flock size:

The averaged values of the interaction effect of stocking density and flock size were non- significant for behavioural activities observed at 28th, 37th and 48th week except for sleeping, drinking and feather pecking (Table 4.26).The birds with space allowance 548cm²/b x 6B showed significantly (P < 0.01) highest sleeping (305.83) behaviour compare to any other treatment groups, whereas, 645cm²/b x 9B recorded significantly lowest sleeping (191.83) activity. The birds with 548 cm²/b x 6B and 645 cm²/b x 6B recorded significantly more sleeping behaviour than their counter parts i.e 548 cm²/b x 9B and 645 cm²/b x 9B. Time spent on drinking was significantly higher (P < 0.01) at both 548cm²/b x 6B and 548cm²/b x 9B treatments when compared to treatment groups of 645 cm²/b x 6B and 645 cm²/b x 9B, though resource allocation i.e availability of nipple drinkers were proportionately same. Feather pecking among the various treatment groups was significantly lower (P< 0.01) at 548cm²/b x 6B when compared to 548cm²/b x 9B and 645cm²/b x 6B but remained non-significant with 645 cm²/b x 9B.

Abrahamsson and Tauson (1997) reported no deteriorative effects on plumage condition or cannibalism/ feather pecking were detected between the different group sizes, and suggested a group of 8 hens may be considered being below a critical group size in furnished cages, regarding outbreaks of feather pecking or cannibalism. The extent of damage on the feather as evident from feather score (Table 4.21) because of feather pecking behavior was non-significant, probably the feather pecks were non-aggressive, though gentle and aggressive feather pecks were not estimated in this trial. Feather pecking may be a type of social grooming with no injury intent and may be a form of recognition between birds.

It could be concluded that the interaction effect of stocking density and flock size brought a significant better sleeping and drinking activities and significant less time spent on the feather pecking for the group 548cm²/b x 6B. The better sleeping behavioural activity at 548cm²/ bird when compared to 645cm²/bird from experiment 1 cannot be neglected as well non-significant difference in feather pecking between 548 and 645cm²/bird. (Table 4.12).

Main effect of Stocking density:

The main effect of stocking density for the averaged values (28th, 37th and 47th week) of behavioural activities was observed significant for standing, sleeping and drinking (Table 4.26). The birds reared with lower stocking density 645cm²/bird had more standing time ($P < 0.05$) compared to the birds reared at 548cm²/bird.

While behavioral patterns for sleeping and drinking was the opposite with significantly ($P < 0.01$) more time was spent by birds for sleeping and drinking in group 548cm²/bird as compared to 645cm²/bird for the main effects of stocking density. This was in contrast to higher drinking behavior for birds reared in 1016 cm²/hen of low stocking density as reported by Albentosa *et al.*, (2007) when compared to high stocking density of 762cm² and 609cm²/bird.

Hughes and Black (1974) reported that the birds spent more time for standing when are at the higher stocking rate ($P < 0.001$) which was confounding to the present result observed with main effects at stocking density $548\text{cm}^2/\text{b}$ which had the higher stocking density but significantly lower standing when compared to $645\text{cm}^2/\text{b}$. Though similar result of experiment 1 where stocking density of 85 sq. inch/ 548cm^2 per bird resulted in lowest standing could not be ignored.

Anderson *et al.*,(2004) also indicated more movement acts ($P < 0.05$) when birds were kept in low density ($482\text{cm}^2/\text{bird}$) than in high density ($361\text{cm}^2/\text{bird}$), in present main effects of stocking density on walking was though non-significant, numerical more movements observed in $645\text{cm}^2/\text{bird}$ as compared to $548\text{cm}^2/\text{bird}$.

It was concluded that the main effect of stocking density gave significant standing behavior at $645\text{cm}^2/\text{bird}$ as compared to $548\text{cm}^2/\text{bird}$, this could be related to result of experiment 1 where stocking density of 85 sq. inch/ 548cm^2 per bird resulted in less standing compare to $645\text{cm}^2/\text{bird}$. The significant higher drinking and sleeping in $548\text{cm}^2/\text{bird}$ for the main effect could be possibly because of social dominance at lower stocking density of $645\text{cm}^2/\text{bird}$ and monopolization of the resources, leading to overall lower drinking time.

Main effect of Flock Size:

The main effect of flock size for the averaged values (28th, 37th and 47th week) of behavioural activities observed significant only for sleeping (Table 4.26) behavior. The treatment group with flock size of 6 birds had significantly higher ($P < 0.01$) sleeping activity as compared to 9 bird flock size.

In the present experiment the main effect of feather pecking for the averaged values were seen higher in the flock size with nine birds as compared to six though it were only numerical.

Bilcik and Keeling (1999) reported significant effect on feather pecking because of group size when birds were maintained in pens in group size of 15, 30,

Fig. 4.12. Interaction effect of Stocking Density and Flock Size on State and Event Behaviors

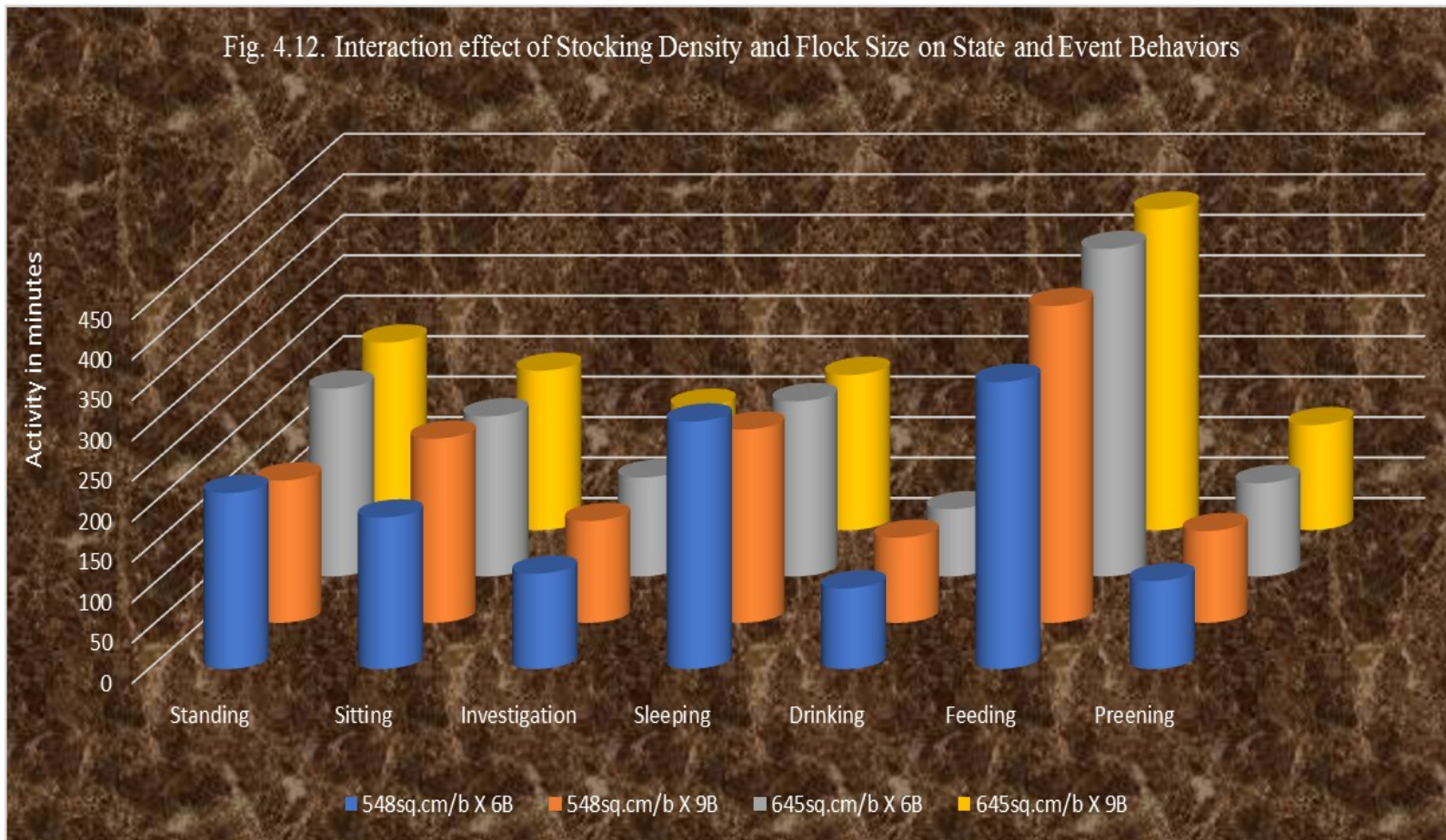
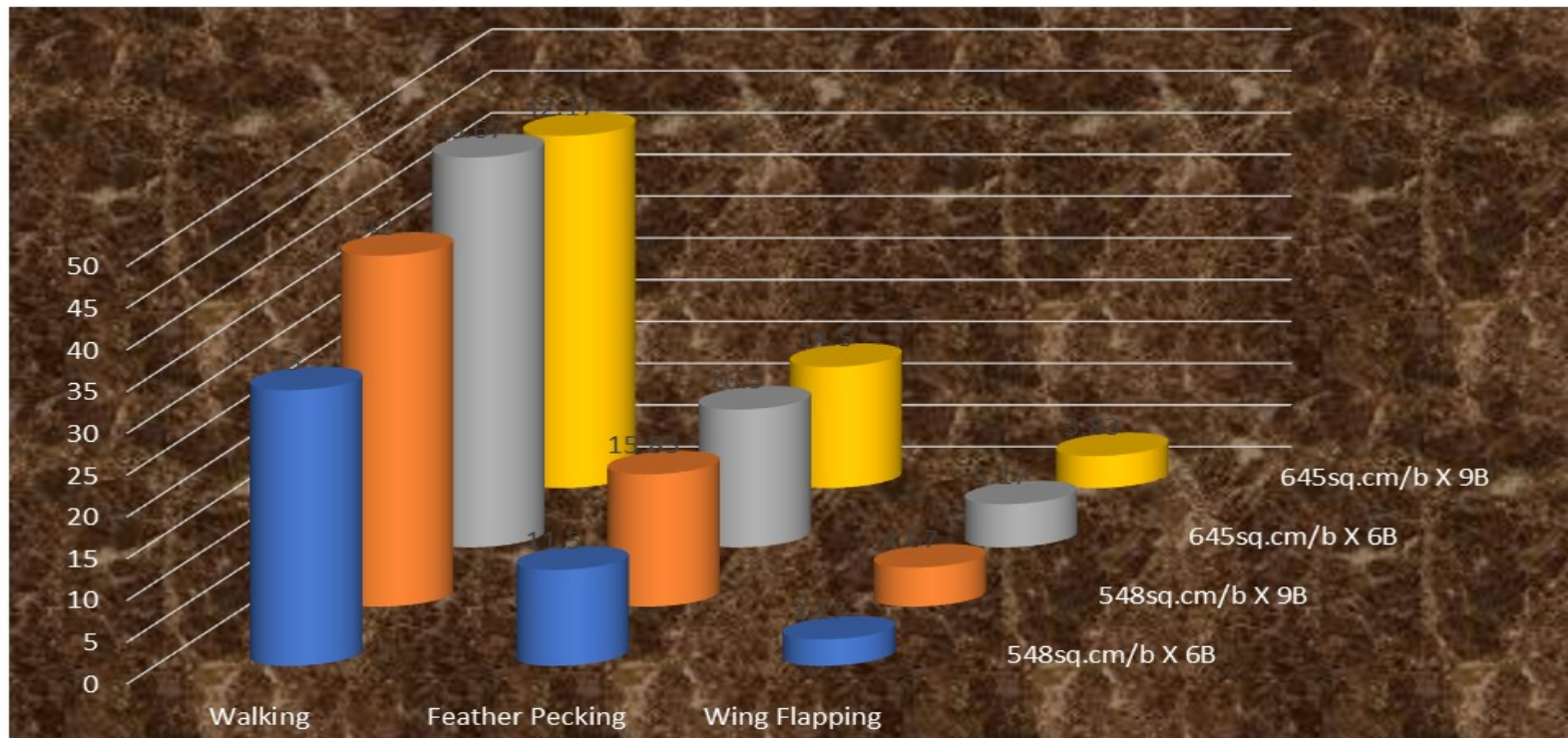


Fig. 4.13. Interaction effect of Stocking Density and Flock size on Walking, Feather Pecking and Wing Flapping Behaviors



60 and 120 birds, each with 4 replicates, behavioral observations were performed at the ages of 22, 27, 32 and 37 weeks which were focused on the number of feather pecks (gentle and severe) and aggressive pecks received. It was concluded that total number of observed gentle feather pecks (273) received was higher than number of severe pecks (137) received, 66.6% Vs 33.4% and only a small proportion of actual feather damage can be attributed to the gentle type of pecks. In contrast, severe feather pecks were found to be strongly related both to feather damage and skin injuries.

Anderson *et al.*, (1989) reported that group size had significant ($P < 0.05$) effects on the amount of standing, crouching, preening, feeding, and comfort movements during the first 5 days post-housing in layer cages. Hens housed four/cage spent significantly less time standing, more feeding and more time preening than hens housed six/cage which is similar to present experiment, though non-significant, but numerically more standing activity was observed for birds with flock size of six as compared to nine. Hens in the four-bird cages could feed simultaneously, whereas those in the six-hen cages had to compete for access to the feeder. Contrasting preening and feeding result was observed in present study with non-significant differences for flock size because the fact better feeding and cage space availability to all flock sizes in the present experiment could not be ignored.

It could be concluded that the main effect of flock size didn't bring any significant changes in behavior activity of birds except, the sleeping behavior with more sleeping in 6 birds flock size as compared to 9 birds which could be possible related to numerical more walking time in 9 bird flock. Overall for the interaction and main effects of stocking density and flock size the treatment group $548\text{cm}^2/\text{b} \times 6\text{B}$ outperformed the others for sleeping, drinking and feather pecking, though the small difference in stocking densities and flock sizes and the interactions negating each other is equally important.

Table 4.23. Interaction effect of Stocking Density(SD) and Flock Size (FS) on state and event behaviour of bird at 28th week of age

Parameters	Interaction Effect of Stocking Density and Flock Size				Effect of Stocking Density (SD)		Effect of Flock Size (FS)		Pooled SEM	Statistical Significance		
	548cm ² /bX 6B	548cm ² /b X 9B	645cm ² /b X 6B	645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Treat - ment	SD	FS
Standing	212.00 ^{ab} ± 8.03	170.17 ^b ± 14.51	247.67 ^a ± 14.56	235.17 ^a ± 28.31	191.08 ^b ± 12.69	241.42 ^a ± 12.69	229.83 ± 2.69	202.67 ± 12.69	8.974	0.03*	**	NS
Sitting	175.83 ± 15.17	208.50 ± 12.81	193.50 ± 4.59	206.33 ± 19.75	192.16 ± 13.18	199.92 ± 13.18	184.67 ± 3.18	207.42 ± 3.18	9.318	NS	NS	NS
Walking	31.50 ± 5.90	49.00 ± 9.22	44.42 ± 5.82	38.67 ± 4.74	40.25 ± 4.69	41.54 ± 4.69	37.96 ± 4.69	43.83 ± 4.69	3.319	NS	NS	NS
Investigation	149.83 ± 24.00	158.67 ± 19.76	117.83 ± 4.64	129.17 ± 7.76	154.25 ± 1.45	123.50 ± 1.45	133.83 ± 1.45	143.92 ± 1.45	8.096	NS	NS	NS
Sleeping	288.33 ^a ± 4.72	207.67 ^b ± 5.83	200.92 ^b ± 7.36	203.50 ^b ± 11.00	248.00 ± 5.38	202.21 ± 5.38	244.63 ^a ± 5.38	205.58 ^b ± 5.38	3.803	**	**	**
Total State	857.50 ± 9.11	794.00 ± 31.47	804.33 ± 1.07	812.83 ± 2.403	825.75 ± 1.489	808.58 ± 14.89	830.97 ± 1.489	803.42 ± 1.489	10.527	NS	NS	NS
Drinking	117.17 ^a ± 5.62	104.00 ^{ab} ± 1.046	89.33 ^{bc} ± 3.82	72.00 ^c ± 1.37	110.58 ± 4.44	80.67 ± 4.44	103.25 ^a ± 4.44	88.00 ^b ± 4.44	3.137	**	NS	**
Feeding	345.00 ± 17.22	419.17 ± 48.85	400.67 ± 2.358	406.67 ± 2.863	382.08 ± 2.252	403.67 ± 22.52	372.83 ± 2.252	412.92 ± 2.252	15.927	NS	NS	NS
Preening	109.67 ± 12.93	95.00 ± 10.20	121.00 ± 1.225	130.17 ± 1.098	102.33 ± 8.23	125.58 ± 8.23	115.33 ± 8.23	112.58 ± 8.23	5.821	NS	NS	NS
Feather pecking	9.33 ^b ± 0.49	19.50 ^a ± 3.51	18.83 ^a ± 2.32	15.00 ^{ab} ± 2.02	14.42 ± 1.66	16.92 ± 1.66	14.08 ± 1.66	17.25 ± 1.66	1.172	0.02*	NS	NS
Wing Flapping	1.33 ^b ± 0.89	4.67 ^{ab} ± 1.61	5.83 ^a ± 1.05	3.33 ^{ab} ± 0.56	3.00 ± 0.77	4.58 ± 0.77	3.58 ± 0.77	4.00 ± 0.77	0.545	0.05*	NS	NS
Total events	582.50 ± 9.11	646.00 ± 31.47	635.67 ± 1.07	627.17 ± 2.403	614.25 ± 1.489	631.42 ± 14.89	609.08 ± 1.489	636.58 ± 1.489	0.085	NS	NS	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.24. Interaction effect of Stocking Density(SD) and Flock Size (FS) on state and event behaviour of bird at 37th week of age

Parameters	Interaction Effect Of Stocking Density & Flock Size				Effect of Stocking Density(SD)		Effect of Flock Size (FS)		Pooled SEM	Statistical Significance		
	548cm ² /b X 6B	548cm ² /b X 9B	645cm ² /b X 6B	645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Treatment	SD	FS
Standing	212.67±21.78	171.67±7.08	207.67±12.99	224.17±26.17	192.17± 13.13	219.13±13.13	210.17±13.13	201.13±13.13	9.61	NS	NS	NS
Sitting	206.50 ^b ± 14.65	256.17 ^a ± 13.69	212.67 ^b ± 5.07	197.50 ^b ± 9.55	231.13 ^a ± 8.05	204.13 ^b ± 8.05	209.58±8.05	225.88±8.05	5.967	0.008**	0.03**	NS
Walking	30.33±1.31	31.50±4.30	45.33±6.16	41.17±5.68	30.92 ^b ± 3.36	42.47 ^a ± 3.36	37.83±3.36	35.55±3.36	2.465	NS	0.00**	NS
Investigation	86.50 ^b ± 6.90	91.17 ^b ± 15.00	124.17 ^b ± 12.71	176.67 ^a ± 12.78	88.83 ^b ± 8.64	148.68 ^a ± 8.64	105.33 ^b ± 8.64	132.18 ^a ± 8.64	6.36	**	0.00**	0.05*
Sleeping	327.83 ^a ± 6.73	273.00 ^b ± 10.07	232.33 ^c ± 6.70	174.17 ^d ± 5.18	300.42±5.22	203.87±5.22	280.03 ^a ±5.22	224.20 ^b ±5.22	3.87	**	NS	**
Total State	863.83±13.33	823.50±10.81	822.17±18.76	813.67±28.77	843.67±13.57	818.28±13.57	843.00±13.57	818.95±13.57	10.089	NS	NS	NS
Drinking	78.67±7.17	102.17±14.52	73.67±9.02	82.33±6.10	90.42±6.90	77.13±6.90	76.17±6.90	91.38±6.90	5.108	NS	NS	NS
Feeding	371.67±11.11	369.50±25.69	418.17±30.22	398.50±39.74	370.58±20.24	409.68±20.24	394.92±20.24	385.39±20.24	15.026	NS	NS	NS
Preening	109.50 ^{ab} ± 7.85	133.50 ^a ± 12.91	102.50 ^b ± 2.40	131.33 ^a ± 9.42	121.50±6.35	116.15±6.35	106.00 ^b ±6.35	131.65 ^a ±6.35	4.704	0.05*	NS	**
Feather pecking	11.33 ^b ±0.21	9.33 ^b ±0.49	20.00 ^a ±2.08	12.50 ^b ±0.34	10.33 ^b ± 0.77	16.30 ^a ± 0.77	15.67 ^a ±0.77	10.97 ^b ±0.77	0.572	**	**	**
Wing Flapping	3.17±0.87	2.00±0.58	3.50±0.56	1.67±0.61	2.58±0.47	2.45±0.47	3.33 ^a ±0.47	1.7 ^b ±0.47	0.344	NS	NS	*
Total events	576.17±13.33	616.50±10.81	617.83±18.76	626.33±28.77	596.33±13.57	621.72±13.57	597.00±13.57	621.05±13.57	10.089	NS	NS	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.25. Interaction effect of Stocking Density(SD) and Flock Size (FS) on state and event behaviour of bird at 47th week of age

Parameters	Interaction Effect Of Stocking Density & Flock Size				Effect of Stocking Density (SD)		Effect of Flock Size (FS)		SEM	Statistical significance		
	548cm ² /b X 6B	548cm ² /b X 9B	645cm ² /b X 6B	645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Tr.	SD	FS
Standing	227.83±13.79	185.17±9.15	240.67±12.81	236.17±23.94	206.50±11.24	238.42±11.24	234.25±11.24	210.67±11.24	7.951	NS	NS	NS
Sitting	179.17±1.80	216.67±11.12	187.33±12.16	187.17±14.25	197.92±7.73	187.25±7.73	183.25±7.73	201.92±7.73	5.905	NS	NS	NS
Walking	37.17±3.26	45.17±2.85	50.33±4.84	46.67±3.53	41.17±2.61	48.5±2.61	43.75±2.61	45.92±2.61	1.847	NS	NS	NS
Investigation	117.17±14.19	126.83±12.87	124.50±7.33	151.17±6.92	122.00±7.65	137.83±7.65	120.83±7.65	139.00±7.65	5.412	NS	NS	NS
Sleeping	301.67 ^a ± 5.78	235.33 ^b ± 6.06	216.33 ^c ± 5.35	197.83 ^d ± 7.39	268.50 ^a ±4.38	207.08 ^b ±4.38	259.00 ^a ±4.38	216.58 ^b ±4.38	3.097	**	**	**
Total State	863.00±10.68	809.17±17.63	819.00±17.46	819.00±22.37	836.08±12.40	819.00±12.40	841.00±12.40	814.08±12.40	8.799	NS	NS	NS
Drinking	103.17 ^a ± 1.22	108.83 ^a ± 5.76	86.50 ^b ± 5.43	87.17 ^b ± 4.17	106.00±3.19	86.83±3.19	94.83±3.19	98.00±3.19	2.258	**	NS	NS
Feeding	348.17±12.13	385.17±11.94	395.17±28.09	383.33±30.79	366.67±15.92	389.25±15.92	371.67±15.92	384.25±3.19	11.256	NS	NS	NS
Preening	106.83±7.63	111.50±2.46	122.50±6.40	128.00±9.87	109.17±5.03	125.25±5.03	114.67± 5.03	119.75±5.03	3.559	NS	NS	NS
Feather pecking	13.83 ^{ab} ±0.31	18.67 ^a ±1.73	10.50 ^b ±3.31	15.83 ^{ab} ±1.30	16.25 ^a ± 1.40	13.17 ^b ± 1.40	12.17 ^b ± 1.40	17.25 ^a ± 1.40	0.992	*	*	*
Wing Flapping	5.00±0.63	7.33±0.61	6.17±0.95	6.50±0.56	6.17±0.50	6.33±0.50	5.58±0.50	6.92±0.50	0.353	NS	NS	NS
Total events	577.00±10.68	631.50±17.55	620.83±17.48	620.83±22.27	604.25±12.37	620.83±12.37	598.92±12.37	626.17±12.37	8.745	NS	NS	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.26. Interaction effect of Stocking Density(SD) and Flock Size (FS) on state and event behaviours of bird for 28, 37 and 47 week (average) of age

Parameters	Interaction Effect of Stocking density and Flock Size				Effect of Stocking Density (SD)		Effect of Flock Size (FS)		SEM	Statistical Significance		
	548cm ² /b X 6B	548cm ² /b X 9B	645cm ² /b X 6B	645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Tr.	SD	FS
Standing	217.50±14.49	175.67±9.11	232.00±13.24	231.83±25.90	196.58 ^b ±11.93	231.92 ^a ±11.93	224.75±11.93	203.75±11.93	8.436	NS	*	NS
Sitting	187.17±0.48	227.50±12.17	197.83±13.47	197.00±14.20	207.33±8.15	197.42±8.15	192.50±8.15	212.25±8.15	5.764	NS	NS	NS
Walking	33.00±3.44	42.00±3.38	46.67±5.26	42.17±4.59	37.50±3.00	44.42±3.00	39.83±3.00	42.08±3.00	2.121	NS	NS	NS
Investigation	117.83±14.75	125.67±11.62	122.17±7.55	152.33±8.11	121.75±7.71	137.25±7.71	120.00±7.71	139.00±7.71	5.450	NS	NS	NS
Sleeping	305.83 ^a ± 5.31	238.67 ^b ± 3.28	216.50 ^c ± 4.24	191.83 ^d ± 5.97	272.25 ^a ± 3.40	204.17 ^b ± 3.40	261.17 ^a ± 3.40	215.20 ^b ± 3.40	2.406	**	**	**
Total State	861.33±8.17	809.50±17.54	815.17±15.23	815.17±24.41	835.42±12.29	815.17±12.26	838.25±12.26	812.33±12.26	8.668	NS	NS	NS
Drinking	99.67 ^a ±0.71	105.00 ^a ±7.43	83.17 ^b ±5.72	80.50 ^b ±3.52	102.22 ^a ±3.55	81.83 ^b ±3.55	91.42±3.55	92.75±3.55	2.510	**	**	NS
Feeding	354.67±13.11	391.33±11.91	404.67±27.11	396.17±32.55	373.00±16.24	400.42±16.24	379.67±16.24	393.75±16.24	11.480	NS	NS	NS
Preening	108.67±8.00	113.33±2.49	115.33±5.78	129.83±10.04	111.00±5.06	122.58±5.06	112.00±5.06	121.58±5.06	3.574	NS	NS	NS
Feather pecking	11.50 ^b ± 0.22	15.83 ^a ± 1.72	16.50 ^a ± 1.09	14.50 ^{ab} ± 1.06	13.67±0.82	15.50±0.82	14.00±0.82	15.17±0.82	0.576	**	NS	NS
Wing Flapping	3.17±0.65	4.67±0.61	5.17±0.48	3.83±0.48	3.92±0.40	4.50±0.40	4.17±0.40	4.25±0.40	0.281	NS	NS	NS
Total events	578.67±8.17	630.50±17.54	624.83±15.23	624.83±24.41	604.58±12.26	624.84±12.26	601.75±12.26	627.67±12.26	8.668	NS	NS	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

4.2.7 Cost of Production Per Egg (CPPE) on the basis of recurring expenses and space utilization:

The interaction effect of stocking density and flock size on the interrelation between cost of production per egg and space utilization is presented in Table 4.27. The CPPE was estimated on the basis of egg production recurring expenses mainly i.e feed, pullet, medicine, vaccine and miscellaneous incurred during this experimental period (20 weeks). There was almost no cost involved in increasing the cage size for keeping the flock of six and nine birds as only the partition between the cages was fabricated so that the birds can accommodate in the required flock size with the stocking density of 548cm²/b or 85 sq. inch/b and 645cm²/b or 100 sq. inch/b. CPPE in relation to space utilization was calculated considering CPPE x Space allowance x number of birds.

The cost of production per egg estimated considering the recurring expenses for the interaction effect as well main effect of stocking density and flock size was non-significant between the various treatments. The main effect of stocking density and flock size could not bring any significant effect, probably because there was no significant difference in the egg production performance for the entire 20 week period and the different recurring expenses for the two different flocks was masked by the egg production in each flock size. However, the CPPE on basis of space utilization, which was mainly dependent on the space and number of birds were statistically different among treatment groups. As expected the CPPE on basis of space utilization was significantly ($P < 0.01$) different among all the groups with the lowest value in the small flock size of 6 birds with high stocking density (548cm²/b x 6B) and highest in flock size with 9 birds and lower stocking density (645cm²/b x 9B), for the interaction effect and main effect of flock size and stocking density.

Koelkebeck and Cain (1984) reported that when birds were maintained in flock size 5B they gave most economical (lowest cost/dozen eggs) among the various group where the birds were maintained small cages in single, double, three and in large cages four, five and six birds. Although egg production was

lower for cage with five or six hens flock size than for those with one or two hens. The cost of housing (cage) contributed significantly to the production costs making lower flock size, less competitive.

Table 4.27. Interaction effect of stocking density and flock size on Cost of Production Per Egg (CPPE) and inter-relation between CPPE and Space Utilization (SU)

TREATMENT	CPPE on the basis of recurring expenses (₹)	CPPE on the basis of space utilization (CPPE on basis of recurring expenses X space allowance X number of birds)
548cm ² /b X 6B	2.44 ± 0.02	1246.35 ^d ± 8.53
548cm ² /b X 9B	2.47 ± 0.04	1885.86 ^b ± 28.58
645cm ² /b X 6B	2.43 ± 0.03	1459.17 ^c ± 20.28
645cm ² /b X 9B	2.50 ± 0.03	2243.93 ^a ± 26.70
Effect of stocking density		
548cm ² /bird	2.45 ± 0.02	1566.11 ^b ± 17.00
645cm ² /bird	2.46 ± 0.02	1851.55 ^a ± 17.00
Effect of flock size		
6 Bird	2.44 ± 0.02	1352.76 ^b ± 17.00
9 Bird	2.48 ± 0.02	2064.90 ^a ± 17.00
Pooled SEM	0.015	81.02
Statistical significance		
Treatment	NS	**
Stocking density	NS	**
Flock size	NS	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

4.3 Experiment III : Interrelationship between best stocking densities (548 and 645cm²/b) and flock Size (6 and 9 birds) of conventional cage and modified/ furnished cage rearing of commercial laying hens on production, welfare and behaviour.

The III Experiment was conducted with the aim of rearing the commercial White Leghorn (BV 300) layers in modified furnished cages at the best of stocking density 85 sq. inch/548 cm² and 100 sq. inch/645 cm² per bird and flock size with 6 and 9 birds concentration in concern to production, welfare and behaviour of the birds. The furnished cages (FC) are provided with perches, nesting area and scratch pad to meet the natural behaviour of the birds. This experiment was carried out with an specific objective to test the comparative performance of laying hens reared in conventional California (II Exp) cages Vs furnished California (III Exp) cages with regards to production, welfare and behaviour of the birds. A total of 180 birds were reared for a 20 week period in the furnished cages with earlier mentioned stocking densities.

There were four different treatments FC-548cm²/b x 6 birds, FC-548cm²/b x 9 birds, FC-645cm²/b x 6 birds and FC-645cm²/b x 9 birds to see the effect of different stocking density and flock size in furnished cages. The performance of the laying hens reared in furnished cages were recorded and compared with findings of birds reared in conventional California cages (CC) at 548 and 645 cm²/b with flock size 6 birds. On the basis of CC trial (II Exp) and all furnished cage results (III Exp) the data generated from FC with flock size 9 birds (III Exp) were not used for further comparison with CC. The below mentioned results are of various treatment groups of FC of Experiment III and are comparison in between CC x 6B (II Exp) and FC x 6B (III Exp).

4.3.1 Hen Day Egg Production (HDEP%):

The data concerning to HDEP % during the entire experimental (20-week period) from all the treatment groups of furnished cages (FC) are presented in Table 4.28 and its comparison with CC is presented in Table 4.29. The total twenty week trial were comprised of 28 to 31st, 32 to 35th, 36 to 39th, 40 to 43rd and 44 to 47th week periods.

The interaction effect of stocking density and flock size in furnished cages showed highly significant differences among themselves. FC-548cm²/b x 9B group had a higher flock size and higher stocking density produced significantly less eggs 78.36 ± 4.11 than the rest of the groups. Next to that group with little higher HDEP % was FC-645cm²/b x 9B, 84.50 ± 2.12 which had lesser stocking density but higher flock size. A decreasing gradient in HDEP % of the order FC-645cm²/b x 9B, FC-645cm²/b x 6B and FC-548cm²/b x 6B for the interaction effect of stocking density and flock size was observed (Table 4.28) but the treatment groups were non-significant among themselves. The main effect of stocking density on HDEP % in furnished cages was non-significant for all the periods while the main effect due to flock size was significant ($P < 0.05$) for 32 - 35th week and highly significant for 36- 39th, 40 - 43rd, 44 - 47th and entire 28 – 47th week of age. The initial four weeks 28 – 31st week the main effect due to flock size for HDEP % was non-significant the rest periods were significantly better for 6 birds. The entire period (20 weeks) the main effects due to flock size

showed that the smaller flock size (6 birds) had a significantly better HDEP % as compared to the larger flock (9birds), Table 4.28.

Table 4.28. Interaction effect of stocking density and flock size in furnished cages on hen day egg production %

Treatment	Experimental duration/birds age					
	28- 31 wk	32-35 wk	36-39 wk	40-43wk	44-47 wk	28-47 wk
FC-548cm ² /b X 6B	84.64 ± 2.85	90.04 ^{a±} 2.02	90.91 ^{a±} 1.38	91.72 ^{a±} 0.35	90.13 ^{a±} 1.17	89.29 ^{a±} 1.11
FC-548cm ² /b X 9B	77.31 ± 4.78	77.38 ^{b±} 5.55	77.98 ^{b±} 3.84	81.15 ^{b±} 4.05	78.50 ^{b±} 3.26	78.36 ^{b±} 4.11
FC-645cm ² /b X 6B	86.73 ± 2.11	89.98 ^{a±} 1.35	86.51 ^{ab±} 3.03	93.45 ^{a±} 1.37	89.48 ^{a±} 2.31	89.15 ^{a±} 1.47
FC-645cm ² /b X 9B	82.34 ± 2.70	85.32 ^{ab±} 2.97	83.26 ^{ab±} 2.73	88.76 ^{a±} 2.23	83.17 ^{ab±} 2.41	84.50 ^{ab±} 2.12
Effect of stocking density						
548cm ² /b	80.97 ± 2.31	83.71 ± 2.39	84.44 ± 2.04	86.43 ± 1.71	84.32 ± 1.70	83.83 ± 1.76
645cm ² /b	84.53 ± 2.31	87.65 ± 2.39	84.88 ± 2.04	91.11 ± 1.71	86.33 ± 1.70	86.82 ± 1.76
Effect of flock size						
6 Birds	85.68 ± 2.31	90.01 ^{a±} 2.39	88.71 ^{a±} 2.04	92.58 ^{a±} 1.71	89.81 ^{a±} 1.70	89.22 ^{a±} 1.76
9 Birds	79.82 ± 2.31	81.35 ^{b±} 2.39	80.62 ^{b±} 2.04	84.96 ^{b±} 1.71	80.84 ^{b±} 1.70	81.43 ^{b±} 1.76
Pooled SEM	1.634	1.687	1.444	1.208	1.203	1.245
Statistical significance						
Treatment	NS	*	*	**	**	*
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	NS	*	**	**	**	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.29. Comparison of hen day egg production % in furnished cages vs conventional California cages

Treatments	Experimental duration/birds age					
	28- 31 wk	32-35 wk	36-39 wk	40-43 wk	44-47 wk	28-47 wk
FC-548cm ² /b X 6B	84.64 ± 2.85	90.04 ± 2.02	90.91 ± 1.38	91.72 ± 0.35	90.13 ± 1.17	89.49±1.02
FC-645cm ² /b X 6B	86.73 ± 2.11	89.98 ± 1.35	86.51 ± 3.03	93.45 ± 1.37	89.48 ± 2.31	89.23±1.46
CC-548cm ² /b X 6B	91.89 ± 1.80	92.39 ± 1.30	92.49 ± 1.24	91.59 ± 1.78	93.22 ± 1.40	92.31±0.82
CC-645cm ² /b X 6B	89.11±1.46	93.28±1.71	93.77 ± 1.76	94.57 ± 1.73	92.19 ± 1.82	92.58±1.50
SEM	1.137	0.814	1.087	0.713	0.866	0.659
P-value	NS	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P<0.05, **P< 0.01.

Appleby *et al.*, (2002) reported egg production was not significantly affected by cage design and flock size for the different conventional and furnished cage. There were two type of Conventional cages one with 4 birds maintained at stocking density 625cm²/bird and another was 5 birds maintained at stocking density of 500cm²/bird and different designs of furnished cages. Furnished cages (type- F) had roll away nest and no dust baths and Furnished cages (type- FD) had both roll away nests and dust bath and Furnished cages (type-H) had two nest hollows, these three types had different combination of flock size. Furnished cages, type -F and FD had three different flock size -5 birds maintained at space allowance of 1000cm²/bird, 7 hens maintained at space allowance of 715cm²/bird and 8 hens maintained at space allowance of 625 cm²/bird. Furnished cages (type H) had flock size of 4 birds with space allowance of 1250cm²/bird, 5 hens with space allowance 1000cm²/bird and 7 hens with space allowance of 715cm²/bird. Rate of lay did not vary significantly between cage designs, ranging only from 82 to 84% over the period from 20 to 72 weeks. However, rate of lay was higher with fewer birds per cage (P<0.05) from a minimum 81% in F cages with 7 birds to a maximum of 84.9% in FD cages with 5 birds, which was similar to present experiment for the main effect of flock size with highly significant difference (P<0.01) between flock size of 6 (89.22 HDEP%) and 9 birds (81.43 HDEP%).

Non-significant difference in egg production between conventional cages of 5 birds at stocking density of 550cm² per hen and in enriched cages with 22 hens equipped with perch, nest box and litter areas as per EU directives was demonstrated by Karkulin (2006). A very little improvement in hen-day egg production was seen in enriched cages by 88.72% as compared to conventional cages of 87.57%, which is in contrast to present experiment where numerically higher number of eggs were seen in conventional cages. Wall and Tauson (2007) compared performance of hens in furnished cages having eight hens with conventional cages of four hens and production was recorded from 20th week to 80th week of age and found no significant difference. The furnished cages differed from each other by perch arrangements and perch space provided per bird into FC- A, B and C, the egg production recorded were 87.9% for conventional cages, 87.8% in FC-A, 88.3% in FC-B and 87.6% in FC-C with no statistical difference.

Tactacan *et al.*, (2009) reported non-significant effect on hen-day egg production between conventional battery cages and in enriched cages, but in period 58 to 61 week, the birds conventional cages had higher production than in enriched cages as seen in 28 to 47 week production for the present study.

Shimmura *et al.*, (2010) also reported non-significant effect of egg production in birds reared in conventional cages - Small (450cm²/hen stocking density) and large (600 cm²/hen stocking density); and Furnished cages –Small (604.5 cm²/hen) and large (658.3 cm²/hen of stocking density). Guo *et al.*, (2012) also reported non-significant difference in laying rate of birds housed in conventional (398cm²/hen) and in small furnished (586 cm²/hen) and large furnished (543 cm²/hen) cages. Windowski *et al.*, (2017) demonstrated that by providing two space allowance 520 cm² and 748cm² and 2 cage size – Smaller furnished cages and Larger Furnished cages could not bring any significant effect on hen day egg production.

Meng *et al.*, (2014) had reported a significant improvement in egg production which is contradictory to the present experimental result on egg production. There was a significant reduction in egg production in birds maintained in Large Furnished cages (stocking density of 750 cm²/bird with 40

birds as group size) as compared to Small Furnished cage (stocking density of 750 cm²/bird with 8 birds as group size) and Conventional cages (stocking density of 528 cm²/hen with 12 birds as group size) as possibly the large furnished cages provided the hens more space, allowing them more activity and higher utilization of energy to activities other than egg production.

Most of the reports are from European countries, hens in furnished cage systems have productivity equal to those in conventional cage systems this is consistent with our results. However many studies have also shown that the productivity in conventional cage systems is better than alternative systems such as free-range, outdoor-run, non-caged indoor, or furnished cage systems (Abrahamsson *et al.*, 1996). It can be concluded that furnishing the cages led did not bring any significant change in HDEP% compared to conventional California cages as the space provided was similar in this study, however the numerical higher egg production were recorded in CC reared birds. This could possibly due to the layer birds was reared in cage system since the chick stage to liquidation stage (0-80 week). Hence birds did not have any exposure of the perch, nesting and dust bath from their early stage of life and adopted for conventional cage system that could be the reason there was non significant difference in production performance in conventional and furnished cages.

4.3.2 Feed Intake and Feed Conversion Ratio:

A weighed quantity of feed 110 g was offered to all the treatment groups as per standard specification. At the end of each week the residual feed from the feeding trough of each cage box was collected quantitatively weighed and divided by the number of birds in that cage which was merely 0.5 gm/b. Therefore, the actual feed intake of birds was considered to be 110gm/bird for all the experimental birds.

The data pertaining to Feed Conversion ratio (per dozen egg and per kg egg mass) obtained from the interaction effect of stocking density and flock size in furnished cages (FC) is presented in Table 4.30 and 4.31 for the 20 week

experimental period. The total twenty week trial were comprised of 28 to 31, 32 to 35, 36 to 39, 40 to 43 and 44 to 47 week period.

The interaction effect of stocking density and flock size on FCR (per kg egg mass) for the entire 20-week period among the furnished cages showed significant difference ($P < 0.01$) with the poorest in FC-548cm²/b x 9B when compared to all other treatment groups (Table 4.31), though FCR per dozen eggs failed to elicit any significant difference in the overall 20 week period (Table 4.30). FC-548cm²/b x 6B, FC-645cm²/b x 6B and FC-645cm²/b x 9B were non-significant among themselves though the numerically best value was seen in FC-548cm²/b x 6B, followed by FC-645cm²/b x 6B and FC-645cm²/b x 9B (per kg egg mass basis).

The trend for difference because of interaction effect between FC-548cm²/b x 9B and rest of the furnished cages was significantly noticeable in 40–43 and 44 - 47 weeks and entire 20 weeks for FCR per kg egg mass; and in 32 – 35 and 44 – 47th weeks for FCR per dozen eggs. The main effects for stocking density on FCR per dozen egg was non-significant for all the four week periods and entire 20 weeks but FCR per kg egg mass was shown to have significant differences in 40 – 43rd and 44 – 47th week with the better FCR in 548cm²/b. The main effect of flock size on FCR per dozen eggs and per kg egg mass shown significant differences except for the 28 - 31 week and 40 - 43 weeks with the best FCR in flock size of six birds.

A comparison of the FCR per dozen eggs and per kg egg mass for birds raised in furnished and conventional cages is presented in Table 4.32 & 4.33. FCR when compared between conventional and furnished cages at same stocking density were non-significant for the entire 20 week period. FCR per dozen eggs at the first four weeks 28-31, produced some significant ($P < 0.05$) change with CC-548cm²/b x 6B being the best, but it couldn't be carried to the later weeks and in the overall 20 weeks, no significant changes could be produced.

Table 4.30. Interaction effect of stocking density and flock size in furnished cages on feed conversion ratio (per dozen eggs)

Treatment	Experimental duration/ birds age					
	28- 31 wk	32-35 wk	36-39 wk	40-43wk	44-47 wk	28-47 wk
FC-548cm ² /b X 6B	1.48±0.03	1.47 ^b ±0.03	1.49±0.04	1.48±0.03	1.47 ^b ± 0.03	1.49±0.04
FC-548cm ² /b X 9B	1.61±0.09	1.75 ^a ±0.13	1.72±0.09	1.61±0.09	1.75 ^a ± 0.13	1.72±0.09
FC-645cm ² /b X 6B	1.46±0.03	1.47 ^b ±0.02	1.54±0.06	1.46±0.03	1.47 ^b ± 0.02	1.54±0.06
FC-645cm ² /b X 9B	1.52 ± 0.03	1.56 ^{ab} ±0.06	1.60±0.06	1.52±0.03	1.56 ^{ab} ± 0.05	1.60±0.06
Effect of stocking density						
548cm ² /b	1.55 ± 0.04	1.61 ± 0.05	1.60 ± 0.04	1.55 ± 0.04	1.61 ± 0.05	1.60 ± 0.04
645cm ² /b	1.49 ± 0.04	1.51 ± 0.05	1.57 ± 0.04	1.49 ± 0.04	1.51 ± 0.05	1.57 ± 0.04
Effect of flock size						
6 Birds	1.47 ± 0.04	1.47 ^b ± 0.05	1.51 ^b ± 0.04	1.47 ± 0.04	1.47 ^b ± 0.05	1.51 ^b ± 0.04
9 Birds	1.57 ± 0.04	1.66 ^a ± 0.05	1.66 ^a ± 0.04	1.57 ± 0.04	1.66 ^a ± 0.05	1.66 ^a ± 0.04
Pooled SEM	0.026	0.037	0.031	0.026	0.037	0.031
Statistical significance						
Treatment	NS	*	NS	NS	*	NS
Stocking density	NS	NS	NS	NS	NS	NS
Flock size	NS	*	*	NS	*	*

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05.

Table 4.31. Interaction effect of stocking density and flock size in furnished cages on feed conversion ratio (per kg egg mass)

Treatment	Experimental Birds age/period					
	28- 31 wk	32-35 wk	36-39 wk	40-43wk	44-47 wk	28-47 wk
FC-548cm ² /b X 6B	2.30±0.04	2.23±0.04	2.21±0.06	2.13 ^b ± 0.01	2.13 ^b ± 0.01	2.21 ^b ±0.02
FC-548cm ² /b X 9B	2.55±0.14	2.64±0.20	2.54±0.13	2.47 ^a ± 0.11	2.47 ^a ± 0.11	2.55 ^a ±0.13
FC-645cm ² /b X 6B	2.32±0.04	2.30±0.03	2.29±0.09	2.10 ^b ± 0.04	2.10 ^b ± 0.04	2.24 ^b ±0.04
FC-645cm ² /b X 9B	2.40±0.06	2.34±0.09	2.38±0.08	2.20 ^b ± 0.06	2.20 ^b ± 0.06	2.34 ^b ±0.06
Effect of stocking density						
548cm ² /b	2.42 ± 0.06	2.44 ± 0.08	2.37 ± 0.07	2.30 ^a ± 0.05	2.30 ^a ± 0.05	2.38 ± 0.05
645cm ² /b	2.36 ± 0.06	2.32 ± 0.08	2.34 ± 0.07	2.15 ^b ± 0.05	2.15 ^b ± 0.05	2.29 ± 0.05
Effect of flock size						
6 Birds	2.31 ^b ± 0.06	2.26 ± 0.08	2.25 ^b ± 0.07	2.11 ^b ± 0.05	2.11 ^b ± 0.05	2.22 ^b ± 0.05
9 Birds	2.48 ^a ± 0.06	2.49 ± 0.08	2.46 ^a ± 0.07	2.33 ^a ± 0.05	2.33 ^a ± 0.05	2.44 ^a ± 0.05
Pooled SEM	0.041	0.057	0.047	0.033	0.033	0.036
Statistical significance						
Treatment	NS	NS	NS	**	**	**
Stocking density	NS	NS	NS	*	*	NS
Flock size	*	NS	*	**	**	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.32. Comparison of feed conversion ratio (per dozen eggs) in furnished cages vs conventional California cages

Treatment	Experimental Birds age/period					
	28- 31 wk	32-35 wk	36-39 wk	40-43wk	44-47 wk	28-47 wk
FC-548cm ² /b X 6B	1.48 ^a ± 0.03	1.47 ± 0.03	1.49±0.04	1.48 ± 0.03	1.47 ± 0.03	1.49 ± 0.04
FC-645cm ² /b X 6B	1.46 ^{ab} ± 0.03	1.47 ± 0.02	1.54±0.06	1.46 ± 0.03	1.47 ± 0.02	1.54 ± 0.06
CC-548cm ² /b X 6B	1.38 ^c ± 0.01	1.43 ± 0.02	1.43±0.02	1.45 ± 0.03	1.42 ± 0.02	1.42 ± 0.01
CC-645cm ² /b X 6B	1.41 ^{bc} ± 0.01	1.42 ± 0.03	1.41±0.03	1.40 ± 0.03	1.43 ± 0.03	1.42 ± 0.02
SEM	0.013	0.013	0.021	0.014	0.013	0.020
P-value	*	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.33. Comparison of feed conversion ratio (per kg egg mass) in furnished cages vs conventional California cages

Treatment	Experimental Birds age/period					
	28- 31wk	28- 31wk	28- 31wk	28- 31wk	28- 31wk	28- 31wk
FC-548cm ² /b X 6B	2.30 ± 0.04	2.23 ± 0.04	2.21 ± 0.06	2.13 ± 0.01	2.13 ± 0.01	2.21 ± 0.02
FC-645cm ² /b X 6B	2.32 ± 0.04	2.30 ± 0.03	2.29 ± 0.09	2.10 ± 0.04	2.10 ± 0.04	2.24 ± 0.04
CC-548cm ² /b X 6B	2.33 ± 0.03	2.28 ± 0.03	2.28 ± 0.03	2.19 ± 0.05	2.17 ± 0.04	2.25 ± 0.02
CC-645cm ² /b X 6B	2.27 ± 0.04	2.24 ± 0.05	2.18 ± 0.04	2.12 ± 0.04	2.18 ± 0.04	2.20 ± 0.03
SEM	0.018	0.020	0.030	0.018	0.018	0.014
P-value	NS	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Abrahamsson and Tauson (1997) observed the effect on feed consumption in laying birds by keeping them in furnished cages along with conventional cages from 20th week until 80th week. Average feed consumption per hen day was 121.6g in the furnished and 121.9 g in the conventional cage. Feed conversion ratio (per kg eggs mass) was 2.27 and 2.23 in the furnished and in the conventional cage, respectively which were non-significant similar to present experiment possibly because of non-significant effect with egg production and egg weight in the two system of housing. Appleby *et al.*, (2002) observed no significant difference in feed consumption when kept various group size birds of 4 to 8 birds in conventional and furnished cages, though there was a trend for higher feed consumption in groups with fewer birds. More eggs per bird were collected when there were fewer birds per cage but food consumption also then tended to be higher, therefore the reason for non-significant effect on food conversion efficiency.

Non-significant results were observed for feed consumption and feed efficiency by Karkulin (2006) for hens maintained at enriched and conventional cages. Also, non-significant effect in feed efficiency between furnished and battery cages was reported by Pohle and Chang (2009). Though, Tactacan *et al.*, (2009) observed in certain ages 21-24 week and 33-36 week, feed consumption

was significantly more in conventional cages than in enriched cages and less in 49-52 week and 52-56 week.

In contrast with the current study for significant differences in feed efficiency was noticed by Guo *et al.*,(2012) and Meng *et al.*,(2014) for hens housed in large furnished cages and small furnished cages had a poorer feed efficiency ($P < 0.01$) than that of conventional battery cages. Feed conversion can be influenced by the housing system and in alternative housing systems, hens have to use some of their energy for heat production and movement, because of lower stocking densities and sometimes lower temperatures in these systems. This leads to higher feed consumption and unfavorable feed conversion. In the present investigation due to similar stocking density there could be non-significant difference in feed conversion ratio in the laying hens reared at FC and CC housing systems. Another point to ponder is that it might have been expected that feed conversion has been unfavorable in groups of 40 and 60 hens because of the larger floor space available for movement, this was not the case in the present experiment.

4.3.3 Live Body Weight (LBW) and Body Weight Gain (BWG):

The result of the effect of modified/furnished cages on the live body weight changes in the layers are presented in Table 4.34. The results revealed that body weight change in layers did not differ significantly among the various groups of furnished cages because of interaction effect of stocking density and flock size and main effect of stocking density; when maintained in different flock size of six and nine birds and at stocking density of 548 sq.cm and 645 sq.cm per bird. Though the main effect due to flock size brought about a significant change ($P < 0.01$) in weight gain with higher weight gain in the bigger flock size of nine birds as compared to six birds was noticed.

Comparison of live body weight and weight gain for laying birds maintained in modified furnished cages and conventional California cages is present in Table 4.35. On comparison of body weight of birds in furnished cages

Table 4.34. Interaction effect of stocking density and flock size in furnished cages on Live Body Weight (LBW) and Body Weight Gain (BWG)

Treatment	Initial (LBW) (28 th week of birds age)	Final LBW (47 th week of birds age)	BWG
FC-548cm ² /b X 6B	1309.00 ± 19.96	1402.00 ± 19.36	93.00 ± 18.48
FC-548cm ² /b X 9B	1236.50 ± 33.14	1399.00 ± 22.86	162.50 ± 30.83
FC-645cm ² /b X 6B	1333.83 ± 31.09	1410.17 ± 27.77	76.33 ± 18.57
FC-645cm ² /b X 9B	1334.17 ± 45.32	1447.17 ± 32.14	113.00 ± 26.09
Effect of stocking density			
548cm ² /b	1272.75 ± 23.76	1400.50 ± 18.38	127.75 ± 17.02
645cm ² /b	1334.00 ± 23.76	1428.67 ± 18.38	94.67 ± 17.02
Effect of flock size			
6 Birds	1321.42 ± 23.76	1406.08 ± 18.38	84.67 ^b ± 17.02
9 Birds	1285.33 ± 23.76	1423.08 ± 18.38	137.75 ^a ± 17.02
Pooled SEM	16.81	12.99	12.04
Statistical significance			
Treatment	NS	NS	NS
Stocking density	NS	NS	NS
Flock size	NS	NS	*

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05.

Table 4.35. Comparison of Live Body Weight(LBW) and Body Weight Gain (BWG) in furnished cages vs conventional California cages

Treatment	Initial live Body weight (28 th week of birds age)	Final live Body weight (47 th week of birds age)	BWG
FC-548cm ² /b X 6B	1309.00±19.96	1402.00 ± 19.36	93.00 ± 18.48
FC-645cm ² /b X 6B	1333.83±31.09	1410.17 ± 27.77	76.33 ± 18.57
CC-548cm ² /b X 6B	1246.33±34.50	1378.50 ± 30.51	132.17 ± 38.93
CC-645cm ² /b X 6B	1248.50±28.05	1408.67 ± 37.60	160.17 ± 25.80
SEM	15.639	14.026	14.239
P-value	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant.

with that of conventional cages maintained in same stocking density (548 cm²/hen and 645 cm²/hen) no significant difference was noticed.

Non-significant difference in body weight of birds maintained in conventional Vs furnished cages, and non-significant effect of flock size (5 to 8) maintained in furnished cages was reported by Abrahamsson and Tauson (1997), similar to the present experiment partly on comparison of furnished and conventional cage, but in the present experiment flock size resulted in a significant change. Heckert *et al.*,(2002) also reported a non-significant difference in body weight for birds reared in furnished cages with and without perches maintained at different stocking density (10, 15, and 20 birds/m²).

Significant body weight differences in birds provided with perches (1.82kg) as compared to non-perch birds (1.89kg) in conventional cages was reported by Tauson (1998) which was thought to be because more energy was directed for perching rather than production. Similarly, Hester *et al.*,(2013) observed differences in body weight when cages were furnished with perches at different stages of growing and laying as compared to control with no perches. Further they observed that chickens with access to perches during the pullet phase had heavier body weight, consumed more feed, and had poorer feed efficiency during egg laying than control chickens without access of perches as pullets.

In the present study non-significant differences in body weight for conventional cages and furnished cages may be because of non-significant difference in feed consumption and the entire energy being directed towards egg production and egg mass. Also, a moderate group of 6 to 9 birds did not influence the body weight due to lesser competition for resources. The difference in body weight gain due to main effect of flock size was better in 9 birds was unknown but could result of lower HDEP%, poorer FCR and lower egg weight in flock size of 9 bird as compared to 6 birds. The energy may get diverted towards weight gain instead of egg production in 9 birds flock group could be possible explanation.

4.3.4 External and Internal Egg Quality Parameters:

The results of the interaction effect of stocking density and flock size in furnished cages on external egg quality traits like egg weight, shape index, percentage of dirty and broken eggs, and internal egg quality traits like albumen index, yolk index, Haugh unit, shell weight, yolk colour, shell thickness and on the percentage of nested eggs for layer hens maintained in different stocking density for the experimental period of 20 week. At each fifteen days of the experimental period twelve eggs per treatment groups, hence total 480 eggs were used to assess the egg quality of the experimental birds, the average detail of which is presented in Table 4.36.

The interaction effect of stocking density and flock size for the various treatments in furnished cages showed significant difference for egg weight only, the rest of the egg quality parameters along with percentage of nested eggs were non-significant for the various treatments. Egg weight was heaviest for FC-548cm²/b x 6B group for the interaction effect of stocking density and flock size with significant difference (P=0.05) with FC-645cm²/b x 9B group, while non-significant main effect with FC-548cm²/b x 9B and FC-645cm²/b x 6B, though numerically it was higher. There is evidence for main effect of flock size on the egg weight with significant difference (P < 0.05) and best for flock size of six bird as compared to 9 bird. Same trend followed for albumen index also, with significant difference (P < 0.05) between six and nine birds. There was no main effect of stocking density on the various egg quality parameters. The number of eggs that were laid on nests neither showed any significant difference due to the main effects of stocking density and flock size nor due to interaction effect. The percentage nested eggs varied from 32 to 39 % and was lesser than the eggs rolling out from other sides of the cages. The occupancy of the nested area by the social dominant birds could have been one of the factor and needs to be explored.

Table 4.36. Interaction effect of stocking density and flock size in furnished cages on external and internal egg quality and percentage of nested eggs

Treatment	Egg Weight	Shape Index	Albumen Index	Yolk Index	Shell Thickness	Haugh unit	Shell weight	Yolk score	Dirty eggs	Broken eggs	% of Nested Eggs
FC-548cm ² /b X 6B	55.00 ^a ±0.54	77.13±0.38	0.138±0.00	0.406±0.00	0.352±0.00	94.66±0.32	5.41±0.06	5.38±0.07	0.73±0.52	0.13±0.07	36.37±3.22
FC-548cm ² /b X 9B	53.72 ^{ab} ±0.18	76.88±0.40	0.134±0.00	0.405±0.00	0.349±0.01	94.75±1.53	5.50±0.10	5.50±0.07	0.55±0.26	0.18±0.11	39.26±5.32
FC-645cm ² /b X 6B	53.91 ^{ab} ±0.36	77.70±0.24	0.137±0.00	0.412±0.00	0.339±0.00	95.34±0.34	5.51±0.06	5.35±0.08	0.44±0.26	0.01±0.00	31.82±2.96
FC-645cm ² /b X 9B	53.08 ^b ±0.59	77.41±0.16	0.128±0.00	0.406±0.00	0.358±0.00	92.90±1.41	5.40±0.042	5.38±0.04	1.22±0.54	0.13±0.05	37.47±4.77
Effect of stocking density											
548cm ² /b	54.36 ± 0.32	77.00±0.22	0.136±0.00	0.406±0.00	0.351±0.00	94.70±0.75	5.46±0.05	5.44±0.05	0.64±0.30	0.16±0.005	37.81±2.96
645cm ² /b	53.50 ± 0.32	77.55±0.22	0.133±0.00	0.409±0.00	0.348±0.00	94.12±0.75	5.46± 0.05	5.37±0.05	0.83±0.30	0.07±0.05	34.65±2.96
Effect of flock size											
6 Birds	54.45 ^a ± 0.32	77.41±0.22	0.137 ^a ±0.00	0.409±0.00	0.346±0.00	94.99±0.75	5.46±0.05	5.37±0.05	0.58±0.30	0.07±0.05	34.09±2.96
9 Birds	53.40 ^b ±0.32	77.14±0.22	0.131 ^b ±0.00	0.406±0.00	0.353±0.00	93.83±0.75	5.45± 0.05	5.44±0.05	0.88±0.30	0.16±0.05	38.36±2.96
Pooled SEM	0.209	0.035	0.224	0.155	0.001	0.002	0.002	0.533	0.035	0.033	2.095
Statistical significance											
Treatment	P=0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Flock size	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05.

Table 4.37. Comparison of external and internal egg quality in furnished cages vs conventional California cages

Treatment	Egg Weight	Shape Index	Dirty eggs	Broken eggs	Albumen index	Yolk Index	Shell Thickness	Haugh unit	Shell weight	Yolk score
FC-548cm ² /b X 6B	55.00 ± 0.54	77.13 ± 0.38	0.73 ± 0.52	0.13 ± 0.07	0.14 ± 0.00	0.41 ± 0.00	0.35 ± 0.00	94.66 ± 0.32	5.41 ± 0.06	5.38 ± 0.07
FC-645cm ² /b X 6B	53.91 ± 0.36	77.70 ± 0.24	0.44 ± 0.26	0.01 ± 0.00	0.14 ± 0.00	0.41 ± 0.01	0.34 ± 0.00	95.34 ± 0.34	5.51 ± 0.06	5.35 ± 0.08
CC-548cm ² /b X 6B	54.13 ± 0.56	77.44 ± 0.21	2.03 ± 1.09	0.07 ± 0.04	0.14 ± 0.00	0.41 ± 0.00	0.34 ± 0.00	95.18 ± 0.75	5.40 ± 0.04	5.25 ± 0.08
CC-645cm ² /b X 6B	54.57 ± 0.52	76.93 ± 0.26	0.31 ± 0.07	0.18 ± 0.14	0.13 ± 0.00	0.41 ± 0.00	0.35 ± 0.01	93.79 ± 0.60	5.49 ± 0.08	5.28 ± 0.05
SEM	0.251	0.143	0.322	0.040	0.001	0.002	0.002	0.280	0.031	0.034
P-value	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

A comparison of the external and internal egg quality parameter for birds raised in modified furnished and conventional California cages is presented in Table 4.37. There was no any significant differences for the egg quality parameters for birds raised in modified furnished and conventional California cages.

Appleby *et al.*, (2002) observed no significant differences on cage type that is conventional and various design of furnished cages on egg weight measured at 50 and 72 weeks of age, though at 60 weeks one of the furnished cages had significantly better egg weight ($P < 0.01$). There were no significant effects of stocking density on any of the egg quality characteristics. Karkulin (2006) also reported non-significant effects of cage system (conventional and furnished) on various egg quality except for egg weight. Hens housed in enriched cages laid significantly ($P < 0.05$) heavier eggs compared to hens housed in conventional cages in contrast to present experiment.

Wall and Tauson (2007), Tactacan *et al.*, (2009), Pohle and Chang (2009) and Meng *et al.*, (2014) reported non-significant difference on egg weight when birds were kept in conventional and enriched cages. Also, Meng *et al.*, (2014) observed non-significant differences in egg weight due to flock size of 40 and 8 birds in furnished cages, though in present experiment flock size produced a change in egg weight and by interaction effect of flock size and stocking density. Effect of flock size in furnished cages did not affect any of the parameter except egg shell weight. Albumen index and Haugh unit were reported to be better in furnished cages than in conventional cages which were in contrast to present experiment. The better egg shell thickness in furnished cages by Meng *et al.*, (2014) was thought to be due to lower egg production in furnished cages as compared to conventional while the feed consumption remained the same. The similar calcium intake with lower egg production would convert more blood calcium into eggshell, making the egg shell thicker. Widowski *et al.*, (2017) observed no changes in egg weight between conventional cages and furnished cages, though a significant ($P < 0.01$) increase in egg weight was noticed with age. The birds in furnished cages were maintained in various stocking density

ranging from 516 to 746cm²/hen and in flock size of 80, 40, 55 and 28 had an equal average egg weight 60.0 ± 0.38 and were non-significant among themselves, in contrast to present experiment.

Hester *et al.*, (2013) found no significant differences on the various egg quality parameter for the furnished cages with availability of perches either in pullet or laying phase. One of the reasons for better egg weight in perches during pullet and no perches in laying phase is thought to be related with higher body weight of this group and larger skeletal frame. Appleby *et al.*, (1993) reported non-significance difference for dirty and cracked eggs in hens reared in conventional cages (stocking density of 675 cm²/hen) and furnished cages (stocking density 1050 to 1155 cm²/hen) equipped with perches, dust baths and nest boxes. Though the cracked eggs were numerically higher in furnished cages as most of them were laid from the perched which dropped in the cage floor resulting more percentage of cracked eggs and more dirty eggs in furnished cages because of the nest boxes were contaminated with faeces.

Karkulin (2006) also reported non-significant effect of furnished and conventional cages on percentage of dirty and cracked eggs, though downgraded eggs were low in enriched cages. It was reported that low amount of downgraded eggs in enriched cages were reached because of the plastic curtain fixed round the nest which effectively reduce the rolling out speed of egg from the nest and so the risk of its breakage was lower. Non-significant effect of cage type that is between conventional and enriched cages on cracked eggs was reported by Tactacan *et al.*, (2009) probably because of the egg savior employed, however there was significant more dirty eggs in enriched cages as more eggs were laid at the scratch pad region of the cage. Non-significant difference on cracked and dirty eggs was also reported by Widowski *et al.*, (2017) for different floor space (516, 522, 750 and 746 cm²/hen) and group size (80,40,55 and 28).

In contrast significant difference in percentage of dirty and cracked egg is reported by Abrahamsson and Tauson (1997) for birds raised in conventional and furnished cages with higher cracked eggs and lower dirty eggs in furnished cages, though the various group size of 5, 6, 7 and 8 birds in furnished cages were non-

significant among themselves for dirty and cracked eggs. Similarly, Tauson (1998) also observed proportions of cracked and dirty eggs were higher and lower, respectively, in modified and enriched cages (9.2% and 2.0%) in comparison with Conventional (5.0% and 6.0%). It was concluded that the increased proportion of cracked eggs in fully furnished cages may be due to accumulation of eggs outside the nest in the cradle of the cage floor. Appleby *et al.*, (2002) also reported significant effect of cage type (conventional vs different designs of furnished cages) on the percentage of cracked and dirty eggs. Dirty eggs were also seen more in cages with increase in group size but the increase was non-significant.

Wall and Tauson (2007) reported significantly ($P < 0.001$) low proportion of cracked eggs in conventional cages as compared to furnished cages, but dirty eggs were non-significant on cage type but numerically higher in conventional cages. Wall (2011) found significant difference in flock size in furnished cages for percentage dirty and cracked eggs with a better result in smaller flock size of 8 to 10 birds as compared to bigger group of 20 and 40 birds in the furnished cages. The group size of 8 and 10 birds were non-significant, which was similar to present experiment of smaller flock size of 6 and 9 birds. Li *et al.*, (2017) reported no significant differences in the nesting behavior for birds kept in various types of modified cages.

It was concluded that maintaining the layers in furnished cages at different stocking density (548 and 645cm²/bird) and flock size (six and nine birds) no any significant interaction effects were found for the various external and internal egg quality parameters excepting for egg weight. The egg weight was significantly better in FC- 548cm²/bird x 6B compared to FC- 645cm²/bird x 9B, also main effect of flock size on egg weight was observed better for 6 birds than 9 birds. Same better trend in 6 bird flock size was observed for albumen index also, with significant difference ($P < 0.05$) between six and nine birds. A comparison of external and internal egg quality in furnished and conventional California cages did not bring any significant difference probably because of same feed, space and flock allotment, furnishing couldn't bring any significant change.

4.3.5 Physical and physiological welfare study.

The results of the interaction effect of flock size and stocking density in furnished cages on the physical welfare indicators like feather score, gait score and physiological welfare indicators like heterophil-lymphocyte ratio, serum corticosterone and immune status is presented in Table 4.38 and 4.39. The comparison of the physical and physiological indicator parameters for modified furnished cages Vs conventional California cages is presented in Table 4.40 and 4.41. Figure 4.14 depicts the comparison of H/L ratio and corticosterone level of laying birds raised in furnished Vs conventional California cages.

Feather score and gait score of 12 birds from each treatment group were measured on 28, 37 and 47th week of age that is at the start, mid and end of the experiment is presented in the Table 4.38. Similarly, Physiological indicators like H/L ratio, corticosterone value (ng/ml) and immune status were also recorded. The immune response was judged by employing HI test to detect the antibody titer against New Castle Disease (ND) and the results are represented as log₂ values.

The interaction effect of flock size and stocking density in furnished cages for the physical indicators of welfare like feather score and gait score means and the antibody titers means are presented as log₂ value were non-significant for the various furnished cages groups. The mean feather score, gait score and antibody titre neither differ significantly due to interaction effect of stocking density and flock size nor was affected by the main effect of stocking density and by the main effect of flock size.

Neither nest boxes and dust baths in furnished cages nor flock size of 8 and 16 and stocking density could bring any change in feather score as reported by Barnett *et al.*, (1997). Moinard *et al.*, (1998) also reported non-significant effect of feather condition due to different cage type- conventional (stocking density of 450, 600 and 800cm²/bird) with furnished (stocking density of 800 cm²/bird) with perches. Appleby and Huges (1995) found no difference in feather

condition when compared birds in modified cages with provision for nest boxes, perches and sand baths with that of conventional cages.

Widowski *et al.*, (2017) reported significant effect ($P=0.048$) of space allowance only but not flock size in furnished cages to affect the feather condition score. Also, reported significant ($P<0.0006$) interaction effect of space allowance and flock size for hens housed with large space allowance ($746 \text{ cm}^2/\text{bird}$) and flock size of 28 had the best feather score.

Shimmura *et al.*, (2010) reported significant different ND antibody titer between different housing system, small and large conventional cages and small and large furnished cages and indicated lower ND antibody titer in large furnished cages. Stress decreases the number of lymphocytes, and the decline in white blood cell response to viruses is largely measured by Lymphocytes. However, in present experiment no changes observed for ND antibody titer.

Table 4.38. Interaction effect of stocking density and flock size in furnished cages on immune status, feather and gait score

Treatment	Feather Score	Gait score	Antibody titer against ND (\log_2 values)		
			28 th week	37 th week	47 th week
FC-548cm ² /b X 6B	6.78±0.20	8.00±0.00	2.74 ± 0.13	2.84 ± 0.06	2.84 ± 0.06
FC-548cm ² /b X 9B	7.00±0.23	8.00±0.00	2.79 ± 0.09	2.81 ± 0.14	2.81 ± 0.14
FC-645cm ² /b X 6B	6.67±0.39	8.00±0.00	2.80 ± 0.05	2.91 ± 0.14	2.82 ± 0.12
FC-645cm ² /b X 9B	6.78±0.32	8.00±0.00	2.93 ± 0.06	3.16 ± 0.07	3.08 ± 0.08
Effect of stocking density					
548cm ² /b	6.88 ± 0.21	8.00 ± 0.00	2.77 ± 0.06	2.82 ± 0.08	2.82 ± 0.07
645cm ² /b	6.72 ± 0.21	8.00 ± 0.00	2.87 ± 0.06	3.03 ± 0.08	2.95 ± 0.07
Effect of flock size					
6 birds	6.72 ± 0.21	8.00 ± 0.00	2.77 ± 0.06	2.87 ± 0.08	2.83 ± 0.07
9 Birds	6.89 ± 0.21	8.00 ± 0.00	2.86 ± 0.06	2.99 ± 0.08	2.94 ± 0.07
Pooled SEM	0.019	0.009	0.011	0.044	0.055
Statistical significance					
Treatment	NS	NS	NS	NS	NS
Stocking density	NS	NS	NS	NS	NS
Flock size	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, * $P < 0.05$, ** $P < 0.01$.

Table 4.39. Interaction effect of stocking density and flock size in furnished cages on heterophil: lymphocyte ratio and corticosterone

Treatment	H/L Ratio at 28 th week	H/L Ratio at 37 th week	H/L Ratio at 47 th week	Corticoster one (ng/ml) at 28 th week	Corticoster one (ng/ml) at 37 th week	Corticoster one (ng/ml) at 47 th week
FC-548cm ² /b X 6B	0.36±0.03	0.24 ^{ab} ±0.03	0.23 ^c ±0.02	1.73 ^{ab} ±0.14	0.87 ^b ±0.08	0.86 ^b ±0.10
FC-548cm ² /b X 9B	0.27±0.03	0.30 ^a ±0.02	0.35 ^a ±0.01	2.09 ^a ±0.16	1.16 ^a ±0.07	1.22 ^a ±0.08
FC-645cm ² /b X 6B	0.30±0.05	0.31 ^a ±0.02	0.35 ^a ±0.02	1.76 ^{ab} ±0.11	1.09 ^a ±0.02	0.67 ^b ± 0.10
FC-645cm ² /b X 9B	0.31±0.04	0.23 ^b ± 0.02	0.29 ^b ±0.01	1.54 ^b ±0.08	0.82 ^b ±0.07	0.95 ^{ab} ±0.11
Effect of stocking density						
548cm ² /b	0.32±0.03	0.27±0.02	0.29±0.01	1.91 ^a ±0.09	1.01±0.05	1.04 ^a ±0.07
645cm ² /b	0.31±0.03	0.27±0.02	0.32±0.01	1.65 ^b ±0.09	0.96±0.05	0.81 ^b ±0.07
Effect of flock size						
6 Birds	0.33±0.03	0.28±0.02	0.29±0.01	1.74±0.09	0.98±0.05	0.76 ^b ±0.07
9 Birds	0.29±0.03	0.27±0.02	0.32±0.01	1.82±0.09	0.99±0.05	1.08 ^a ±0.07
Pooled SEM	0.052	0.000	0.146	0.072	0.042	0.062
Statistical significance						
Treatment	NS	*	**	*	**	**
Stocking density	NS	NS	NS	*	NS	*
Flock size	NS	NS	NS	NS	NS	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.40. Comparison of immune status, feather and gait score in furnished cages vs conventional California cages

Treatment	Feather Score	Gait score	Antibody titer against ND (log ₂ values)		
			28 th week	37 th week	47 th week
FC-548cm ² /b X 6B	6.78±0.20	8.00±0.00	2.74±0.13	2.84±0.06	2.84±0.06
FC-645cm ² /b X 6B	6.67±0.39	8.00±0.00	2.80±0.05	2.91±0.14	2.82±0.12
CC-548cm ² /b X 6B	6.89±0.22	8.00±0.00	2.64±0.12	2.99±0.09	2.95±0.11
CC-645cm ² /b X 6B	7.00±0.61	8.00±0.00	2.59±0.20	3.01±0.09	2.92±0.11
SEM	0.185	0.00	0.065	0.050	0.049
P-value	NS	NS	NS	NS	NS

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01

Fig. 4.14. Comparison of H/L Ratio and Corticosterone (ng/ml) of laying birds raised in Furnished Vs conventional California Cages

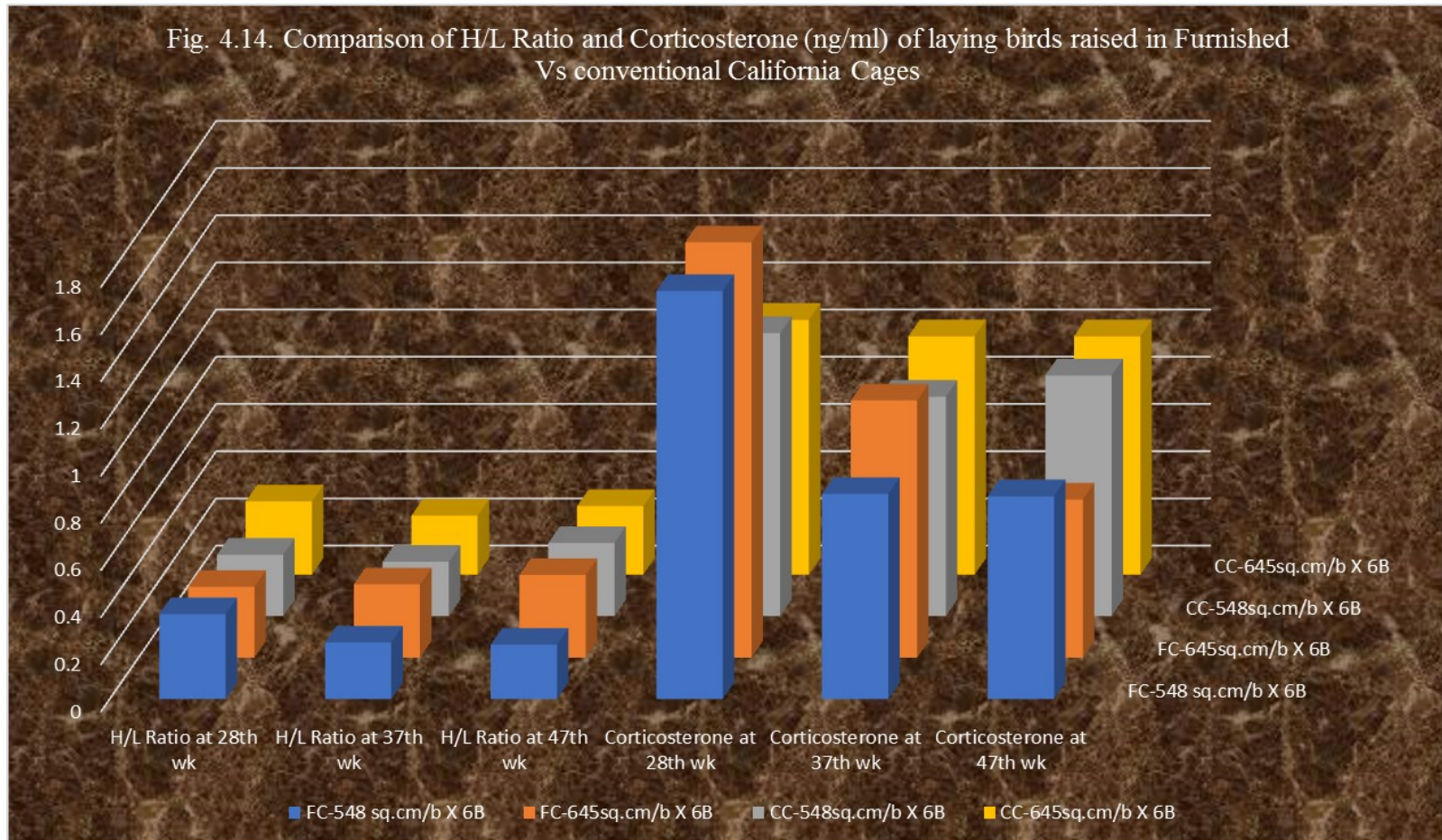


Table 4.41. Comparison on heterophil: lymphocyte ratio and corticosterone in furnished cages vs conventional California cages

Treatment	H/L Ratio at 28 th week	H/L Ratio at 37 th week	H/L Ratio at 47 th week	Corticoster one (ng/ml) at 28 th week	Corticoster one (ng/ml) at 37 th week	Corticoster one (ng/ml) at 47 th week
FC-548cm ² /b X 6B	0.36±0.03	0.24±0.03	0.23 ^b ±0.02	1.73 ^a ± 0.14	0.87 ± 0.08	0.86 ^{ab} ± 0.10
FC-645cm ² /b X 6B	0.30±0.05	0.31±0.02	0.35 ^a ±0.02	1.76 ^a ± 0.11	1.09±0.02	0.67 ^b ± 0.10
CC-548cm ² /b X 6B	0.26±0.02	0.23±0.01	0.31 ^a ±0.03	1.20 ^b ± 0.01	0.93 ± 0.09	1.02 ^a ± 0.06
CC-645cm ² /b X 6B	0.31±0.04	0.25±0.02	0.29 ^{ab} ±0.02	1.08 ^b ± 0.07	1.01 ± 0.04	1.01 ^a ± 0.06
SEM	0.018	0.011	0.015	0.078	0.034	0.048
P-value	NS	NS	*	**	NS	*

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

There was significant (P < 0.001) interaction effect of stocking density and flock size in furnished cages on the heterophil/lymphocyte ratio and corticosterone level during the 37th and 47th week of bird's age that is at the mid and end of the experimental period (Table 4.39). Significant difference (P<0.05) was noticed for corticosterone level at 28th week, but not for the H/L ratio, that is the start of the experimental period, immediately after placing the pullets in the furnished cages (Table 4.39). At the end of the experimental period FC-548cm²/b x 6B group has the significant lowest H/L ratio as compared to the rest of the furnished cages. FC-548cm²/b x 9B and FC-645cm²/b x 6B were non-significant among themselves but were significantly different from FC-548cm²/b x 6B and FC-645cm²/b x 9B at 47th week. H/L ratio observed at 37th week of bird's age was significantly (P<0.05) lowest in FC-645cm²/b x 9B group as compared to FC-548cm²/b x 9B and FC-645cm²/b x 6B, but remain non-significant with FC-548cm²/b X 6B. At 47th week of bird's age FC-548cm²/b x 6B and FC-645cm²/b x 6B had significantly (P<0.001) lowest corticosterone level than FC-548cm²/b x 9B and FC-645cm²/b x 9B, though FC-548cm²/b x 6B, FC-645cm²/b x 6B and FC-645cm²/b x 9B were non-significant among themselves and also FC-548cm²/b x 9B and FC-645cm²/b x 9B were non-significant.

There was changes in the main effect of stocking density during the period of 28th and 47th week of bird's age, the mean corticosterone value was found significant better in ($P < 0.01$) at 645cm² reared birds as compared to 548cm² per hen, though H/L values did not produce any significant difference for the main effect of stocking density. Main effect due to flock size was non-significant for both corticosterone level and H/L ratio measured at 28th, 37th and 47th week of birds age except at 47th week for corticosterone level and significantly higher at group of 9 bird flock size.

Barnett *et al.*, (1997) reported non-significant effect on plasma corticosterone because of addition of nest boxes, dust baths and perch in furnished cages, also group size of 16 and 8 birds in furnished cages had non-significant effect on plasma corticosterone, which is contrast to present experiment where individual effect of flock size of six and nine birds had significant effect, also, an interaction effect of stocking density and flock size was noticeable with the lowest value of corticosterone in FC-645cm²/b x 6B at 47th week (Table 4.39).

The comparison of the various physical and physiological indicators of welfare like feather score, gait score and antibody titer (except for 28th week) for modified furnished cages and conventional California cages were non-significant. Non-significant difference for feather score at 72 weeks of age among conventional and different arrangements of furnished cages similar to present experiment result was observed by Appleby *et al.*, (1993), although there was a tendency for feather damage to be worse in conventional cages of score 5.4 as compared to 3.0 in experimental furnished cages which is contradictory to present experiment probably because of the length of the trial. Moreover, non-significant difference in plumage for conventional and modified cages was reported by Tauson (1998).

However, Abrahamsson and Tauson (1997) observed significant difference ($P < 0.01$) in body plumage condition between furnished (having cage floor area of 600cm²/per bird excluding nest and sand-bath) and conventional cages (cage floor area of 600cm²/hen), that is with different space allowance in contrast to present experiment, but there was no effect of flock size on body

plumage. They concluded that there was no effect on plumage condition or cannibalism in flock size of 8 birds, and flock size of 8 bird is suitable in furnished cages, because provision of a perch, nest and sand bath decreases stress and therefore gave a general decrease in feather -pecking.

Appleby *et al.*, (2002) also reported significant differences ($P < 0.001$) between conventional and furnished cages for feather damage with conventional cages tended to be worse than the various furnished cage types of different designs, also feather damage was more pronounced where the flock size was bigger among the various treatments of conventional and furnished cages.

Tactacan *et al.*, (2009) observed significant ($P < 0.05$) better wing feathers in conventional cages than in enriched cages. In the enriched cages because of greater space available the birds had the opportunity to increase the flapping of their wings which may be increased the possibility of wing abrasions on the bars of the cages. Also the higher number of birds housed in cages (24) compared to conventional cages (5) may have increased the feather pecking and have contributed to the observed greater wing deterioration. Poorly feathered birds not only present a problem for welfare but also affect overall production economics. Birds with highly deteriorated feather cover can show increased feed consumption due to increased heat loss resulting in higher maintenance energy requirement. Meng *et al.*, (2014) observed significant better feather score and gait score for birds reared in large and small furnished cages when compared to conventional cages. This study showed that feather condition in large furnished cages was better than in small furnished and traditional cages, the poor feather condition of hens in conventional cages is attributable to narrow space and crowded conditions where the hens are unable to preen.

Similarly, Li *et al.*, (2016) reported that hens in conventional cages had poorer feather and gait condition than hens in small and medium furnished cages. In conventional cages, a hen's feathers are easily damaged because of the abrasion by cages and space limitation for combing feathers, also higher stocking density could be contributed to a worse feather condition as feather condition was downgraded as stocking density was increased from small furnished cages to

medium furnished cages and hens in furnished cages showed better gait score than the hens in conventional cage. In this experiment though the values of feather score were non significantly differ but slightly inclined towards FC. In furnished cages, because of the larger space and existence of perch, hens performed more walking and perching, and less standing behaviours which could exercise the muscle and bone of hen's legs, as a result, the leg or claw condition was improved. Conversely, the hens in conventional cages performed more standing and less walking potentially because of the narrow space, which eventually induced a lower gait score which was in contrast to present experiment.

The heterophil/lymphocyte ratio observed for the three different periods start, mid and end, shown significant differences among the furnished (FC) and conventional (CC) cages only at the end of the experiment with significantly lowest ($P < 0.05$) value in FC-548cm²/b x 6B as compared to other furnished and conventional cage though it remained non-significant with CC-645cm²/b x 6B, having a better stocking density. At stocking density of 548cm²/b the difference in H/L ratio between furnished and conventional cage indicated that furnished cages were in lower level of stress than conventional California cages. Significant different corticosterone value between furnished and conventional California cages was observed only during the start or 28th week and at the end of the experiment that is 47th week of bird's age. At the end of the experiment significant differences ($P < 0.05$) were observed for birds maintained in furnished cages had lower corticosterone (ng/ml) value indicating lower level of stress as compared to conventional California cages.

Barnett *et al.*, (1997) reported non-significant effect on heterophil-lymphocyte ratio because of group size (8 and 16) and addition of furniture like perch, nest box and dust-baths in furnished cages and the ratio ranged between 1.62 to 1.97. Also, Tactacan *et al.*, (2009) reported non-significant difference in H/L ratio for birds in furnished and conventional cages.. In contrast Shimmura *et al.*, (2010) reported highly significant difference in H/L ratio between furnished cages and conventional cages with different flock size. In the present experiment H/L ratio and plasma corticosterone (ng/ml) value comparison of conventional

cage with furnished cages varied differently for various age or periods; but towards the end of the experimental period bird's in furnished cages had lower values of H/L ratio and corticosterone (ng/ml) indicating a less stressful condition in furnished cages. In poultry, population differences exist in response to various stressors and social interaction among chickens can be a serious source of stress.

The findings of Cheng and Muir (2004) support the hypothesis that chickens need social attachments within a group but prefer a low-density social environment and within a low density and stable group, chickens are less stressed by following the established social rank order. In the present experiment non-significant differences for few periods of the experiment between furnished and conventional cages could be related to low density of birds and stable group. As authors Pohle and Chang (2009) reported non-significant difference in battery and furnished cages on the plasma corticosterone value with flock size of 6 and 10 hens and floor space per hens was 645 and 610 cm²/hen respectively, apart from the dust-bath and nest box area. Tactacan *et al.*, (2009) also reported non-significant differences in corticosterone value in the birds reared at conventional and furnished cages.

4.3.6 Behavioral Activity:

The interaction effect of stocking density (548 cm² and 645cm² per bird) and flock size (6 and 9 birds) on various behaviors (standing, sitting, sleeping, investigating, walking, drinking, feeding, preening, feather pecking, scratching, wing flapping, fighting etc.) of the experimental birds are presented phase wise (Start-28, Mid-37 and End-47 week) in Table 4.42, 4.43 and 4.44.

The various activities of the birds behavior were recorded by a CCTV camera as illustrated in the Materials and methods. The specific 24 hour (1440 minute) behavioral observations collected at three different period (28, 37 and 47 week) of experiment were averaged and presented in Table 4.45 and depicted in Figure 4.15 and 4.16. The average of three different stages/weeks of each behavioral activity for the interaction effect and main effect of stocking density

and flock size and the comparison of behavioral activity for birds in furnished and conventional cages is discussed as below.

Interaction Effect of Stocking Density and Flock Size in Furnished Cages:

The interaction effect of the average values of the various behavioral activity collected at different period was significantly different ($P < 0.05$) for standing, walking, sleeping, feeding, preening, feather pecking, perching, wing flapping and total state and total event behavior are presented in Table 4.45.

The interaction effect of stocking density and flock size was significantly ($P < 0.01$) different for perching with the lowest time (less utilized) observed for FC-548cm²/b X 9B compared to the rest of the furnished cages which is thought to be because of social dominance by few birds, letting not others (selected for observations) to perch resulting in a decline in average perching activity or this group have been active for other activities like standing or walking. Li *et al.*, (2017) reported low perching activity with the lowest perching space in small furnished cages (perch allowance 16 cm/hen and area of 1000cm²/hen) when compared to three types of medium furnished cages, MFC-I, II and III (perch allowance of 22.3 cm, 21.3 cm, 26.7cm and area of 1000, 1200,1400 cm²/hen respectively). Cooper *et al.*, (2004) studied the 24 hours activity of hens in furnished cages in groups of ten with stocking density of 1219 cm² per bird, it was observed at night 86% of resting birds were on a perch usually next to another bird and not all hens could fit on one perch, the maximum number observed resting on a perch was seven. Hens were observed to rest on the floor of the cage on 14% of occasions, of which 8%, were close to a perching bird and 5% at the front of the cage next to the food trough. For the 8 hours of darkness from 9 PM to 5AM in present experiment, birds that stayed perching ranged from 36 to 55% of time as all observation birds did not perch, FC-548cm²/b X 6B-53% for six birds and FC-548cm²/b X 9B- 36% for nine birds, as stocking density improved from 100 sq. inch, there was linear increase time in perching FC-645cm²/b X 6B- 55% for six birds and FC-645cm²/b X 9B- 46% for nine bird flock size.

FC-645cm²/b X 9B group had significantly higher values for preening and wing flapping when compared to other furnished cages FC-548cm²/b X 6B, 9B and FC-645cm²/b X 6B with similar or different flock size and stocking density, but this did not follow the finding of Guo *et al.*, (2012) who found non-significant differences for preening, wing flapping pecking, feeding, drinking, standing, and sleeping in small and large furnished cages. For preening in corroboration are the results of Li *et al.*, (2017) who concluded stocking density affected socializing and preening behavior of hens. They suggested that low stocking density (MFC-I, II, and III) seemed to result in more socializing and preening behavior in hens than high stocking density as in FC and CC. It also may indicate hens in low stocking density cages feel more comfortable than in high stocking density cages. Also, FC-645cm²/b x 9B had statistically higher standing and walking but was non-significant with FC-645cm²/b x 6B group for standing; FC-548cm²/b x 9B for walking and FC-548cm²/b x 6B for feather pecking. Non-significant differences in feather pecking between various groups of birds furnished cages was related by Abrahamsson and Tauson (1997), due to provision of a perch, nest and sand-bath, these furniture decreases stress and therefore gave a general decrease in feather pecking and cannibalism compared with conventional cages, which is not evident in present experiment.

Table 4.42. Interaction effect of Stocking Density (SD) and Flock Size (FS) in furnished cages on behaviour activity at 28th week of bird's age

Parameters	Interaction effect of SD and FS				Main Effect of SD		Main effect of FS		Pooled SEM	Statistical significance		
	FC548cm ² /b X 6B	FC-548cm ² /b X 9B	FC-645cm ² /b X 6B	FC-645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Treatment	SD	FS
Standing	127.50 ^a ±7.78	84.50 ^b ±6.63	137.17 ^a ±7.66	154.67 ^a ±17.32	106.00 ^b ±7.61	145.92 ^a ±7.61	132.33±7.61	119.58±7.61	7.363	**	**	NS
Sitting	139.33 ^c ±3.15	185.17 ^b ±9.85	203.50 ^a ±3.62	173.17 ^b ±3.23	162.25 ^b ±4.04	183.33 ^a ±4.04	171.42 ± 4.04	179.17±4.04	5.559	**	**	NS
Walking	29.83±4.42	34.17±5.57	22.67±4.03	41.67±6.56	32.00±3.71	32.17±3.71	26.25 ^b ± 3.71	37.92 ^a ±3.71	2.834	NS	NS	*
Investigation	90.50±10.67	88.17±9.42	100.33±14.47	110.50±10.73	89.33±8.12	105.42±8.12	95.42 ± 8.12	99.33±8.12	5.661	NS	NS	NS
Sleeping	234.83 ^a ±3.69	170.67 ^b ±3.38	138.33 ^c ±6.84	159.17 ^b ±2.54	202.75 ^a ±3.13	148.75 ^b ±3.13	186.58 ^a ± 3.13	164.92 ^b ±3.13	7.790	**	**	**
Perching	193.17 ^b ±4.04	183.67 ^b ±6.17	229.33 ^a ±12.74	196.00 ^b ±8.64	188.42 ^b ±6.04	212.67 ^a ±6.04	211.25 ^a ± 6.04	189.83 ^b ±6.04	5.363	**	**	*
Total State	815.17 ^a ±4.46	746.33 ^b ±3.06	831.33 ^a ±12.74	835.17 ^a ±5.26	780.50 ^b ±5.23	833.25 ^a ±5.23	823.25 ^a ± 5.23	790.75 ^b ±5.23	8.228	**	**	**
Drinking	64.83 ^{ab} ±4.56	78.67 ^a ±9.05	79.83 ^a ±8.44	53.67 ^b ±3.23	71.75±4.80	66.75±4.80	72.33 ± 4.80	66.17±4.80	3.880	*	NS	NS
Feeding	409.67 ^a ±9.22	468.33 ^a ±20.68	402.17 ^b ±19.93	374.67 ^b ±11.18	439.00 ^a ±11.37	388.42 ^b ±11.37	405.92±11.37	421.50±11.37	10.337	**	**	NS
Preening	76.17 ^{bc} ±5.10	83.17 ^b ±8.15	61.83 ^c ±4.81	104.67 ^a ±2.54	79.67±3.91	83.25±3.91	69.00 ^b ± 3.91	93.92 ^a ±3.91	4.124	**	NS	**
Feather pecking	16.17 ^a ±2.74	10.17 ^{bc} ±1.82	6.33 ^c ±0.84	12.50 ^{ab} ±0.85	13.17 ^a ±1.24	9.42 ^b ±1.24	11.25 ± 1.24	11.33±1.24	1.104	**	*	NS
Scratching	56.50±3.16	50.00±2.54	56.33±2.60	51.00±0.93	53.25 ± 1.74	53.67 ± 1.74	56.42 ^a ± 1.74	50.50 ^b ± 1.74	1.302	NS	NS	*
Wing Flapping	1.50 ^b ±0.34	3.33 ^b ±1.41	2.17 ^b ±0.54	7.50 ^a ±1.06	2.42 ^b ± 0.66	4.83 ^a ± 0.66	1.83 ^b ± 0.66	5.42 ^a ± 0.66	0.653	**	*	**
Total events	624.83 ^b ±4.46	693.67 ^a ±3.06	608.67 ^b ±12.74	604.67 ^b ±5.25	659.25 ^a ± 5.23	606.67 ^b ± 5.23	616.75 ^b ± 5.23	649.17 ^a ± 5.23	8.234	**	**	**

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.43. Interaction effect of Stocking Density(SD) and Flock Size (FS) in furnished cages on behaviour activity at 37th week of bird's age

Parameters	Interaction effect of SD and FS				Main Effect of SD		Main effect of FS		Pooled SEM	Statistical significance		
	FC-548cm ² /b X 6B	FC-548cm ² /b X 9B	FC-645cm ² /b X 6B	FC-645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Treatment	SD	FS
Standing	75.33±2.54	96.00±11.53	94.50±3.32	104.17±9.11	85.67±5.40	99.33±5.40	84.92±5.40	100.08±5.40	4.189	NS	NS	NS
Sitting	185.67±4.24	160.67±10.33	181.67±11.07	180.00±12.94	173.17±7.20	180.83±7.20	183.67±7.20	170.33±7.20	5.158	NS	NS	NS
Walking	18.17±2.36	23.83±2.83	18.50±1.65	22.33±1.33	21.00±1.50	20.42±1.50	18.33 ^b ±1.50	23.08 ^a ±1.50	1.114	NS	NS	*
Investigation	55.83±8.90	71.83±9.80	45.50±9.76	56.33±8.25	63.83±6.51	50.92±6.51	50.67±6.51	64.08±6.51	4.716	NS	NS	NS
Sleeping	226.00 ^b ±12.34	268.67 ^a ±5.42	233.50 ^b ±3.71	258.67 ^a ±8.68	247.33±5.82	246.08±5.82	229.75 ^b ±5.82	263.67 ^a ±5.82	5.298	**	NS	**
Perching	298.50 ^a ±27.54	160.67 ^b ±7.11	282.17 ^a ±25.09	208.17 ^b ±18.14	229.58±14.86	245.17±14.86	290.33 ^a ±14.86	184.42 ^b ±14.86	15.222	**	NS	**
Total State	859.50 ^a ±17.82	781.67 ^b ±11.34	855.83 ^a ±22.29	829.67 ^a ±2.25	820.58±10.89	842.75±10.89	857.67 ^a ±10.89	805.67 ^b ±10.89	9.671	**	NS	**
Drinking	44.00±7.31	55.17±7.45	51.33±1.05	51.33±4.00	49.58±3.97	51.33 ± 3.97	47.67±3.97	53.25±3.97	2.750	NS	NS	NS
Feeding	357.17 ^b ±16.13	424.33 ^a ±1.78	337.17 ^b ±16.40	365.67 ^b ±5.30	390.75 ^a ±8.37	351.42 ^b ±8.37	347.17 ^b ±8.37	395.00 ^a ± 8.37	8.729	**	**	**
Preening	105.17 ^{bc} ±8.02	97.50 ^c ±0.34	121.67 ^a ±5.25	114.17 ^{ab} ±2.83	101.33 ^b ±3.54	117.92 ^a ± 3.54	113.42±3.54	105.83±3.54	3.009	*	**	NS
Feather pecking	7.00 ^a ±1.10	1.17 ^b ±0.31	0.83 ^b ±0.54	0.33 ^b ±0.21	4.08 ^a ±0.45	0.58 ^b ±0.45	3.92 ^a ±0.45	0.75 ^b ±0.45	0.639	**	**	**
Scratching	67.17±1.28	77.17±3.27	71.00±3.47	75.67±2.12	72.17±1.90	72.33±1.90	69.08 ^b ±1.90	76.42 ^a ±1.90	1.499	NS	NS	**
Wing Flapping	0.00 ^b ±0.00	3.00 ^a ±0.00	2.17 ^a ±0.60	3.17 ^a ±0.31	1.50 ^b ±0.24	2.67 ^a ±0.24	1.08 ^b ±0.24	3.08 ^a ±0.24	0.306	**	**	**
Total events	580.50 ^b ±17.82	658.33 ^a ±11.34	584.17 ^b ±22.29	610.33 ^b ±2.25	619.42±10.89	597.25±10.89	582.33 ^b ±10.89	634.33 ^a ±10.89	9.671	**	NS	**

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.44. Interaction effect of Stocking Density(SD) and Flock Size (FS) in furnished cages on behaviour activity at 47th week of bird's age

Parameters	Interaction effect of SD and FS				Main Effect of SD		Main effect of FS		Pooled SEM	Statistical significance		
	FC-548cm ² /b X 6B	FC-548cm ² /b X 9B	FC-645cm ² /b X 6B	FC-645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Treatment	SD	FS
Standing	92.67±3.57	113.33±15.53	104.83±4.04	92.67±7.98	103.00±6.46	98.75±6.46	98.75±6.46	103.00±6.46	4.634	NS	NS	NS
Sitting	134.17±4.59	136.83±18.90	131.17±29.69	88.00±6.24	135.50±12.74	109.58±12.74	132.67±12.74	112.42±12.74	9.382	NS	NS	NS
Walking	22.67 ^b ±1.71	25.00 ^b ±2.61	25.67 ^b ±3.86	41.00 ^a ±3.15	23.83 ^b ±2.08	33.33 ^a ±2.08	24.17 ^b ±2.08	33.00 ^a ±2.08	2.041	**	**	**
Investigation	93.50±8.57	96.67±9.07	86.17±6.33	86.00±8.64	95.08±5.82	86.08±5.82	89.83±5.82	91.33±5.82	3.955	NS	NS	NS
Sleeping	220.17 ^a ±6.26	214.67 ^a ±4.98	188.00 ^b ±7.65	208.50 ^a ±2.36	217.42 ^a ±4.00	198.25 ^b ±4.00	204.08±4.00	211.58±4.00	3.660	**	**	NS
Perching	266.00 ^a ±15.99	179.00 ^b ±8.98	276.50 ^a ±21.52	262.33 ^a ±11.18	222.50 ^b ±10.75	269.42 ^a ±10.75	271.25 ^a ±10.75	220.67 ^b ±10.75	10.788	**	**	**
Total State	829.17 ^a ±14.47	765.50 ^b ±8.16	812.33 ^a ±10.85	778.50 ^b ±7.52	797.33±7.50	795.42±7.50	820.75 ^a ±7.50	772.00 ^b ±7.50	7.264	**	NS	**
Drinking	62.00±4.55	68.67±5.94	65.67±3.11	69.00±3.81	65.33±3.17	67.33±3.17	63.83±3.17	68.83±3.17	2.168	NS	NS	NS
Feeding	376.17 ^b ±8.57	419.83 ^a ±7.69	365.83 ^b ±14.97	365.50 ^b ±11.91	398.00 ^a ±7.89	365.67 ^b ±7.89	371.00±7.89	392.67±7.89	6.987	**	**	NS
Preening	97.50±9.07	97.50±9.07	97.50±9.07	97.50±9.07	95.17 ^b ±4.55	109.00 ^a ±4.55	101.75±4.55	102.42±4.55	3.378	NS	*	NS
Feather pecking	12.33 ^b ±2.14	11.67 ^b ±1.94	9.83 ^b ±0.70	21.17 ^a ±2.57	12.00±1.39	15.50±1.39	11.08 ^b ±1.39	16.42 ^a ±1.39	1.294	**	NS	*
Scratching	60.83±6.05	76.83±1.92	77.83±9.53	82.83±5.18	68.83±4.44	80.33±4.44	69.33±4.44	79.83±4.44	3.398	NS	NS	NS
Wing Flapping	2.00 ^b ±0.68	4.67 ^b ±1.02	2.33 ^b ±0.67	9.83 ^a ±1.64	3.33 ^b ±0.76	6.08 ^a ±0.76	2.17 ^b ±0.76	7.25 ^a ±0.76	0.824	**	*	**
Total events	610.83 ^b ±14.47	674.50 ^a ±8.16	627.67 ^b ±10.85	661.50 ^a ±7.52	642.67± 7.50	644.58±7.50	619.25 ^b ±7.50	668.00 ^a ± 7.50	7.264	**	NS	**

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.45. Interaction effect of Stocking Density(SD) and Flock Size (FS) on behavioural activity observed on 28, 37 and 47 week (average) of bird's age

Parameters	Interaction effect of SD and FS				Main Effect of SD		Main effect of FS		Pooled SEM	Statistical Significance		
	FC-548cm ² /b X 6B	FC-548cm ² /b X 9B	FC-645cm ² /b X 6B	FC-645cm ² /b X 9B	548cm ² /b	645cm ² /b	6 birds	9 birds		Treatment	SD	FS
Standing	98.50 ^b ±4.17	98.00 ^b ±8.26	112.17 ^{ab} ±3.91	117.00 ^a ±3.08	98.25 ^b ± 3.71	114.58 ^a ±3.71	105.33±3.71	107.50±3.71	2.626	*	*	NS
Sitting	153.00±3.22	161.17±11.89	172.33±12.62	147.17±4.92	157.08±6.48	159.75±6.48	162.67±6.48	154.17±6.48	4.578	NS	NS	NS
Walking	23.50 ^b ±2.77	27.67 ^{ab} ±3.62	22.33 ^b ±1.52	35.00 ^a ±2.68	25.58±1.95	28.67±1.95	22.92 ^b ±1.95	31.33 ^a ±1.95	1.376	*	NS	**
Investigation	80.17±9.02	85.50±9.17	77.33±8.77	84.17±8.76	82.83±6.32	80.75±6.32	78.75±6.32	84.83±6.32	4.467	NS	NS	NS
Sleeping	227.00 ^a ±5.06	218.00 ^{ab} ±4.16	186.50 ^c ±4.96	208.83 ^b ±3.11	222.50 ^a ±3.11	197.67 ^b ±3.11	206.75±3.11	213.42±3.11	2.201	**	**	NS
Perching	252.50 ^{ab} ±15.8 ₁	174.50 ^c ±6.46	262.67 ^a ±14.07	222.17 ^b ±6.46	213.50 ^b ±8.15	242.42 ^a ±8.15	257.58 ^a ±8.15	198.33 ^b ±8.15	5.763	**	*	**
Total State	834.67 ^a ±11.86	764.50 ^b ±5.53	833.17 ^a ±13.90	814.50 ^a ±4.26	799.58±6.92	823.83±6.92	833.92± 6.92	789.50± 6.92	4.891	**	*	**
Drinking	57.00±4.52	67.50±7.46	65.67±3.33	58.00±3.07	62.25±3.48	61.83±3.48	61.33±3.48	62.75±3.48	2.458	NS	NS	NS
Feeding	381.00 ^a ±10.63	437.50 ^b ±9.01	368.50 ^a ±13.68	368.50 ^a ±6.46	409.25 ^a ±7.23	368.50 ^b ±7.23	374.75 ^b ±7.23	403.00 ^a ±7.23	5.143	**	**	**
Preening	93.00 ^b ±6.61	91.17 ^b ±3.36	96.50 ^b ±3.45	110.33 ^a ±1.78	92.08 ^b ±2.96	103.42 ^a ±2.96	94.75±2.96	100.75±2.96	2.093	*	**	NS
Feather pecking	11.83 ^a ±1.05	7.67 ^b ±1.26	5.67 ^b ±0.42	11.33 ^a ±0.92	9.75±0.68	8.50±0.68	8.75±0.68	9.50±0.68	0.481	**	NS	NS
Scratching	61.50±2.93	68.00±1.18	68.33±3.01	69.83±2.09	64.75±1.71	69.08±1.71	64.92±1.71	68.92±1.71	1.209	NS	NS	NS
Wing Flapping	1.17 ^c ±0.31	3.67 ^b ±0.80	2.17 ^{bc} ±0.48	6.83 ^a ±0.87	2.42 ^b ±0.47	4.50 ^a ±0.47	1.67 ^b ±0.47	5.25 ^a ±0.47	0.329	**	**	**
Total events	605.33 ^b ±11.86	675.50 ^a ±5.53	606.83 ^b ±13.90	625.33 ^b ±4.27	640.42 ^a ±6.92	616.08 ^b ±6.92	606.08 ^b ±6.92	650.42 ^a ±6.92	4.892	**	*	**

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Feeding space of FC-645cm²/b x 9B group was only significantly used of FC-548cm²/b x 9B, others treatment (FC-548cm²/b x 6B and FC-645cm²/b x 6B) remained non-significant probably because of feeder's space, social order and interaction of group size in that stocking density. FC-645cm²/b x 9B did not have the highest perching activity, the best significant higher number was FC-645cm²/b x 6B and FC-548cm²/b x 6B, non-significant among themselves, followed by FC-645cm²/b x 9B and then FC-548cm²/b x 9B. The availability of perch space played an important factor that is indirectly the space allowance per cage as well as the flock size. Sleeping was significantly better in FC-548cm²/b x 6B than FC-645cm²/b x 6B and 9B, but was non-significant with FC-548cm²/b x 9B, time spent on sleeping followed the sequence of FC-548cm²/b x 6B; 9B and FC-645cm²/b x 9B;6B.

Main effect of Stocking density:

The main effects for stocking density on the average values of 28th, 37th and 47th week with statistical significance was seen for standing, sleeping, feeding, preening, perching and wing flapping. The birds reared with 645 cm²/bird enjoyed significantly higher standing, preening, perching and wing flapping than in 548 cm²/bird was observed. Similar balanced wing flapping is observed by Appleby *et al.*, (2002), it was more common where there were 5 birds per cage than where there were 8 (P<0.05), that is within the furnished cages was affected by the stocking density of birds. Zimmerman *et al.*, (2006) reported more preening in the low (P < 0.05) and high (P < 0.001) density pens compared to the medium stocking density pens, when kept birds at three stocking density, low 7 birds /m², medium 9 birds/ m² and high 12 birds/ m². The incidence of preening tended to be higher (P = 0.06) at the high density than at the low stocking density while the opposite of more preening activity in 645 cm²/bird, low density than 548 cm²/bird, high density in the present experiment. Surely, the preening at low and high stocking density has different behavioral intension.

A reverse significant better activity for 548 cm²/bird for sleeping and feeding was observed than in 645 cm²/bird for the main effect of stocking density.

There was no main effect of stocking density on feather pecking which was similar to reports of Anderson *et al.*, (2004) with non-significant difference in feather pecking at stocking density of 361 and 482 cm²/bird. In Contrary, Zimmerman *et al.*, (2006) found some differences in feather pecking and aggression with age, stocking density and flock size, at the initial level feather pecking and aggression was highest in the low stocking density. Later on feather pecking and aggression increased with age but only in the high stocking density treatments. In the high stocking density treatments more aggression, preening and all preening were recorded in small flocks than in large flocks.

Guo *et al.*, (2012) studied effect of furnished cage systems with different group sizes on laying performance and behavior and compared with conventional cages, it was noted that hens spent most time in feeding and standing in all three housing system. Small Furnished cages with lower space allowance had a lower frequency of sitting ($P < 0.001$) and perching behaviors ($P < 0.01$) compared to birds in large furnished cages, this was evident in the present experiment (Table 4.45) for the main effect of stocking density.

Main effect of Flock size:

The main effect for flock size was significantly noticeable for the average values on walking, feeding, perching and wing flapping activities. Smaller flock size of six birds was significantly better for perching as compared to group with nine birds; while 9 bird flock had higher activity for walking, feeding and wing flapping than 6 bird flock. This is contrasting to the perching result indicated by Abrahamsson and Tauson (1997), where no effect of group size was reported for perching. Significant more time ($P < 0.01$) was spent on perching by flock size of 6 birds as compared to 9 birds was seen in present experiment and significant less locomotion by flock size of 6 birds. The possibility of spending more time on perching by flock size of 6 birds might have resulted in less walking by them. Similar observation of more perching activity in furnished cages with smaller flock size as compared to larger flock size was described by Newberry *et al.*, (2001).

Bird's bodies cover a certain area of floor space due to their pure presence referred to as "floor space requirement" (FSR) however, the birds need additional space to perform behaviors eg., to move, to feed, etc., also sitting requires slightly more space than standing. Also interesting is to note that most active behaviors are performed in a standing position such as locomotion, feeding, social behaviors, etc. and when chickens are sitting, they are mostly resting (except dust bathing) and, thus, do not require as much additional space as active chickens. However, additional space is regarded as a key quality parameter as it allows the birds to perform behaviors, and ideally their entire natural behavioral repertoire, as FSR represents only the necessary and immutable space for the birds.

Comparison of Behavioral Activity in Furnished cages Vs conventional

California Cages:

In an attempt to understand the behavioral activity of the laying hens in furnished cages and compare them with conventional California cages without any furniture, the behavioral record is presented in Table 4.46 and Figure 4.15 and 4.16.

In both the system of conventional California and furnished cages, birds spent considerable amount of time in feeding (24-28%), standing (7-16%), sitting (11-14%) and sleeping (13-21%) for the entire 24 hour day activity. Conventional California cages were not furnished with furniture's like perches, scratch-pad or nesting area, so it was quite obvious that the natural behavioral activity like perching and scratching values were non-existent in conventional California cages. There was highly significant ($P < 0.01$) effect of perching and scratching in furnished cages over the conventional California cages. Also, it is interesting to note that in absence of scratch pad and perch in conventional California cages, there is a significant increase for other behavioral activities (standing, sitting, investigation, walking) when compared to same activity of birds reared in furnished cages.

It was noted that the birds in CC-548cm²/b x 6B and CC-645cm²/b x 6B conventional California cages had the significant more standing, sitting, walking,

sleeping, investigation, drinking, feather pecking activities when compared with similar stocking density groups in furnished cages. Though most of the state behavior activity was highest in conventional California cages, the total state behavior did not have any significant difference between the CC and FC groups. The perching activity on furnished cages nullified the total state behavior difference between the CC and FC which was otherwise noticed on individual state behavior like standing, sitting, walking, investigation and sleeping.

Table 4.46. Comparison of behaviour activity of laying birds in Furnished Cages vs Conventional California cage (average value of FC and CC)

Treatment	FC-548cm ² /b X 6B	FC-645cm ² /b X 6B	CC-548cm ² /b X 6B	CC-645cm ² /b X 6B	SEM	P- Value
Standing	98.50 ^b ± 4.17	112.17 ^b ± 3.91	217.50 ^a ±14.49	232.00 ^a ±13.24	13.411	**
Sitting	153.00 ^b ±3.22	172.33 ^{ab} ±12.62	187.17 ^a ± 0.48	197.83 ^a ±13.47	5.605	*
Walking	23.50 ^b ± 2.77	22.33 ^b ±1.52	33.00 ^b ± 3.44	46.67 ^a ± 5.26	2.612	**
Investigation	80.17 ^b ± 9.02	77.33 ^b ±8.77	117.83 ^a ±14.75	122.17 ^a ±7.55	6.493	**
Sleeping	227.00 ^b ±5.09	186.50 ^c ± 4.96	305.83 ^a ± 5.31	216.50 ^b ±4.24	9.472	**
Perching	252.50 ^a ±15.81	262.67 ^a ±14.07	0.00 ^b ± 0.00	0.00 ^b ± 0.00	27.315	**
Total State	834.67±11.86	833.17± 13.90	861.33 ± 8.17	815.17±15.23	6.797	NS
Drinking	57.00 ^c ±4.52	65.67 ^c ± 3.33	99.67 ^a ± 0.71	83.17 ^b ± 5.72	3.904	**
Feeding	381.00±10.63	368.50±13.68	354.67±13.11	404.67±27.11	8.961	NS
Preening	93.00 ± 6.61	96.50 ± 3.45	108.67±8.00	115.33±5.78	3.443	NS
Feather pecking	11.83 ^b ± 1.05	5.67 ^c ± 0.42	12.17 ^b ± 0.60	16.50 ^a ± 1.09	0.895	**
Scratching	61.50 ^b ± 2.93	68.33 ^a ± 3.01	0.00 ^c ± 0.00	0.00 ^c ± 0.00	6.856	**
Wing Flapping	1.17 ^c ± 0.31	2.17 ^{bc} ± 0.48	3.50 ^b ±0.72	5.17 ^a ±0.48	0.395	**
Total events	605.33 ±11.86	606.83 ± 13.90	578.67 ± 8.17	624.83±15.23	6.797	NS

Means bearing superscript within a row differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Battery cages limit the expressions of bird's natural behaviors, there was more sitting and less walking behavior in conventional cages when compared to small and large furnished cages reported by Guo *et al.*, (2012), this is similar to present experiment (Table 4.46) with regard to sitting. Li *et al.*,(2017) in their comparison for furnished and conventional cages reported similarly for the behavior activity for standing as present experiment (but contrasting for walking) and seen that the hens in the furnished cages had significantly lower standing than conventional cages hens (P < 0.05), while their walking was significantly higher

than conventional hens ($P < 0.05$), though only at $645\text{cm}^2/\text{bird}$ for conventional cages walking was higher. The current higher standing and walking in conventional cages in the present comparative study might be due to provision of a perch in the furnished cages which rather led the birds to perch more than standing leading to less standing and walking activity in furnished cages.

Feather pecking in FC- $645\text{cm}^2/\text{b}$ x 6B was significantly lower ($P < 0.01$) than rest of the CC and FC; and non-significant between FC and CC at same stocking density of $548\text{cm}^2/\text{b}$. The highest significant pecks were observed in CC of stocking density of $645\text{cm}^2/\text{b}$ and significant less pecks in FC- $645\text{cm}^2/\text{b}$ x 6B could be probably because that a social order has been established and they were engaged in other natural behaviors like perching and scratching. In Present experiment feather pecking findings are not similar to findings of Appleby *et al.*, (2002) who reported that aggressive behavior was more frequent in furnished cages than in C cages ($P < 0.05$), but this was confounded by number of birds per cage; in furnished cages with 8 hens/ cage there were 1.6 pecks/ hen hour, with 5 hens/cage there were 0.8 and in C cages with 4 hens there were 0.4. Which means stocking density per cage plays major role in feather pecking. Contrasting findings on feather pecking with highest feather pecking in furnished cages is reported by Li *et al.*, (2017) as a result of more socializing in furnished cages. The space allowance provided in these furnished cages range from 1000-1400 cm^2/bird and for conventional cage is $528\text{ cm}^2/\text{hen}$, while in the present experiment the difference in stocking density for conventional and furnished cages is 645 and $548\text{cm}^2/\text{b}$; or almost non-existent FC-548 = CC- $548\text{cm}^2/\text{b}$, FC-645 = CC- $645\text{cm}^2/\text{b}$.

There was no scratching and perching activity in the conventional California group, otherwise birds provided with perches spend 15 to 18 % of 24-hour time sitting on the perches and around 4 to 5% time scratching as evident from the birds in furnished cages. Bennett *et al.*, (1997) shown that presence of a perch in a cage resulted in significant perching activity which was at a higher frequency than in the floor pens ($P < 0.01$) which might be concluded that the large amount of time that birds spent perching in that treatment (46.5% of

Fig. 4.15. Comparison of behavioral activity of laying birds raised in Furnished Vs conventional California Cages

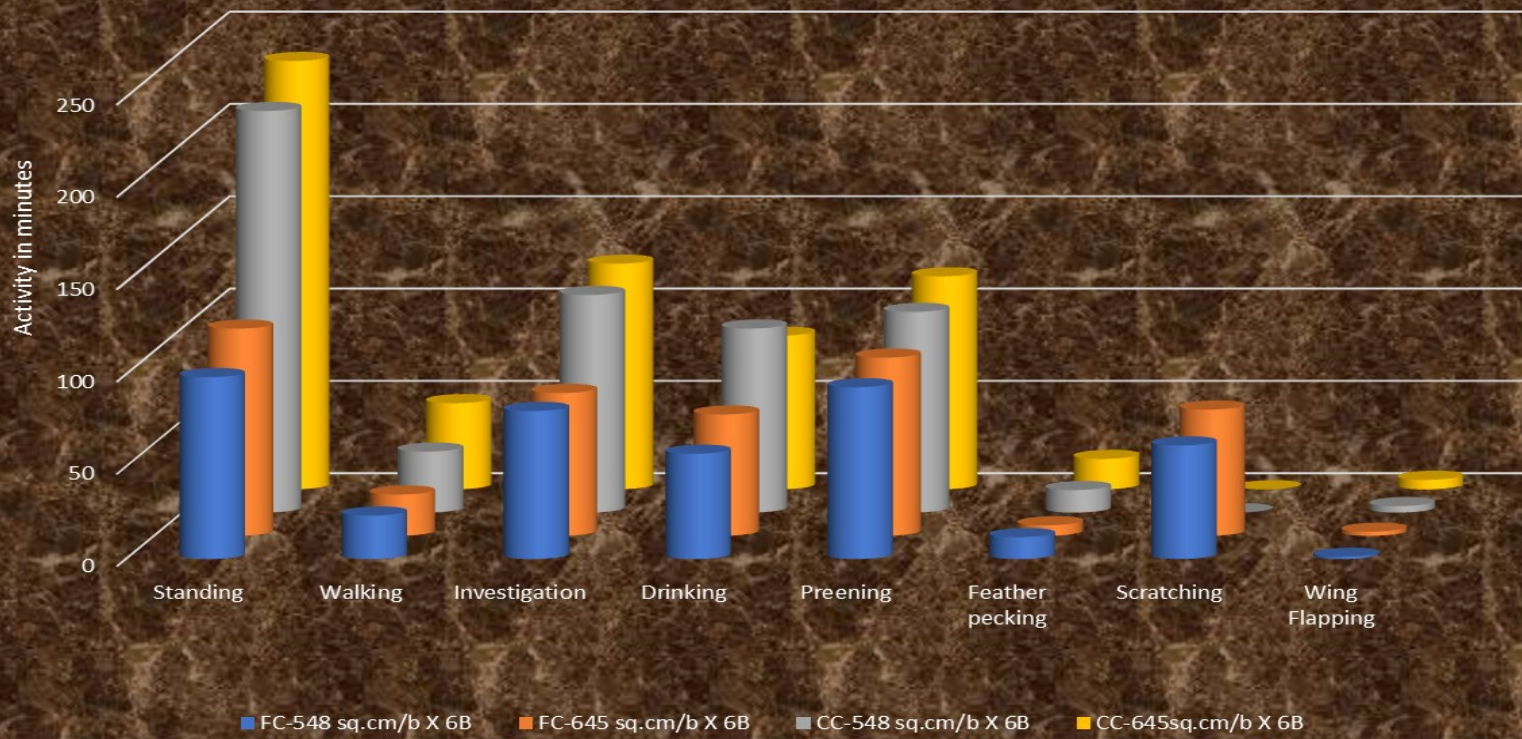
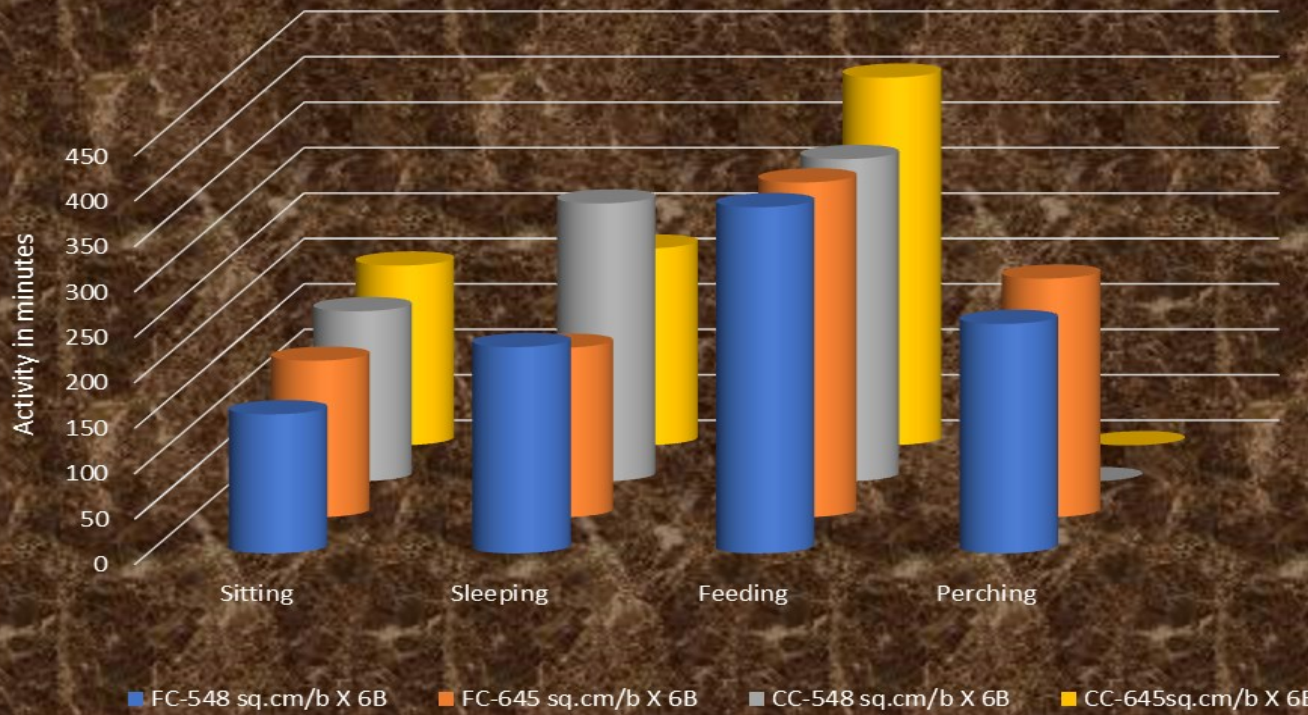


Fig.4.16. Comparison of Sitting, Sleeping, Feeding and Perching activity of laying birds raised in Furnished Vs conventional California Cages.



observation time) might result in improved feather condition and cover through a reduction in pecking behavior. In the present experiment 12 to 18% of the time birds were perched of the total 24 hours activity and 36 to 55% during light off period were perched for birds in furnished cages but did not bring any differences in feather score. Tauson (1998) reported that birds in Modified and Enriched Cages (MEC) used perches 96% during night. Further it is concluded that fully furnished cages for small groups of birds (<10 percentage) may compete well with conventional cages as far as production and mortality are concerned. The smaller group size and, hence, probably, more stable rank order, the improved hygiene due to the presence of perches on only one level, and the greater ease of inspection, are some of the benefits of MEC.

Comparison of wing flapping activity in FC and CC resulted significantly poorest in FC-548cm²/b x 6B and significantly best (P<0.01) in CC-645cm²/b x 6B possibly nest curtains and perches provided a hindrance for wing flapping in furnished cages. Non-significant differences with FC-645cm²/b x 6B and CC-548cm²/b x 6B was evident, though significantly poorer than CC-645cm²/b x 6B. Appleby *et al.*, (1993) indicated that the modified cages had increased area, height and facilities compared to conventional cages, and birds apparently had considerable local freedom of movement; for example, occasional wing-flapping was observed. Clearly, however, they did not have freedom to carry out large-scale locomotion. It remains to be determined whether this behavior is actually important for welfare. No evidence of frustration was seen in these hens, but research on the motivation of hens for locomotion is obviously required. Comfort behaviors like wing flapping, head scratching and tail wagging are more common in furnished cages than in conventional cages as reported by Appleby *et al.*, (1993) while other comfort behavior like aggression and feather pecking did not differ between furnished and conventional cages which is in contrast to present comparison of furnished and conventional cages.

Investigation and feeding activity reported by Barnett *et al.*, (1997) in furnished cages and conventional cages, indicated no effect of furniture (P>0.05) on hen activity as measured by movement of heads or investigation, neither any

effect on feeding activity, similar to the present data comparison for conventional and furnished cages for feeding activity but not so in investigation.

4.3.7 Cost of Production Per Egg (CPPE) on the basis of recurring expenses and space utilization:

The Cost of Production per Egg (CPPE) for the furnished cages was calculated on the basis of egg production, recurring expenses mainly i.e feed, pullet cost, medicine, vaccine and miscellaneous expenditure incurred during this experimental period (20 weeks). To this recurring cost, the cost of furnishing was included for each cage (cage size and furniture specification per bird/cage mentioned in Table 3.8) for fixing perch, scratch pad and nesting curtains to find the CPPE of FC. The furnishing cost was almost similar between the flock size (6 and 9 B) at the two stocking density 548 and 645cm²/b, as the amount of furnishings, that is size of scratch pad or nesting area was directly proportional to the number of birds, though a mere difference arose because of perch length. The furnishing cost per bird for FC-548cm²/b x 6B group is Rs. 42.19; FC-548cm²/b x 9B group is Rs. 40.45; FC-645cm²/b x 6B group is Rs. 43.86 and for FC-645cm²/b x 9B group is Rs. 42.12. All the material required for furnishing were procured from local market.

The interaction effect of stocking density and flock size and the main effect due to flock size on the CPPE on the basis of recurring cost was found to be significant ($P < 0.05$) for birds raised in various furnished cages and is presented in Table 4.47. FC-548cm²/b x 6B and FC-645cm²/b x 6B had the lowest value when compared to other furnished cages, also flock size 6 birds produced the lowest CPPE. But there was no main effect of stocking density-alone on CPPE when recurring cost and furnishing costs were included. The CPPE on basis of space utilization had seen to effect significantly ($P < 0.001$) for the interaction effect and the main effect for flock size and stocking density. As CPPE on basis of space utilization is dependent on the space allowance and the number of birds, therefore a higher value output is obvious for the birds in flock size of nine birds with 645cm²/b stocking density.

Treatment group FC-548cm²/b x 6B were significantly lower for CPPE on basis of space utilization when compared with FC-645cm²/b x 6B ,FC-548cm²/b x 9B and FC-645cm²/b x 9B for the interaction effect of stocking density whereas, the main effect of flock size in 6 birds was significantly lower than in 9 birds and stocking density at 548cm²/b was better than 645cm²/b.

Table 4.47. Interaction effect of stocking density and flock size on Cost of Production Per Egg (CPPE) of laying birds in furnished cages

Treatment	CPPE on the basis of recurring expenses (₹)inclusive of Furnishing cost (FC).	CPPE on the basis of space utilization (CPPE on basis of recurring expenses X space allowance X number of birds)
FC-548cm ² /b X 6B	2.87 ^b ± 0.03	1463.52 ^d ± 16.43
FC-548cm ² /b X 9B	3.23 ^a ± 0.17	2470.45 ^b ± 128.70
FC-645cm ² /b X 6B	2.88 ^b ± 0.05	1725.79 ^c ± 27.04
FC-645cm ² /b X 9B	3.01 ^{ab} ±0.07	2706.49 ^a ± 66.54
Effect of stocking density		
548cm ² /bird	3.05 ± 0.07	1966.98 ^b ± 52.43
645cm ² /bird	2.94 ± 0.07	2216.14 ^a ± 52.43
Effect of flock size		
6 Bird	2.87 ^b ± 0.07	1594.65 ^b ± 52.43
9 Bird	3.12 ^a ± 0.07	2588.47 ^a ± 52.43
Pooled SEM	0.054	112.28
Statistical significance		
Treatment	*	**
Stocking density	NS	**
Flock size	*	**

Means bearing superscript within a column differ significantly. NS- Non-significant, *P < 0.05, **P < 0.01.

Table 4.48. Cost of Production Per Egg (CPPE) of laying birds in furnished cages vs conventional California cage

Treatment	CPPE on the basis of recurring expenses (₹) inclusive of Furnishing cost (FC).	CPPE on the basis of space utilization (CPPE on basis of recurring expenses X space allowance X number of birds)
FC-548cm ² /b X 6B	2.87 ^a ± 0.03	1463.52 ^b ± 16.43
FC-645cm ² /b X 6B	2.88 ^a ± 0.17	1725.79 ^a ± 27.04
CC-548cm ² /b X 6B	2.44 ^b ± 0.02	1246.35 ^c ± 9.18
CC-645cm ² /b X 6B	2.43 ^b ± 0.03	1459.17 ^b ± 21.80
SEM	0.080	36.561
P-value	**	**

A comparison for the CPPE on the basis of recurring expenditure and on the basis of space utilization for conventional and furnished cages is presented in Table 4.48 and Figure 4.17. The CPPE on basis of recurring expenses for the conventional cages at stocking density of 548 and 645cm²/b was significantly lower than its counterpart on the furnished cages as cost of furnishing was non-inclusive in conventional. Similarly, for CPPE on basis of space utilization was significantly lowest in CC-548cm²/b x 6B group as compared to other groups FC-548cm²/b x 6B, FC-645cm²/b x 6B and CC-645cm²/b x 6B which had same flock size of six birds but the additional furnishing costs in each FC group escalated to bring a significant difference for furnished cages. Also, it is interesting to note FC-548cm²/b x 6B and CC-645cm²/b x 6B were non-significant, possibly because of non-significant similar egg production. It was observed that a difference of Rs. 0.44 – 0.45 in CPPE on basis of recurring expenses existed for birds when raised in furnished cages and further concluded that such a difference could be ignored considering the natural behaviors the birds can exhibit in furnished cages.

Tauson (1998) reported a slightly larger group size would reduce the investment cost per hen regarding nest and dust-bath as well as increasing the total available area per bird, although it is already 40% greater per hen in the modified and enriched cages (MEC) than in a conventional cage with 600 cm². Group size is a very important issue because the restricted area in a cage will remain a critical point and more such studies are under way. A future possibility might be a system similar to the MEC, in which the obvious advantages of hygiene and small group sizes in cages can be combined with an increased behavioral repertoire similar to that of hens kept in loose housing systems. Such improvements in housing system will also have associated costs, but may be less expensive than more radical alternatives, while having more predictable benefits for welfare. Magnale (2018) observed the highest profit was obtained for birds maintained in furnished cages of 750cm² of stocking density, although at the same space for battery cages recorded the lowest profit, because of egg production. However, only the working capital was considered but not the actual cost of cages, equipment's, efficiency of space utilization and labour.

SUMMARY AND CONCLUSIONS

In India the poultry sector has undergone colossal transformation in structure and operation from a mere backyard activity into a major commercial agri-based industry over a period of time. The intent of poultry production is to provide safe and healthy animal protein to the ever-growing population, overcoming the challenges cropping up on its sustainability. Egg laying hens are kept traditionally in conventional cages (battery cage) for high intensity egg production so as to maximise egg production per unit area. In the process the hens have to live for the entire production cycle in a very limited space as the sole objective is to increase the productivity and profit. This neglected the welfare state of caged hens which led to introduction of new minimum welfare standards.

Since EU banned cage rearing of birds, the cage rearing become a global issue in poultry sector. Recently in India issues relating to animal welfare and environmental pollution raised by few of the NGOs are of increasing concern. In parallelism the use of battery cages in India is now raising a considerable debate pertaining to the relative effect of the practice on hen well-being.

Better stocking density, optimum flock size and raising birds in furnished cages are some of the attempts brought about in a response to the criticism of raising commercial laying hens in conventional battery cages for egg production. Optimum space along with comfortable flock size and furnished cage systems provide an enriched environment (i.e., facilities) to meet the welfare needs of laying hens.

The Animal Welfare Board of India advised to the Government of India and the State Governments to issue suitable directions to poultry farmers to prohibit the use of battery cages in egg production, so that poultry farms keeping egg laying hens adhere to the provisions of the Prevention of Cruelty to Animals (PCA) Act, 1960. In response, on 29th April 2019 Ministry of Agriculture and Farmers welfare under G.S.R. 335 (E) published a draft of Gazette of India on the space allowance for conventional colony enclosures of commercial egg-laying hens. Draft indicated that the floor space per bird shall not be less than 550 sq. cm

and each cage should accommodate preferably a minimum of 6-8 birds, thus ensuring reasonable space for laying hens for lying down, standing up, flapping wings, turning around and access to feed and water.

In Indian context California cage space standards for rearing commercial laying hens must be tested. There is need to develop furnished cages with little modifications in existing California cages to maintain welfare friendly environment within the routinely used California cage system. The little modification in California cage system involves addition of furnished items (perch, dust bath, scratch pad etc) at different stocking density and flock size. Hypothesis of these furnished cages was, it will be one step ahead to maintain the welfare of the commercial laying hens' as well economically feasible solution for the poultry farmers. Therefore, the present investigation was carried out at Poultry Research and Training Centre, Department of Poultry Science, Nagpur Veterinary College, M.A.F.S.U, Nagpur with an objective to study the effect of stocking density, flock size in conventional California and modified furnished cages on well-being and the performance of commercial layers. In experiment I the effect of different stocking density (60 sq. inch/bird or 387 cm²/bird, 75sq. inch/bird or 484cm²/bird, 85 sq. inch/bird or 548cm²/bird and 100 sq. inch/bird or 645 cm²/bird) and on the basis of I experiment results (best two stocking density) II experiment was carried out to test inter relationship between best stocking densities (548 and 645 cm²/b) and flock Size (06 and 09 birds) on production, welfare and behaviour of laying birds was determined. In experiment III the relationship between best stocking densities (548 and 645 cm²/b) and flock Size (06 and 09 birds) with enhanced or modified furnished cages reared birds was determined on basis of production, welfare and behaviour and further compared with conventional California cages reared birds.

To achieve stated three objectives a total of 600 commercial White Leghorn layers (BV-300) were reared, 240 in experiment I and 180 birds each in experiment II and III. The birds were provided free access to fresh and clean drinking water through nipple system and offered with 110 gm feed/day/ bird as per recommendation. The standard specifications of BV-300 manual were

followed for all feeding and management practices including medication and vaccination. The birds that were dead during the trial period was replaced by layers of same age that was reared in similar condition so as to maintain the stocking density and flock size for the different experiments. The differences among treatment groups were determined by analyzing the data generated, by using Complete Randomized Design (Snedecor and Cochran, 1994). Data produced from experiment I for stocking density and comparison of furnished and conventional California cages from experiment was analysed by one-way ANOVA and interaction effect of stocking density and flock size for experiment II and experiment III was analysed by two-way ANOVA using SPSS software package version 20.0. The replicate means were used for all the parameters to generate the data for statistical analysis.

The experiment I was conducted from October 2018 to March 2019 to study the effect of stocking density on production, welfare and behavior of laying hens in conventional California cages. From experiment I, it was noted that the birds reared with 645 cm²/bird recorded significantly highest ($P < 0.01$) HDEP % as compared to 387 and 484 cm²/bird during 28- 47 week age i.e for 20 weeks of experimental period. However, there was no significant difference in between 645 and 548 cm²/b reared groups. HDEP % followed a linear trend of decreasing as the stocking density increased. High stocking density creates more competition among the birds for food and space and consequently the physiological stress results in lower egg production. Less stress at stocking density of 548 and 645 cm²/bird when compared to higher stocking densities was evident from the results of H/L ratio and corticosterone (ng/ml) measured at the 47th week that is towards the end of the experiment.

As FCR is related to the egg production, the birds reared with space 387 cm²/bird had the lowest egg production with significant poorest (1.58 and 2.38) FCR (per dozen eggs and per kg egg mass) than 484, 548 and 645 cm²/bird during the 20-week experimental period. At the same time treatment group 484, 548 and 645 cm²/birds were non-significant among themselves. The improvement in FCR is attributed to the fact that hens in lower density cages faced less competition for

feed intake and stress free movements resulted into better feed conversion to produce the eggs. There was non-significant effect on live body weight and body weight gain as the birds were offered an optimum amount of feed as per the standards mentioned for the White Leghorn strain.

Stocking density could not bring any changes for the external and internal egg quality parameters except for egg weight, albumen index and yolk index. The eggs laid by the experimental birds reared at highest stocking density (387cm²/bird) were recorded significantly ($P < 0.01$) lowest egg weight (52.44± 0.34) among all the birds reared at different treatment stocking densities. As the stocking density improved (387 to 548 cm²/bird), the egg weight was increased (52.44 to 55.88 g), whereas, there was not much difference in egg weight of the birds reared at stocking density 484 to 645 cm²/bird. As food is partitioned for maintenance, growth, reproduction and health, stressful condition in high density cages will lead to most of the food to be utilized to cope unpleasant conditions, the lower egg weight, egg production and FCR could be related with this.

There was a reverse effect on albumen index, with significantly ($P < 0.05$) better in 387 cm²/bird group as compared to the other treatments, also yolk index was significantly better in 387 cm²/bird group, when compared to 548 cm²/b and 645 cm²/b and statistically same with 484 cm²/b. It may be presumed the protein requirement for egg production was diverted for better albumen and yolk index (HDEP% was significantly lower in 387 and 484cm²/b).

The physical indicators of bird welfare that is feather and gait score determined for the various stocking density showed non-significant effect for gait score, but feather score showed significant differences ($P < 0.05$) between 387 cm²/bird with lowest score when compared to rest of the stocking density treatment groups 484, 548 and 645 cm²/bird. The birds reared at higher stocking density (387 cm²/bird) depicted the frayed feathers at the end of experiment because of feather rubbing on wire cages, few feathers around the base of the neck were also removed off as there was less feeding space to reach the feeder through the cages at higher stocking density, as they tried to synchronize their feeding activities. It was observed that feather pecking behavior was higher in stocking

density of 5 birds or with space allowance of 387cm²/bird than the other stocking densities per bird, which may one of the cause for poor feather score in 387 cm²/bird group.

Stocking density had no influence on the average value of the gait score and the antibody titer against New Castle Disease (ND) measured at different time interval. It was found non-significant for the birds reared at different treatments of stocking density during the entire period of the experiment. Though social stress associated with competition and changing group composition is known to cause decreases in circulating antibody levels, but was not seen in the present experiment probably the stress was not very severe to cause a change in the level of immunity.

In regard to the heterophil-lymphocyte ratio and corticosterone level, the birds reared at 548 and 645cm²/bird had significantly lower heterophil-lymphocyte ratio and less corticosterone level as compared to the birds reared in 387cm²/bird, while when compared with the treatment group 484cm²/b showed non-significant changes at 47th week of bird's age. The birds reared at space 548 cm²/b had recorded the significant lowest corticosterone level as compared to others, indicating less stress upon birds, and highest stress was observed in birds reared with space allotment 387cm²/bird. The elevated concentration of corticosterone in blood circulation in 387cm²/bird group is thought to cause an increase in heterophil count and a decrease in the lymphocyte count, which are the indicators of stress. It could be concluded that by the end of the experimental period that is at 47th week of age of the bird, birds maintained at stocking density of 387 cm²/bird and 484 cm²/bird were seen to be under stress also 645cm²/b had higher corticosterone as compared to 548 cm²/b.

The effect of stocking density on behavioral activity was studied by budgeting the 1440 minutes of the entire day at three different period (28, 37 and 47 week) of experiment and averaged into the various state and event activity as performed by the bird and totaling it. It was noticed that the birds in 548 cm²/b group had significantly ($P < 0.001$) standing activity and had more walking minutes in comparison to 387, 484 and 645 cm²/b. Standing and locomotion

(walking) are inter-related and decrease in locomotion in high density cages is always accompanied by more standing which is not seen in these 548 cm²/b groups. Highest sleeping activities were evident in 548 cm²/b group indicating less disturbances by peers when there is sudden movement or indirectly indicating sufficient space. Grooming and preening, the incidence of maintenance behavior (feeding, drinking, sitting, standing, walking, preening) was best seen in low density cages and the highest significant value ($P < 0.001$) was observed in 548 cm²/b followed by 645 cm²/b, 484cm²/b which were non-significant among themselves for the average preening values of 28th, 37th and 47th week of bird's age (Table 4.12). For comfort/ natural behaviors like wing flapping as the stocking density improved there was a linear significant increase in the wing flapping activity with highest in 645 cm²/b followed by 548 cm²/b which were non-significant, but significantly higher than 484 cm²/b and 387 cm²/b.

The averaged values for feeding and drinking for the various stocking density groups were non-significant with each other. Probably the case of dominant birds to monopolize the limited resources because of less feeder space did not arise in our study. Locomotion is reported to be related with investigations, caged birds show less locomotor activity and consequently more scanning or investigation, such stationary visual exploration substitute for locomotor exploration. The average value collected (Table 21) showed highest significant ($P < 0.001$) investigation in 484 cm²/b, followed by 645 cm²/b, 387 cm²/b and 548 cm²/b with significant differences among each other.

Sitting was observed to be higher in high density cages with linear increase in percentage of time (except 484 cm²/b) as stocking density increased, 25% for 387 cm²/b, 14% for 484 cm²/b, 18% for 548 cm²/b and 17% for 645 cm²/b. On an average 1.1% of the time was engaged on feather pecking activity by the birds reared at 387 cm²/b which was significantly ($P < 0.01$) higher than the rest of the treatment groups. The birds reared at 548 and 645cm²/b showed significantly less feather pecking compared to the birds reared at 387 and 484cm²/b. There was linear trend for feather pecking activity associated with

increase in stocking density which could be due to social facilitation or social recognition among the bird mates resulting in feather pecking more in 387cm²/b.

The cost of production per egg (CPPE) at different stocking density, was observed, because of egg production difference the birds reared at 387cm²/bird had significantly (P<0.01) higher cost of production (₹ 2.74/egg) than the rest of the treatment groups. The CPPE on the basis of recurring expenditure was found non significant among the birds reared with space at 484, 548 to 645 cm²/bird. The CPPE on the basis of space utilization was found to be more with higher stocking density group, as number of birds were more in limited space with less production performance. It was noticed that, as space allowance increased the egg production percentage as well CPPE on the basis of recurring expenses improved, with better welfare points. Therefore, considering the CPPE on the basis of recurring expenses and space utilization it was confirmed that the birds reared at 548 cm²/b emerged as best treatment group.

After the end of first experiment the birds/treatment were assessed for different parameters of stocking density, production and welfare of the birds on the basis of developed well being score card. The top two groups (548 and 645 cm²/b) scored highest marks (84 and 82) were selected for further investigation. The findings of the first experiment were further utilized to conduct the second experiment, on the basis of developed score card. It was confirmed from first experiment that the rearing of commercial laying hens in California cage system at 548 and 645 cm²/b gave best results in terms of production, welfare, behaviour and economics. Hence, second experiment was planned to test the interaction effect of best two space allowances (548 and 645 cm²/b) and flock size (06 and 09 birds) to know the production, welfare and behaviour of commercial laying hens. The experiment was started in October 2019 and continued till March 2020. A total of 180 laying hens were maintained for 20 weeks from 28 week – 47 week of birds age with four different treatment groups of 548 cm²/b with 6 and 9 bird; 645 cm²/b with 6 and 9 bird.

The interaction effect of stocking density and flock size did not bring any significant difference on the HDEP % in entire 28-47 week, but birds maintained

at 548 and 645cm²/b with flock size 6B was noted a trend of better production due to interaction effect of stocking density and flock size, but not enough to elicit a statistical significance among the various treatment groups. The main effect because of stocking density could not produce a change in HDEP% for the entire 20-week period, while main effect of flock size shown significant results (P<0.05) with best performance in 6 bird flock size. It could be concluded that though flock size with different stocking density could not evoke significant differences, but with smaller flock size of 6 birds produced numerically higher eggs at stocking density 548 and 645cm²/b.

Similar to HDEP %, interaction effect of stocking density and flock size did not bring any significant difference for Feed Conversion Ratio (FCR) values for the individual 4 weekly periods and entire 20week experimental period except for 44-47 week of bird's age. The main effects for stocking density on FCR per dozen egg did not lead to any significant differences but main effect for flock size of 6 birds for entire 20 week and in 44 – 47 week proved significantly (P<0.05) better. A trend for a better FCR in small flock size (6B) at stocking density of 548 and 645cm²/b was observed for the interaction effect of flock size and stocking density, as it was seen in main effect for flock size. The poorer efficiency in 9 bird flock size is related with low egg production in 548 and 645cm²/b treatment groups. The performance in different group size is related to development of a stable hierarchical, social structure and a tolerant social system.

The results for Live Body Weight (LBW) and Body Weight Gain (BWG) revealed that initial and final body weight in experimental birds did not differ significantly due to either main effect of stocking density or flock size or interaction when maintained at flock size of six and nine birds with stocking density of 85 sq. inch or 548 cm² and 100 sq. inch or 645 cm² per bird.

The results for external egg quality parameters for the entire period (20-47 weeks) were non- significant for the interaction effects, main effect of stocking density and flock size, except for shape index at interaction. Egg Shape Index was found to be best for birds in 645cm²/b with flock size 9B for the interaction effect with significant differences (P = 0.01) in comparison with 548cm²/b with flock

size 9B and 645cm²/b with flock size 6B, but non-significant with 548cm²/b with flock size 6B. The interaction and main effect of stocking density and flock size were non-significant for all the internal egg quality parameters except for shell weight for stocking density and shell thickness with flock size. The shell weight was significantly better at lower stocking density (100 sq. inch /645 cm² per hen) as compared to higher stocking density (85 sq. inch/ 548 cm² per hen). Shell thickness was significantly better in flock size of nine birds as compared to six birds.

Interaction and main effect of stocking density and flock size were non-significant (Table 4.21) of the physical indicators of welfare parameters viz., feather score, gait score and physiological indicators viz., heterophil-lymphocyte ratio, serum corticosterone and immune status determined at 28th, 37th and 47th week of bird's age. The behavioral activity of the bird due to the interaction effect of stocking density and flock size also had same non-significant differences among the treatment groups for most of the averaged (Table 4.26.) behavioral activities determined from 28th, 37th and 47th week of age. Sleeping, drinking and feather pecking activity were the exceptions; The birds reared at 548cm²/b x 6B group had the most sleeping activity followed by 548cm²/b x 9B, 645cm²/b x 6B and 645cm²/b x 9B and all the treatments were significantly different (P<0.001) from each other. Significant more drinking activities (P < 0.01) were observed for 548cm²/b x 6B and 548cm²/b x 9B as compared with 645cm²/b x 6B and 645cm²/b x 9B. The birds reared in 548cm²/b x 6B group were more comfortable with very less (P< 0.05) feather pecking behavior when compared to the rest groups. The main effects for flock size on averaged behavioral value was observed for only sleeping activity which was for more duration in smaller flock (6B) than larger flock size (9B). The main effects for stocking density on the averaged behavioral values were observed for standing (highest for 645cm²/b), sleeping and drinking (highest for 548cm²/b).

The interaction effect of stocking density and flock size on the CPPE on basis of recurring expenses was non-significant for the all treatment groups. The non-significant effect is attributed due to similar egg production in all the groups

for the 20 weeks experimental period. There was no significant change on CPPE on basis of recurring expenses due to the main effects of stocking density and flock size. The CPPE on basis for space utilization, however a huge significant change could be noticed which was depend upon the space provided and number of birds in that particular space were statistically different among treatment groups. As expected the CPPE on basis of space utilization was significantly ($P < 0.01$) lower in the small flock size ($548\text{cm}^2/\text{b} \times 6\text{B}$ & $645\text{cm}^2/\text{b} \times 6\text{B}$), for the interaction effect and main effect of flock size.

In parallel to experiment II, Experiment III was carried out with the aim of rearing the commercial White Leghorn (BV 300) layers in furnished cages (with facility of nesting area, perch and scratch pad etc) at the best of stocking density 548 cm^2 and 645 cm^2 per bird and flock size with 6 and 9 birds (on the basis of I Experiment) to test the production, welfare and behaviour of the birds. It was carried with an objective to study the comparative laying hens performance in conventional California cages (Experiment II) Vs furnished California cages (Experiment III). The experiment was started in the month of October 2019 and continued till March 2020. A total of 180 laying hens were maintained for 20 weeks from 28 week 47 week of bird's age in four different treatment groups of FC- $548\text{cm}^2/\text{b} \times 6\text{B}$, FC- $548\text{cm}^2/\text{b} \times 9\text{B}$, FC- $645\text{cm}^2/\text{b} \times 6\text{B}$ and FC- $645\text{cm}^2/\text{b} \times 9\text{B}$.

The interaction effect of stocking density and flock size in furnished cages on the overall HDEP% of the birds showed highly significant differences among themselves when maintained at two stocking densities and two flock sizes. The best results were found in FC- 548 and $645\text{ cm}^2/\text{b} \times 6\text{B}$, compare to FC- 548 and $645\text{ cm}^2/\text{b} \times 9\text{B}$ in concern to HDEP %. The birds reared in FC- $548\text{cm}^2/\text{b} \times 9\text{B}$ group which had a higher flock size and higher stocking density produced significantly ($P < 0.05$) less HDEP % than the rest of the groups. There was no changes in HDEP % due to main effect of stocking density while flock size produced a highly significant ($P < 0.01$) change in the furnished cages with smaller flock size (6 birds) when compared to larger flock (9 birds). Comparison of FC with CC had resulted in non- significant differences over the various four weekly

periods and the overall period, though numerical higher HDEP% were observed in Conventional cages maintained birds at 548 and 645 cm²per bird. Furnishing the California cages could not produce a significant response in the egg production. It can be concluded that furnishing the cages did not bring any significant change in HDEP % compared to conventional California cages as the space provided was similar in this study.

The Feed Conversion Ratio (per dozen egg) for the entire 20-week period for the different furnished groups were neither affected by the interaction effect of stocking density and flock size in furnished cages nor by the main effect of stocking density, however main effects of flock size were evident with better FCR (per dozen eggs) in flock size of 6 birds than 9 birds. Similar to HDEP %, comparison of FC with CC had resulted in non- significant differences over the entire 20 week period, though numerical better FCR values were generated in conventional California cages maintained birds at 548 and 645 cm²per bird.

The interaction effect of stocking density and flock size in furnished cages on the overall revealed that live body weight change in layers did not differ significantly among the various treatment groups of furnished cages because of interaction effect, stocking density and flock size. Though the main effect due to flock size brought about a significant change ($P < 0.01$) in higher weight gain in the larger flock size of nine birds as compared to six birds. The reason was unknown but could result from lower HDEP%, poorer FCR and lower egg weight in flock size of 9 bird as compared to 6 birds As a result the energy may get diverted towards weight gain instead of egg production in 9 birds flock group could be possible reason.

On comparison of body weight of birds in furnished cages with that of conventional cages maintained in same stocking density (548 and 645 cm²/hen) no significant difference was noticed. It is thought that non-significant difference in body weight between conventional and furnished cages is because feed consumption was similar and the entire energy being directed towards egg production and egg mass. Also, a moderate group of 6 to 9 birds did not influence the body weight due to lesser competition for resources.

Maintaining the layers in furnished cages at different stocking density (548 and 645cm²/bird) and flock size (six and nine birds) showed non significant interaction effect for the various external and internal egg quality parameters excepting for egg weight. The egg weight was significantly better in FC-548cm²/bird x 6B compared to FC- 645cm²/bird x 9B, moreover, main effect of flock size on egg weight was observed better for 6 birds than 9 birds. Same trend in 6 bird flock size was observed for egg albumen height, with significant difference (P<0.05) between six and nine birds. A comparison of external and internal egg quality in furnished and conventional California cages did not bring any significant difference probably because of same feed, space and flock allotment, furnishing couldn't bring any significant change.

Physical indicators of welfare parameters like feather score and gait score and physiological indicators- antibody titers value were non-significant for the interaction effect for furnished cages and for the main effects of stocking density and flock size. However, there was significant (P < 0.05) interaction effect in furnished cages on the physiological indicators of welfare like heterophil/lymphocyte ratio and corticosterone level during the mid (37 wk) and end (47 wk) of the experimental period. At the end of the experimental period FC-548cm²/b x 6B group has the significant lowest H/L ratio as compared to the rest of the furnished cages. For corticosterone value towards the end of experiment FC-548 and 645 cm²/b x 6B had significantly (P < 0.001) lowest level than FC-548 and 645 cm²/b x 9B. No significant difference for main effect of stocking density and flock size was observed for H/L ratio, though main effect of stocking density and flock size brought changes in corticosterone level.

The comparison of the various physical and physiological indicators of welfare like feather score, gait score and antibody titer (except for 28th week) for modified furnished cages and conventional California cages were non-significant. The heterophil/lymphocyte ratio, shown significant differences among the furnished (FC) and conventional (CC) cages only at the end of the experiment with significantly lowest (P < 0.05) value in FC-548cm²/b x 6B as compared to other furnished and conventional cage though it remained non-significant with

CC-645cm²/b x 6B, having a lower stocking density. At stocking density of 548cm²/b the difference in H/L ratio between furnished and conventional cage indicated that furnished cages were in lower level of stress than conventional California cages. Significant different corticosterone value between furnished and conventional California cages was observed only during the start or 28th week and at the end of the experiment that is 47th week of bird's age. At the end of the experiment significant differences ($P < 0.05$) were observed for birds maintained in furnished cages had lower corticosterone (ng/ml) value indicating lower level of stress as compared to conventional California cages.

The behavioral activities presented as an average for the three evaluated period (viz determined at 28th, 37th and 47th week of bird's age) recorded significant differences for standing, walking, sleeping, feeding, preening, feather pecking, perching, wing flapping, total state and event behavior due to interaction effect of stocking density and flock size (Table 4.26). FC-645cm²/b x 9B group had the highest activity for standing, walking, feeding, preening, feather pecking and wing flapping when compared with the other furnished cages, while sleeping and perching activity for FC-645cm²/b x 9B group was comparatively less. The main effects due to stocking density could be observed only for standing, preening, perching, wing flapping with significant higher values for 645 cm²/bird space than 548cm²/bird; while for sleeping and feeding 548 was better than 645cm²/bird. The main effect due to flock size was noticeable only for walking, feeding, perching and wing flapping activities, with better perching by 6 bird flock size, while flock size of 9 bird had higher walking, feeding and wing flapping activities.

A comparison of behavioral activity of conventional California cages and furnished cages showed highly significant ($P < 0.01$) effect of perching and scratching in furnished cages over the conventional California cages.

It is interesting to note in absence of scratch pad and perch birds in CC-548 and 645 cm²/b x 6B conventional California cages had the significant more standing, sitting, walking, sleeping, investigation, drinking, feather pecking activities when compared with similar stocking density groups in furnished cages.

Battery cages limit the expressions of bird's natural behaviors, there was more sitting in conventional cages. In the present comparative study the higher standing and walking in conventional cages might be due to provision of a perch in the furnished cages which rather gave comfort to the birds to perch more than standing leading to less standing behaviour and walking activity. Feather pecking in FC-645cm²/b x 6B was significantly lower ($P < 0.01$) than rest of the CC and FC; and non-significant between FC and CC at same stocking density of 548cm²/b. The highest significant pecks were observed in CC of stocking density of 645cm²/b and significant less pecks in FC-645cm²/b x 6B. This could be probably because of social order had been established and they were engaged in other natural behaviors like perching and scratching. Comparison of wing flapping activity in FC and CC resulted significantly poorest in FC-548cm²/b x 6B and significantly best ($P < 0.01$) in CC-645cm²/b x 6B possibly nest curtains and perches provided a hindrance for wing flapping in furnished cages. Non-significant differences with FC-645cm²/b x 6B and CC-548cm²/b x 6B was also evident for wing flapping.

The interaction effect of stocking density and flock size on the CPPE on basis of recurring expenses was found to be significant ($P < 0.05$) for birds raised in various furnished cages, but there was no main effect of stocking density alone on the cost of production when recurring cost and furnishing costs were included for the furnished California cages. While the CPPE on basis of space utilization had seen to effect significantly ($P < 0.001$) for the interaction effect and the main effect for flock size and stocking density. As it was dependent on the space allowance and the number of birds, therefore a higher value output is obvious for the birds in flock size of nine birds with 645 cm²/b stocking density. Treatment group FC-548cm²/b x 6B were significantly lower for CPPE on basis of space utilization when compared with FC 645 cm²/b x 6B; FC-548 and 645 cm²/b x 9B for the interaction effect of stocking density. The main effect of flock size on CPPE on the basis of space utilization in 6 birds was significantly lower than in 9 birds and stocking density at 548 cm²/b was better than 645 cm²/b.

Comparison of conventional and furnished cages for the CPPE on basis of recurring expenses, the conventional cages at stocking density of 548 and 645cm²/b was significantly lower than its counterpart on the furnished cages as cost of furnishing was non-inclusive in conventional. Similarly, for CPPE on basis of space utilization was significantly lowest in CC 548 cm² x 6B group as compared to other groups. FC-548 and 645 cm²/b x 6B and CC 645 cm² x 6B which had same flock size of six birds but the additional furnishing costs in each FC group escalated the cost to bring a significant difference for furnished cages. It was observed that a difference of ₹ 0.43 in CPPE on basis of recurring expenses existed for birds when raised in furnished cages. If the ₹ 0.43 difference could be burdened considering the natural behaviors of the birds which can exhibit in furnished cages well.

Conclusions and Recommendation

On the basis of results of present study entitled “Welfare, Behavior and performance of laying hens at different stocking densities and flock size in California and furnished cages” following conclusions can be drawn.

1. The best stocking density for commercial laying hens (White Leghorn) was assessed through developed well-being Scorecard. It was adjudged to 548cm²/b or 85 sq. inch/b with a total score of 84 marks, followed by 82 marks for stocking density of 645cm²/ b or 100 sq. inch/b. The laying hens reared at stocking density 548 and 645 cm²/ b were performed best among all the treatment groups on the basis of stocking density, production, behaviour, welfare and economics of the laying birds.
2. On the basis of first experiment results, best two treatment groups were studied for interaction effect of stocking density of 548cm²/ b or 85 sq. inch/b and 645cm²/ b or 100 sq. inch/b with two flock sizes of six (06) and nine (09) birds. The commercial laying hens (White Leghorn) reared in conventional California cages at 548cm²/ b or 85 sq. inch/b and 645cm²/ b or 100 sq. inch/b with six bird flock size performed best on the basis of production, egg quality and welfare. However, considering the behavior and economics of the rearing

of laying hens at stocking density $548\text{cm}^2/\text{b}$ or 85 sq.inch with 6 birds flock size performed well.

3. Interaction effect of stocking density and flock size resulted in behavioral differences of sleeping, drinking and feather pecking. It could be considered that the interaction effect of stocking density and flock size brought a significant better sleeping, drinking activities and significant less time spent on the feather pecking in the group $548\text{cm}^2/\text{b}$ with flock size of six birds.
4. On the basis of second experiment results, best two treatment groups i.e $548\text{cm}^2/\text{b}$ or 85 sq. inch/b and $645\text{cm}^2/\text{b}$ or 100 sq. inch/b with flock size of six birds reared in conventional California cages and compared with furnished cages at same stocking density and flock size. While comparing conventional and furnished cages the production, egg quality, physical indicators of welfare and immunity parameters were non-significantly different. However, considering the physiological indicators furnished cage birds were stress free while Conventional cages were economically more affordable.
5. Welfare and behavioral parameters like scratching, perching, feather pecking, H/L ratio, corticosterone (ng/ml) level were well expressed by laying hens in furnished cages. A conventional California cage hinders the expressions of natural behaviors of laying hens.

The rearing of commercial laying hens (White Leghorn) in conventional California cages is best suited for minimum stocking density of $548\text{cm}^2/\text{b}$ or 85 sq. inch/b with flock size of six birds considering the production, egg quality, and economics of the layer farming with optimum expression of welfare, behavior of laying hens. The natural behaviors of the laying hens expressed/maintained well in furnished cages of $548\text{cm}^2/\text{b}$ with flock size six birds for cage with dimension of 34'' x 15'' x 16'' (width x depth x height), if the layer farmers bear the expenditure of approx. ₹ 7.0/b as furnishing (Perch, scratch pad and nesting area) cost as one time investment in existing California cages and approx. ₹ 0.43 as additional recurring cost of production per egg.

PROPOSED AREA OF FUTURE RESEARCH

Addressing the welfare of animals and in particular poultry with regards to cage-ban has been pertinent throughout the world. India has left no stone unturned in this regard whether be it Prevention of Cruelty to Animals (PCA) Act 1960 mandated by the Constitution long back or recent publishing a draft on the Space allowance for conventional colony enclosures of egg-laying hens for commercial layer production on 29th April 2019 by The Gazette of India. In an attempt to give the maximum welfare to the layers reared in cages and an environment to exhibit behaviours similar to cage-free bird without compromising production, work on stocking density, flock size and rearing of layers in furnished cages has been focused.

Ministry of Agriculture and Farmers welfare indicated that the floor space per bird shall not be less than 550 sq. cm and each cage should accommodate preferably a minimum of 6-8 birds. Stocking density of 548cm²/b and 645cm²/b in conventional California cages for layers provided the maximum production and welfare for the 20 week experimental period in present experiment. Flock size of six birds at stocking density of 548cm²/b and 645cm²/b resulted the best in comparison to nine birds, however further work on flock size of 12 and 15 birds at different and better stocking density in different permutation combination should be investigated in both California and furnished cages. A further study for the entire production cycle of 72 weeks would generate more substantial data.

Furnished cages have been developed with different size, design and stocking density by various authors so as to allow caged hens to engage in various natural behaviour patterns, however a parity between production and behaviour and welfare is the need of the hour. Studies show the furnished cages can play an important role in improving hens welfare, by decreasing fear, aggression and pecking behavior, and increasing the bone density compared to conventional California cages.

Furnished cages with bigger flock size, better stocking density and deeper cages for more movement of space without disturbing other birds while in locomotion should be investigated.

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APPENDIX- I

MAHARASHTRA ANIMAL & FISHERY SCIENCES UNIVERSITY, NAGPUR

Board of Studies in Poultry Science

NVC/PSC/ 357 /2019/Nagpur

Dt:- 10.05.19

Minutes of 30th meeting held on 10th May, 2019

The 30th meeting of Board of Studies in Poultry Science was held at Poultry Research and Training Centre, Department of Poultry Science, Nagpur Veterinary College, Nagpur on 10th May 2019. Following members were present for the meeting.

1) Dr. A. S. Ranade	Chairman & Associate Dean, M.V.C, Mumbai
2) Dr. S.J.Manwar	Member, PGIVS, Akola
3) Dr. M.V.Dhumal	Member, COVAS, Parbhani
4) Dr. D. N. Desai	Member, BVC, Mumbai
5) Dr. D. B. Bhaisare	Co-opted Member, NVC, Nagpur
6) Dr. M. M. Kadam	Member Secretary

At onset of the meeting Dr. M. M. Kadam, Member Secretary, welcomed the Chairman Dr. A. S. Ranade, Associate Dean, M.V.C Mumbai and all the members of BOS in Poultry Science.

SUBJECT: Confirmation of minutes of 29th meeting of BOS in Poultry Science.

Chairman, Dr. A.S. Ranade & Associate Dean M.V.C Mumbai welcomed all the BOS members. Dr. Kadam read out the subjects and their resolutions of 29th BOS held on 04th Jan 2019 at the PRTC, Dept of Poultry Science, NVC, Nagpur.

Chairman, Dr. A.S. Ranade pointed out that the evaluation of UG examination answer sheet of our university is not being done in proper manner due to shortage of time available for the external examiner, if possible this system should corrected. Moreover the BOS Poultry Science record twelve names of examiners for evaluation of UG answer sheet with an attempt to insure proper evaluation of answer sheet within available time.

The names of the external examiners to evaluate the UG answer sheet are as below

The List of experts for Department of Poultry Science especially for Undergraduate examinations

Sr. No.	Name of faculty	Designation	Qualification	Specialization	Willingness to act as	Address
1.	Dr. Amitav Bhattacharya	Assistant Professor	PhD	Poultry Science	UG Examiner	Dept. of Poultry Science, DDD Farms Campus (Dairy Farm campus), Veterinary College, Mathura-281 001 Uttar Pradesh. Email:- amitav6@yahoo.com, Amitav16@rediffmail.com ; Mobile:- 09411217515
2.	Dr. R. M. Rajpura	Assistant Professor	PhD	Poultry Science	UG Examiner	Deptt. of Animal science B A college of Animal Anand Agriculture University Anand, 388 110 Email:-raisvet@gmail.com; Phone:- 09408423121
3.	Dr. Vasanthi B.	Assistant Professor	PhD	Poultry Science	UG Examiner	Veterinary University Training and Research Centre, Melmaruvathur – 603 319 Tamil Nadu Email:- vasdivi@yahoo.co.in, vasanthi.b@tanuv.ac.in Phone:- 91-44-27529548
4.	Dr. Santosh Marandi	Assistant Professor	PhD	Poultry Science	UG Examiner	ILFC, College of Vet. Sci. & A.H. Junagadh (Gujarat State) Email:-drsantoshlpm@gmail.com; Mobile:- 7814259561
5.	Dr. M. Hanumanth Rao	Assistant Professor	PhD	Poultry Science	UG Examiner	Assistant Professor /C.V.Sc, R'Nagar, Hyderabad; Email:-hanumanthrao54@gmail.com Mobile: -09866318361
6.	Dr. E. Tirupathy Reddy	Assistant Professor	MVSc	Poultry Science	UG Examiner	C.V.Sc, Proddatur, Chittoor Dist Email:- tiruvety@gmail.com; Mobile:- 09603137597
7.	Dr. O.P. Dinani	Assistant Professor	PhD	Poultry Science	UG Examiner	College of Veterinary Science & AH Anjora Durg, 491001, Chattisgarh Email:- drdinani@rediffmail.com; Mobile:- 09926947821
8.	Dr. Vimal Antony	Assistant Professor	MVSC	Poultry Science	UG Examiner	Assistant Professor, College of Avian Sciences and Management, Kerala Veterinary and Animal Sciences University, Thiruvazhamkundu, Palakkad-678601, Kerala Email:- vimal@kvasu.ac.in; Mobile :- 9447770207, 9497140207
9.	Dr. Jaydeep Rokade	Scientist	PhD	Poultry Science	UG Examiner	Division of Poultry Breeding & Genetics ICAR-CARI; Izatnagar 243122; Email:- jaydeepvet@gmail.com; Mobile:- 09758703488

10	Dr. Goutam Kallouri	Scientist	PhD	Poultry Science	UG Examiner	Division of Avian Physiology and Reproduction ICAR-CARI Izatnagar Bareilly 243122 Uttar Pradesh Email:- drgau_vet@yahoo.com Mobile:- 09536153139
11	Dr. Laxmi Chauhan	Assistant Professor	PhD	Poultry Science	UG Examiner	College of Veterinary Science & Animal Husbandry, Mhow - 453 446, Nanaji Deshmukh Veterinary Science University, Madhya Pradesh Email:- drlaxmimhow@gmail.com; Mobile:- 8827521670
12	Dr. Girraj Goyal	Assistant Professor	Ph.D.	Poultry Science	UG Examiner	College of Veterinary Science & Animal Husbandry, Rewa, Madhya Pradesh; Email:- drgirrajivri@gmail.com Mobile:-9713241057

Further Chairman, Dr. A.S. Ranade added that Bos meeting should be conducted twice in year, among that one should be one day before JASRC of MAFSU, Nagpur

RESOLUTION NO. 1: With above additional remarks the Minutes of 29th BOS meeting which was held on 04th Jan 2019 at the PRTC, Dept of Poultry Science, NVC, Nagpur were unanimously approved.

SUBJECT: Equivalence of degrees for the subject of Poultry Science.

The chairman narrated the purpose of special BOS meeting is equivalence of the degrees for various recruitments in MAFSU, we are here to discuss especially for the subject of Poultry Science. All members actively participated in the subject discussion. After through discussion it was decided that basic qualification for requirement of Assistant professor/Associate Professor/Professor in the subject of Poultry Science in MAFSU, Nagpur must possess the qualification of MVSc Poultry Science from any recognized SAU/SVU of India. The candidates, possess MVSc Poultry Science and completed his PhD from recognized foreign university with PhD Research work on Poultry species are also eligible for recruitment of Assistant professor and above.

RESOLUTION NO. 2: BoS resolves to equate M.V.sc. degree in Poultry Science for recruitment of Academic staff in the subject of Poultry Science in MAFSU as below.

Sr. No.	Name of the post	Essential Educational Qualification
1.	Assistant Professor	1. M.V.Sc. in Poultry Science 2. Preferably Ph.D. in Poultry Science or PhD from recognized foreign university with PhD Research work on Poultry species
2.	Associate Professor	1. M.V.Sc. in Poultry Science 2. Preferably Ph.D. in Poultry Science or PhD from recognized foreign university with PhD Research work on Poultry species 3. Dr. M.G. Nikam, Assistant Professor, COVAS, Parbhani, MAFSU, Nagpur possess the MVSC degree in Poultry Production and appointed as Assistant Professor, in MAFSU on 25 th Sep 2006 on regular basis. Hence his case should treat as exceptional case for consideration of his promotion on higher post Associate Professor
3.	Professor	1. M.V.Sc. in Poultry Science 2. Mandatory Ph.D. in Poultry Science or PhD from recognized foreign university with PhD Research work on Poultry species 3. Dr. M. G. Nikam, Assistant Professor, COVAS, Parbhani, MAFSU, Nagpur possess the MVSC degree in Poultry Production and appointed as Assistant Professor, in MAFSU on 25 th Sep 2006 on regular basis. Hence his case should treat as exceptional case for consideration of his promotion on higher post Professor

*

SUBJECT: Modification in title of ORW of Ph.D. student Mrs. Pinky Roy, Enrolment No. V/16/418 entitled "Welfare behavior and performance of laying hens at different stocking density flock size and in enhanced conventional California cages". The topic was thoroughly discussed and approved with following corrections

Dr. Kadam, requested to Chairman and BoS member to give suggestions to modify the ORW title of his PhD student Mrs. Pinky Roy, Enrolment No. V/16/418. The title of PhD ORW was "Welfare behavior and performance of laying hens at different stocking density flock size and in enhanced conventional California cages" which was again discussed and house suggested the following modified title

"Welfare, behavior and performance of laying hens at different stocking densities and flock size in california and furnished cages"

RESOLUTION NO. 3: The modified title of ORW of Mrs. Pinky Roy, Enrolment No. V/16/418 entitled "Welfare, behavior and performance of laying hens at different stocking densities and flock size in california and furnished cages"

As there was no other subject for discussion, the meeting ended with the vote of thanks. With the approval of Chairman, BOS, Poultry Science.

Sd/-

Dr. M. M. Kadam
Member Secretary
Board of Studies in Poultry Science
MAFSU, Nagpur

Copy submitted for information and necessary action to:-

1. The Dean, Faculty of Veterinary Science, MAFSU, Nagpur.
2. Dr. A. S. Ranade Chairman BOS & Professor Poultry Science, BVC, Mumbai
3. Dr. M. V. Dhumal, Member, Board of studies and Prof. of PSc, COVAS, Parbhani.
4. Dr. S. J. Manwar, Member, Board of studies and Prof. of PSc, PGIVAS, Akola.
5. Dr. D. N. Desai Member, Board of studies and Ass. Prof. of PSc, BVC, Mumbai
6. Dr. R. C. Kulkarni, Member, Board of studies and Asst. Prof. of PSc, COVAS, Udgir.
7. Dr. Prasanna Pedgaonkar, Member, BOS & GM, Venkateswara Hatcheries Pvt. Ltd., Pune
8. Controller of Examination, MAFSU, Nagpur
9. Dr. M. M. Kadam, Member Secretary, Board of studies and Asst. Prof. of PSc, NVC, Nagpur.

APPENDIX- II

Form B(per rule8(a))

APPLICATION FOR PERMISSION FOR ANIMAL EXPERIMENTS

Application to be submitted to sent either to the CPCSEA (address in form A above) or Institutional Animal Ethical committee (IAEC).

*(1) Name and address of the Establishment. : Nagpur Veterinary College, Seminary Hills, Maharashtra, 440006

*(2) Registration Number and date of registration.
244/ CPCSEA, 1st August 2000.

(3) Name, address and registration number of breeder from which animals acquired (or to be acquired) for experiments mentioned in part B & C.

M/S. Venkateshwara Hatcheries, Hyderabad and Government Hatchery, Seminary Hills, Nagpur

(4) Place where the animals are presently kept (for the purpose): NA

(5) Place where the experiment is to be performed (Please provide CPCEA Reg number).

Poultry Research and Training Centre, Nagpur Veterinary College, Nagpur
244/ CPCSEA, 1st August 2000.

(6) Date on which the experiment is to commence and duration of experiment.

May 2019. For a period of 5 months

(7) Type of research involved (basic research/educational purpose/regulatory).

Basic research

Signature

Name and designation

Investigator
Department of Poultry Science
Nag. Vet. College, Nagpur

Date: 25/03/19

Place: NAGPUR

*Applicable only for application to be submitted to CPCSEA

Part-B

Protocol form for research proposals to be submitted to the Committee / Institutional Animal Ethics Committee, for new experiments or extensions of ongoing experiments using animals other than non-human primates.

1. **Project / Dissertation / Thesis Title:** Stocking Density, Flock size and Modified management effects on laying Hens Production, Behaviour and Welfare in California cages.

2. **Principal Investigator / Research Scholar / Research Guide /Advisor:**
 - a. Name : RS- PINKY ROY, RG and Advisor – Dr. M.M KADAM
 - b. Designation :Assistant Professor
 - c. Dept / Div / Lab : Department of Poultry Science
 - d. Telephone No : 8888886374
 - e. Experience : 10 years

3. **List of names of all individuals authorized to conduct procedures under this proposal Co-guides**
 - a. Name : Dr.N.V.Kurkure, Dr. J.P. Korde, Dr. S.P. Chaudhari,
Dr. R. K. Ambadkar,

 - b. Address : Nagpur Veterinary College, Seminary Hills
 - c. Experience: 15 years

4. **Funding source with complete address (Please attach the proof):** Nagpur Veterinary College

5. **Duration of the project**
 - a. Number of month : 5 month
 - b. Date of initiation(Proposed) : June 2019
 - c. Date of completion : November 2019

6. **Detailed study plan may be given (Not more than one page):** The experiment will be conducted on BV 300 commercial layer strain up to 20 weeks of period. The program of research work consists of three research trials to test the hypothesis if given modified cages, birds at higher density will also perform

better. The aim of the first trial is to test the different space requirement (Standard stocking density -60; Average stocking density -75; Low stocking density-85 Lowest stocking density 100 sq. inch/bird) in California cage system for optimum production considering immune response, welfare, behavior and economics parameters.

TRIAL NO: 1 : Completed, Permission taken from IEAC

No. of birds - 270 birds

No. of treatments – 04 stocking density (Standard-60; Average-75; Low-85 and Lowest 100 sq. inch/bird)

No. of replicates - 18

Cage Size 300 sq inch per box		Treatment	No. of Replicate	No. of bird per treatment
60 sq. inch per bird (5 birds per box)	Standard	A	18	90
75 sq. inch per bird (4 birds per box)	Average	B	18	72
85 sq. inch per bird (3 birds per box)	Low	C	18	54
100 sq inch per bird (3 birds per box)	Lowest	D	18	54

One cage box (300 Sq. inch) will act as one replicate

Trial Number: - 02

Number of treatment : 4

Number of replicate: 7 for each treatment.

Number of birds: 315 birds

On the basis of result of Trial No.1, the best **TWO** lead performing (on the basis of score card which will be prepared considering all important parameters with due weightage) laying hens groups will be reared at different flock size.

On the basis of first trial result ,TWO lead treatment of below will be used in II trial

Space per bird	Bird X Box	Flock Size each box	FLOCK SIZE	Treatment	Replicate	No. of birds
A.60 sq. inch per bird (5 birds per)387cm ² /bird	5x2	10 birds	Double /2X	A	7	70
	5x3	15 birds	Triple /3X	B	7	105
B. 75 sq. inch per bird (4 birds) or 483.87cm ² /bird	4x2	08 birds	Double /2X	I	7	56
	4x3	12 birds	Triple /3X	II	7	84
C. 85 sq. inch per bird (3 birds) or 548.39 cm ² /bird	3x2	06 birds	Double/ 2X	1	7	42
	3x3	09 birds	Triple/ 3X	2	7	63
D.100 sq inch per bird (3 birds) or 645cm ² /bird	3x2	06 birds	Double /2X	*	7	42
	3x3	09 birds	Triple /3X	**	7	63

Trial Number: - 03

Number of treatment : 4

Number of replicate : 7 for each treatment

Number of birds: 315

The third trial will be based to provide an enriched environment in the conventional cages with a perch, scratch pad and nesting area to the TWO lead performing group (of Trial 1) and will be compared with performance parameter of Trial 2 in terms of egg production, egg quality, immune response, economics, welfare and behavioral changes. The flock size will be assessed with maximum **double** and **triple** stocking density of the TWO lead performing groups of trial-I in the modified California cages.

On the basis of first trial result ,TWO lead treatment of below will be used in III trial

Space per bird	Box size	Flock Size each box	Total Space/bird Including	Actual space/bird	Treatment	Replicate	No. of Birds
60 sq. inch per bird (5 birds)	3 cages	10 birds	580 cm ²	387 cm ²	A	7	70
	3 cages	15 birds	387 cm ²	258 cm ²	B	7	105
75 sq. inch per bird (4 birds)	3 cages	08 birds	725 cm ²	483.75cm ²	1	7	56
	3 cages	12 birds	483cm ²	322.5cm ²	2	7	84
85 sq. inch per birds (3 birds)	3 cages	06 birds	822.58 cm ²	500cm ²	I	7	42
	3 cages	09 birds	548.39 cm ²	333.33cm ²	II	7	63
100 sq inch per bird (3 birds)	3 cages	06 birds	967cm ²	645 cm ²	*	7	42
	3 cages	09 birds	645cm ²	430 cm ²	**	7	63

Methodology: 1) Birds production and performance parameter

2) Haematology and Biochemical parameter

3) Welfare and behavioural study.

4) Mineral estimation of Bone at the end of the experimental period by slaughtering the bird, 10 birds from each treatment. A total of 50 birds per treatment in each trial.

7. Animals required

- Species / common name :Poultry/ layers
- Age / weight / size : 22week and above
- Gender : Female
- Number to be used (Year-wise breakups and total figures needed to be given).:
 - First Trial- 5 months :384 layers
 - Second and Third Trial- 5months , 560- 720, depending on the outcome of first trial.
- Number of days each animal will be housed: Laying period
- Proposed source of animals. :M/S. Venkateshwara Hatcheries, Hyderabad

8. Rationale for animal usage

- a. Why is animal usage necessary for these studies?: As the experiment is designed to study on the layers welfare
- b. Why are the particular species selected required?: To study the welfare on layers.
- c. Why is estimated number of animals essential?: For statistical applications and data analysis.
- d. Are similar experiments conducted in the past? If so, the number of animals used and results obtained in brief. : NO
- e. If yes, why new experiment is required? : NA
- f. Have similar an experiment has been made by any other organization agency? If so, there results in your knowledge. : NO

9. Description of the procedures to be used.

List and describe all invasive and potential stress full non invasive procedures that animals will be subjected to in the course of the experiments : None

Furnish details of injections schedule

Substances: NA

Doses: NA

Sites: NA

Volumes: NA

Blood withdrawal:

Volume: 2ml

Sites: Wing/ Jugular vein

Radiation:

10. Please provide brief description of similar studies from invitro / invivo (from other animal's models) on same/Similar test component or line of research. If enough information is available, justify the proposed reasons.

11. **Does the protocol use of anesthetic or analgesic for the conduct of painful procedure (any which cause more pain than that associated with routine injection or blood withdrawal)? If yes, explanation and justification.** : NA

12. **Will survival, surgery to be done?** : NA

If yes, the following to be described.

- a. List and description of all such surgical procedures (including methods of asepsis).
- b. Names, qualification and experience levels of operators.
- c. Description of post-operative care.
- d. Justification if major survival surgery is to be performed more than once on single individual animal.

- b. Names, qualification and experience levels of operators.
- c. Description of post-operative care.
- d. Justification if major survival surgery is to be performed more than once on single individual animal.

13. **Methods of disposal post experimentation.** : Birds will maintained till the end of laying period

- a. Euthanasia (Specific method): NA
- b. Method of carcass disposal: NA
- c. Rehabilitation: Birds will be maintained at poultry for further production.

14. **Animal transportation methods if extra-institutional transport is envisaged:**
Birds will be transported in open van with due care.

15. **Use of hazardous** (use of recombinant DNA-based agent or potential human pathogen requires documented approval of institutional Biosafety Committee (IBC). For each category, the agent and the biosafety level required, appropriate therapeutic measure and the mode of disposal of contaminated food, and water, animal waste and carcasses must be identified : NA

- a. Radio nucleotide
- b. Microorganisms / biological infectious Agents
- c. Hazardous chemicals drugs
- d. Recombinant DNA
- e. Any other (give name)

If, your project involved use of any above, attach copy of the minutes of IBC granting approval.

Investigator's declaration.

1. I certify that I have determined that the research proposal herein is not unnecessarily duplicative of previously reported research.
2. I certify that, I am qualified and have experience in the experimentation on animals.
3. For the procedures listed under item 11, I certify that I have reviewed the pertinent scientific literature and have found no valid alternative to any other procedure described herein which may cause less pain or distress.
4. I will obtain approval from the IAEC/CPCSEA before initiating any significant changes in this study.
5. Certified that performance of experiment will be initiated only upon review and approval of scientific intent by appropriate expert body (Institutional Scientific Advisory Committee / funding agency / other body to be named)
6. Institutional Biosafety Committee (IBC) certification of review and concurrence will be taken (Required for studies utilizing DNA agents of human pathogens).
7. I shall maintain all the records as per format (Form D).
8. I certify that, I will not initiate the study unless approval from CPCSEA received in writing. Further, I certify that I will follow the recommendation of CPCSEA.
9. I certify that I will ensure the rehabilitation policies are adapted.

Date: 25/3/19



Signature
Name of Investigator
Head
Department of Poultry Science
Nag. Vet. College, Nagpur

NAGPUR VETERINARY COLLEGE, NAGPUR

Outward No : NVC/IAEC/.....29.....1.....2019.....

Date :12.04/2019

CERTIFICATE

16. This is certify that the project title “Stocking Density, Flock size and Modified management effects on laying Hens Production, Behaviour and Welfare in California cages” has been approved by the IEAC.

Dr. R. K. Ambadkar

Name of chairman / ~~Member Secretary~~ IEAC:

Dr. Anil S. More

Name of CPCSEA nominee

Signature with date

Chairman/~~Member Secretary~~ of IEAC:

CPCSEA nominee

(Kindly make sure that minute of the meeting duly signed by all the participants are maintained by office)

VITA

The author Mrs. Pinky Roy was born on 10th October, 1976 at Dibrugarh, Dist. Dibrugarh (Assam State.). She has passed S.S.C. with first class from Government Higher Secondary School, Tezu, Arunachal Pradesh in the year 1991 and H.S.S.C. examination with first class from DHS Kanoi College in year 1993. She has completed B.V.Sc. & A.H. degree course with first division from the College of Veterinary and Animal Sciences, Khanapara in year 1999 and subsequently after clearing ICAR examination joined M.V.Sc. in Poultry Science and passed out in first division from JNKVV, Jabalpur in year 2001 with university gold medal. During M.V.Sc. and Ph.D she was recipient of Dr B. V Rao scholarship to carry her research work.

After completion of M.V.Sc., she has worked as Production Incharge of Poultry Feed production unit, Rohini Minerals, Hyderabad and later in Pet and Vet clinic, from 2005-2008. She joined Vosterman Ventilations in the year 2010 and worked as Business Development Manager, India. She has joined her Ph.D. degree program in the discipline Poultry Science as Regular candidate in the year 2016 at NVC, Nagpur.

THESIS ABSTRACT

- | | |
|--|--|
| a) Title of the thesis | “WELFARE, BEHAVIOR AND PERFORMANCE OF LAYING HENS AT DIFFERENT STOCKING DENSITIES AND FLOCK SIZE IN CALIFORNIA AND FURNISHED CAGES” |
| b) Full name of student | PINKY ROY |
| c) Name and address of Major Advisor | Dr. M.M. Kadam
Assistant Professor and Head I/c
Dept. of Poultry Science,
Nagpur Veterinary College, Nagpur |
| d) Degree to be awarded | Ph.D. |
| e) Year of award of degree | 2021 |
| f) Major subject | Poultry Science |
| g) Total number of pages in the thesis | 212 |
| h) Number of words in the abstract | 498 |
| i) Signature of Student | |
| j) Signature, Name and Address of forwarding authority | Associate Dean
Nagpur Veterinary College,
Nagpur |

ABSTRACT

The present investigation was planned to study the welfare, behavior and performance of laying hens at different stocking densities and flock size in California and furnished cages. Three experimental trials were conducted on commercial White

Leghorn layer (strain BV 300) for a period of twenty weeks each, starting at 28th week. A total of 600 layers were reared under standard managemental practices, 240 in experiment I (16 replicates); and 180 birds (6 replicates) each in experiment II and III. In experimental trial I- effect of stocking density (387, 484, 548 and 645 cm²/b), in trial II-interaction effect of two best stocking density (548 and 645cm²/b) and flock size (06 and 09 birds) in conventional cages and in Trial III same interaction effect as in trial II but in furnished cages was conducted along with comparison of production, welfare and behavior of commercial laying hens in conventional California and Furnished cages. The cages in trial III were furnished with scratch pad (483.87cm²/cage, perch (14.39 cm/b and 16.93 cm/b at higher and lower stocking density, respectively) and nesting area (265cm²/b). The birds were offered standard commercial layer feed and maintained under standard managemental practices (BV 300 manual).

Trial I resulted in the best production performance, physical and physiological indicators of welfare (HDEP%, FCR, Egg weight, Feather score, H/L ratio) at stocking density of 548 and 645cm²/b with non-significant difference between the two. However, Cost of Production per Egg (CPPE) on basis of space utilization and corticosterone (ng/ml) another indicator of stress was significantly better at 548 cm²/b when compared with others. Though total state and event behaviour were non-significant among the groups, but better sleeping, wing flapping, walking, preening and less feather pecking was observed for 548cm²/b with significant differences in above behaviors with rest of the treatments and sometimes non-significant with 645cm²/b stocking density was recorded. Interaction effect of stocking density and flock size from Trial II resulted in non-significant effect on all of the production performance, welfare, CPPE on basis of recurring expenditure and total state and event behavioral parameters excepting significantly better sleeping, drinking and least feather pecking was observed in 548cm²/b x 6B. The CPPE on basis of space utilization was significantly better for flock size of 6 birds at both stocking density, also flock size of 6 birds shown significant better HDEP%, FCR and for sleeping

behaviour, therefore, it could be concluded a flock size of 6 birds at stocking density 548 and 645cm²/b led to the best result.

Interaction effect of stocking density and flock size in furnished cages were best seen for birds maintained at stocking density of 548cm²/b with flock size of 6 birds have numerical highest value (statistically higher than one or rest of the other treatment groups) for HDEP, egg weight, CPPE on basis of space utilization and behaviors like sleeping, feeding and total state. A comparison of production, welfare and behaviour for the furnished and conventional California resulted in non-significant difference for production and physical indicators of welfare, only the H/L ratio and corticosterone (ng/ml) proved to be lower in furnished cages when determined at the end of the experiment, also, there was significant differences for behavior like perching and scratching which was observed only in furnished cages. In absence of perching and scratching in conventional cages, other behaviors like sitting, standing, walking, sleeping to name a few were higher in conventional cage, but total state and total event behaviors were nullified as for example in absence of perching the birds kept on standing or walking. The CPPE was significantly higher for furnished cages, a difference of Rs. 0.44 – 0.80 in CPPE on basis of recurring expenses was observed but such a difference could be ignored considering the less stress and natural behaviors the birds can exhibit in furnished cages.

Thus, it may be concluded that rearing of commercial layer in conventional California cages is best suited for stocking density of 548cm²/b or 85 sq. inch/b. Further a flock size of 6 birds at stocking density of 548cm²/b or 85 sq. inch/b was best when interaction effect was tested. To allow commercial layer birds to exhibit natural behaviors similar to cage free birds like perching and scratching and a stress-free environment, furnished cages can be a solution, ignoring the facts that production parameters does not improve in furnished cages and cost of production per egg is higher in furnished cages when compared to conventional California cages.

प्रबंध सारांश

अ.	प्रबंधाचे शिर्षक	:	कॅलिफोर्निया व सुसज्ज पिंजऱ्यात अंडी देणाऱ्या कोंबड्याचे वेगवेगळ्या कळपात व जागेतिल कल्याण वर्तणूक व कामगिरी
ब.	विद्यार्थ्यांचे पुर्ण नांव	:	पिंकी रॉय
क.	मार्गदर्शकाचे नांव व पत्ता	:	डॉ. एम. एम. कदम स. प्राध्यापक व विभाग प्रमुख, कुकुटपालनशास्त्र विभाग, नागपूर पशुवैद्यकीय महाविद्यालय नागपूर
ड.	प्रदान करण्यात येणारी पदवी	:	पी. एच. डी.
इ.	पदवी प्रदान करण्याचे वर्ष	:	२०२१
फ.	मुख्य विषय	:	कुकुटपालनशास्त्र
ग.	प्रबंधातील एकूण पृष्ठे	:	२१२
ह.	सारांशातील एकूण शब्द	:	४९८
ई.	विद्यार्थ्यांची सही	:	
		:	
ज.	अग्रेषित करणाऱ्या अधिकाऱ्याची सही, नांव आणि पत्ता	:	सहयोगी अधिष्ठाता नागपूर पशुवैद्यक महाविद्यालय नागपूर

सारांश

पारंपारिक कॅलिफोर्निया कजेस आणि सुसज्ज पिंजरे मध्ये वेगवेगळ्या संखेच्या कळपात अंडी देणाऱ्या कोंबड्यांचे कल्याण, वर्तन आणि कामगिरीचा अभ्यास करण्यासाठी सदरिल

संशोधनाची योजना आखली गेली. उद्दिष्टे सत्यापित करण्यासाठी, व्यावसायिक पांढऱ्या रंगाच्या लेगॉर्न लेयर (स्ट्रेन बीव्ही 300) वर प्रत्येकी वीस आठवड्यांसाठी संशोधनाच्या तीन चाचण्या घेण्यात आल्या. पहिल्या प्रयोगात २४० आणि दुसऱ्या व तिसऱ्या प्रयोगात १८० पक्षी उपयोगात आणले होते. संशोधना दरम्यान सर्व अंडी देणाऱ्या कोंबड्यांचे व्यवस्थापन मानका प्रमाणे होते. संशोधनात प्रथम कळप घनता म्हणजेच, ३८७ (६० चौरस इंच), ४८४ (७५ चौरस इंच), ५४८ (८५ चौरस इंच) आणि ६४५ सेमी स्क्वेअर प्रति पक्षी (१०० चौरस इंच) चा अभ्यास केला गेला. उत्पादन, वर्तणूक, कल्याण आणि अर्थशास्त्र यावर आधारित विकसीत स्कोअरकार्डद्वारे व्यावसायिक अंडी देणाऱ्या कोंबड्यांसाठी उत्कृष्ट कळप घनतेचे मूल्यांकन केले गेले. ज्या पक्षी गटाने एकूण १०० गुणांपैकी ८४ व ८२ गुण प्राप्त केले अशा ५४८ व ६४५ सेमी स्क्वेअर प्रति पक्षी कळप घनतेच्या पक्षी गटाने उत्पादन, कल्याण, रोग प्रतिकार शक्ति, वर्तन आणि अर्थशास्त्र सर्वोत्कृष्ट कार्य करून अव्वल स्थान मिळवले. पहिल्या प्रयोगाच्या निकालांच्या आधारे, ५४८ आणि ६४५ सेमी स्क्वेअर प्रति पक्षी जागा प्रमाणे कळप घनता परिणाम व सहा आणि नऊ पक्षी संख्या व त्याचा परिणाम दुसऱ्या संशोधन चाचणी मधून तपासून घेतला. अंडी देणाऱ्या कोंबड्यांचे उत्पादन ५४८ आणि ६४५ सेमी स्क्वेअर प्रति पक्षी जागा प्रमाणे सहा पक्षी संख्या असलेल्या गटाने अंडी उत्पादन व त्यांची गुणवत्ता आणि पक्षी कल्याण यांच्या आधारे उत्कृष्ट प्रदर्शन केले. तथापि, सहा पक्षी संख्या असलेल्या ५४८ सेमी स्क्वेअर प्रति पक्षी गटाने वर्तन आणि अर्थशास्त्र लक्षात घेता उत्तम प्रदर्शन केले. पक्षी कळप घनता आणि पक्षी संख्या याचा परस्पर परिणाम पक्षी वर्तणुकीतील झोप, पाणी पिने आणि चोच मारण्यावर होतो. ५४८ सेमी स्क्वेअर प्रति पक्षी कळप घनता व सहा पक्षी संख्या याचा पक्षी वर्तणुकीतील झोप, पाणी यावर लक्षणीय बदल झाला तसेच पक्षांमध्ये कमीत कमी पंख टोचने दिसून आले. दुसऱ्या प्रयोगाच्या निकालांच्या आधारावर, दोन सर्वोत्तम गट म्हणजे ५४८ व ६४५ सेमी स्क्वेअर प्रति पक्षी व सहा पक्षी संख्या बरोबर पारंपारिक कॅलिफोर्निया कजेस आणि सुसज्ज पिंजरे यांचे तुलनात्मक तपासणी केली. पारंपारिक कॅलिफोर्निया कजेस आणि सुसज्ज पिंजरे यांचे तुलना करताना अंडी गुणवत्ता, वर्तणूक व रोग प्रतिकारक शक्ति मध्ये लक्षणीय बदल दिसून आले नाहीत. तथापि, सुसज्ज पिंजरे मधील पक्षी तणाव मुक्त होते तर पारंपारिक कॅलिफोर्निया कजेस ची किमत अर्थिक दृष्ट्या परवडणारी होती. कल्याण व वर्तणूक आणि पक्ष्यांची उडून बसण्याची सवय हि चांगल्या प्रमाणे सुसज्ज पिंजऱ्यामध्ये जोपासली गेली. अंडी देणाऱ्या कोंबड्यांना पारंपारिक पिंजऱ्यात कमीतकमी ५४८ सेमी स्क्वेअर प्रति पक्षी तसेच सहा पक्षी संख्या ठेवून पिंजरा आकार ३४X१५X१६ इंच दिला तर त्या उत्तम उत्पादन, अंडी गुणवत्ता अर्थशास्त्र व त्यांचे कल्याण वर्तणूक चांगल्या प्रकारे टिकून ठेवतात. जर पक्ष्यांना ५४८ सेमी स्क्वेअर प्रति पक्षी व सहा पक्षी संख्येत पाळले व जर कुकूट पालकाने एका वेळीचा अधिकचा खर्च पिंजरे सुसज्ज (पक्षी बसण्याकरिता, स्क्रॅच करण्याकरिता जागा आणि अंडी जागा) करण्यासाठी रुपये ७.० तसेच रुपये ०.४३ हा प्रति अंडी सहन केला तर अंडी देणाऱ्या कोंबड्यांचे नैसर्गिक गुण हे चांगल्या प्रकारे पिंजऱ्यात जोपासले जातात.