"EFFECT OF PROBIOTIC FEED SUPPLEMENT IN COMMERCIAL LAYER CHICKENS"

By

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THESIS SUBMITTED TO THE
P.V.NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY
IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE AWARD OF THE DEGREE OF
MASTER OF VETERINARY SCIENCE

DEPARTMENT OF POULTRY SCIENCE
COLLEGE OF VETERINARY SCIENCE
P.V.NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY
RAJENDRANAGAR, HYDERABAD – 500 030.

DECEMBER, 2019

CERTIFICATE

Mr. G.RAVI SUDHAKAR has satisfactorily prosecuted the course of research and that the thesis entitled **"EFFECT OF PROBIOTIC FEED SUPPLEMENT IN COMMERCIAL LAYER CHICKENS"** submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date: (**KRISHNA DAIDA**)

Place: Hyderabad Chairman of Advisory Committee

CERTIFICATE

This is to certify that the thesis entitled **"EFFECT OF PROBIOTIC FEED SUPPLEMENT IN COMMERCIAL LAYER CHICKENS"** submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** of the **P.V.NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY**; Hyderabad is a record of the bonafide research work carried out by **G.RAVI SUDHAKAR** under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted for any other degree or diploma or has been published. The published part has been fully acknowledged. All the assistance and help received during the course of investigation have been duly acknowledged by the author of the thesis.

20/12/2019

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DECLARATION

I, **Mr. G. RAVI SUDHAKAR**, hereby declare that the thesis entitled **"EFFECT OF PROBIOTIC FEED SUPPLEMENT IN COMMERCIAL LAYER CHICKENS"** submitted to P .V .Narsimha Rao Telangana Veterinary University for the degree of **MASTER OF VETERINARY SCIENCE** is a result of the original research work done by me. I also declare that the thesis or part thereof has not been published earlier elsewhere in any manner.

Date: (**G. RAVI SUDHAKAR**)

Place: Hyderabad

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LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
<	:	Less than
>	:	Greater than
/	:	per
Avg	:	Average
BD	:	Basal diet
BW	:	Body weight
Ca	:	Calcium
CF	:	Crude fibre
CFU	:	Colony Farming Unit
CP	:	Crude protein
d	:	Days
FC	:	Feed Consumption
FCR	:	Feed conversion ratio
g	:	Gram
g/d	:	Gram/day
g/kg	:	Gram per kilogram
g/t	:	Gram per Tonne
HDEP	:	Hen Day Egg Production
IU	:	International Units
hr.	:	Hour
k.cal.	:	Kilo calorie
kg	:	Kilogram
ME	:	Metabolizable Energy
mg	:	Milligram
N	:	Newton
P	:	Phosphorus
ppm	:	Parts per million
Rs.	:	Rupees
T	:	Treatment
Wks	:	weeks

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ACKNOWLEDGEMT

*My profound etiquette, deep sense of gratitude and abysmal thanks to **Dr. Krishna Daida** Associate Professor & Head, Department of Poultry Science, College of Veterinary Science, Korutla, Jagtial for his meticulous guidance and patience. He was a source of inspiration to me throughout the period of investigation. I am grateful to him for his constant fomenting, punctilious and impeccable advice. Above all, his affectionate way of dealing with the things throughout the course of my study has helped me to consummate the research work with a grand success. I take this opportunity to express my heartfelt gratitude towards him. I had really a great pleasure and privilege to be associated with him during this course of study.*

I also thank **Dr. V. Chinni Preetam** *Professor & Head, Department of Poultry Science, Rajendranagar, Hyderabad. I thank him for helping correct my thesis and putting it to its current shape. It has been an amazing experience to learn from a man with such deep and profund knowledge. I also wish to thank him for allowing me to work in his laboratory whenever necessary and providing the materials to work with. I will always be indebted to him for the opportunities he gave me.*

Dr. B. Prakash, *Senior Scientist, Directorate of Poultry Research, Rajendranagar, Hyderabad and member of my Advisory Committee for his support and valuable suggestions during my research work.*

*It gives me great pleasure to place on record my deep sense of gratitude to **Dr.S.V.Rama Rao,** Principal Scientist, Directorate on Poultry Research, Rajendranagar, Hyderabad. For his unstinted patience, indefatigable guidance, constant encouragement and his sense of humanity and helping me in this investigation.*

I am also thankful to ***Dr. D. K. Vijaya laxmi*** *, Professor, Department of Poultry Science, College of Veterinary Science, Hyderabad for her help during my course work.*

I thank ***Dr.G. srinivasulu and Dr. Hanumanth Rao*** *Assistant Professors of Department of Poultry Science for their help and kind cooperation and guidance during my course of work.*

I am extremely grateful to ***M/S Sanzyme biologicals Private Limited, Hyderabad.*** *for funding ,providing the product and constant observation during entire my research work and extending the necessary help and support at the right time in successful completion of research work.*

With respectful regards and immense pleasure, I am in dearth of words to express my unboundful gratitude and genuflect love to my beloved parents ***Shri. G.Venkataramana & G.Anuradha*** *for their affectionate love, blessings and dedicated efforts in moulding me to the present position and whose backup brought out the best in me. My verbosity utterly fails to express my love and affection towards my beloved sister* ***Dr. G.Varalakshmi*** *without whose instilling aspiration I would not have reached this stage.*

I extol my sincere feelings of gratitude and heartfelt thanks to my Seniors Drs, ***Bala sundaram sir*** *,* ***K. Sridhar, Purshotham shirisha ,Prasanth, RP, Faruk , Satya ,radhika and my collegues Bala Krishna , Muthaym reddy*** *for their help, transcendent suggestions and endless co-operation during the prosecution and presentation of this research work.*

I sincerely thank to my beloved great buddies ***Ashok , Vishnu, Raju, Rajesh, prasanna, srujan, pavan.s pavan Manoranjan Hala And My Beloved Juniors rajini, kalish ashok ,*** *for their help, co-operation and constant support.*

I am thankful to ***Pradeep , Anusha , Laxmi*** *Research Associates, Directorate of Poultry Research, ICAR, Hyderabad for their amazing support during my research work.*

I would like to extend my thanks to ***Non-teaching staff Hari , Naresh, Vikram, Sri Sailam, Lalithamma of Department of poultry science.*** *College of Veterinary Science, Rajendranagar, Hyderabad,* *I would like to express my sincere gratitude and appreciation to many people who helped me throughout my course of study in* ***Department Of Poultry Science, Rajengranagar, Hyderabad.***

It gives me immense pleasure to express my profound sense of gratitude and sincere thanks to ***Dr. L. Atchamamba*** *, Assistatnt Professor,* ***Rajesh*** *and* ***Kishore*** *of College Library for their help in providing the necessary facilities and to carry out research work.*

(G.Ravi sudhakar)

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Title of the Thesis	:	**EFFECT OF PROBIOTIC FEED SUPPLEMENT IN COMMERCIAL LAYER CHICKENS**
Degree to which it is submitted	:	**MASTER OF VETERINARY SCIENCE**
Faculty	:	**VETERINARY SCIENCE**
Discipline	:	**POULTRY SCIENCE**
Major Advisor	:	**Dr. DAIDA KRISHNA**
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Year of submission	:	**2019**

ABSTRACT

The study was conducted to assess the three types of probiotic (100 & 200g/ton) and bacitracin methylene disalicylate (50g/ton) supplemented in diet on *is caloric* and *iso nitrogenous* basis. A total of 288 White Leghorn layers were allocated to each of the eight treatments in a replicated manner, six replicates per treatment. The diets were fed from 16 week of age (six birds/replicate) and evaluated production performance from 22 to 37 week of age.

The trial results revealed that, the per cent hen day egg production for four laying periods in White Leghorn layers (BV300) from 22 to 37 weeks of age was significantly ($P<0.05$) influenced by supplementation of probiotics and bacitracin methylene disalicylate (BMD). The higher per cent egg production was noticed in probiotic-2 @100g/ton (91.77%) compared to all other treatments.

The mean feed intake and feed conversion ratio per dozen eggs were significantly ($P<0.05$) influenced by supplementation of probiotics and BMD. The feed intake was significantly ($P<0.05$) lower in control group (112.16g) and highest in probiotic-3 @200g/ton of feed (118.79g). The better feed conversion ratio per dozen eggs was noticed in probiotic-2 @ 100g/ton (1.527) and poor in probiotic-2 & 3 @200 g per ton of feed (1.633 & 1.600) during 22-37 weeks of age.

A significant difference ($P<0.05$) was observed in egg weights . The higher egg weight in probiotic-1@100g/ton(58.84g). The internal and external egg quality parameters of haugh unit, yolk index, shell thickness, shell percentage and shell strength were significantly ($P<0.05$) influenced by supplementation of probiotics and BMD at different graded levels. Whereas albumen index, shell weight and density were not influenced by all the dietary supplementation groups. The mortality was within the limits during 22-37 weeks of age.

The probiotic at (100 & 200g/ton) supplemented in diet influenced Net profit per dozen eggs and is highest in probiotic -2 @100 g/ton of feed (Rs.9.43) compared with control (Rs 8.67) during the 22-37 weeks of age.

It can be concluded that, multi strain probotic-2 @ 100g/ton of feed can be supplemented as an alternative to antibiotic (BMD) which show better performance compared to the single strain probiotic-1 @ 100g/ton of feed and control. Probotic-2 @ 100g/ton of feed has exhibited better production performance in commercial White Leghorn layer birds during the 22-37 weeks of age.

CHAPTER – I

INTRODUCTION

India with an annual production (BAHS, 2015) of around 73.21 billion eggs and 3.72 million metric tons of poultry meat ranks 3rd in egg production and 7th in broiler meat production, respectively, in the world.

Probiotic is live microbial feed supplement, which beneficially affects the host by improving its intestinal microbial balance. Their mode of action is by "competitive exclusion of harmful pathogens" in the gut. The use of sub-therapeutic levels of antibiotic as routine feed additive has been banned in many countries because of public concern, over possible antibiotic residual effects and the development of drug resistant bacteria. This has led to the development and application of many non-antibiotic substance performance enhancers.

Gut micro biota stimulate the mucosal immune system, help to maintain intestinal homeostasis, and play an important role in digestion and absorption (Hara and Shanahan, 2007; Dankowiakowska *et al.,* 2013). Probiotic compete with pathogenic bacteria for binding sites and nutrients, thus supporting a healthy gut microbial ecosystem (Mizak *et al.*, 2012). Probiotic bacteria affect the composition and activity of cecal micro flora (Willis and Reid, 2008; Vila *et al.*, 2009). Probiotic contained in laying hen diets contribute in improving egg quality, increasing laying rates and reducing feed costs (Panda *et al.*, 2003; Kurtoglu *et al.*, 2004; Panda *et al.*, 2008; Youssef *et al.*, 2013; Chung *et al.*, 2015).

Continuous use of sub-therapeutic level of antibiotics in animal feeds may result in not only bacterial resistance (Adams, 2004) but also accumulation of antibiotic residues in various tissues of the birds resulting in development of drug resistant microorganisms in humans upon consumption of such poultry products (Jin *et al.*, 1996).

The increasing awareness among consumers for the poultry products without antibiotic residue encouraged the utilization of suitable alternatives for antibacterial compounds. Several non-antibiotic feed additives like probiotic and prebiotic have been identified as promising sources in minimizing the disease incidence and achieving better performance in chicken.

Hence, the present study is designed to explore the influence of dietary supplementation of probiotic on Layer performance with following objectives:

1. To study the effect of dietary supplementation of probiotic on the performance and production of commercial layer birds.
2. To study the effect of probiotic on the egg quality parameters
3. To study the effect of probiotic on Economics of production

CHAPTER II

REVIEW OF LITERATURE

Antibiotics are added to feeds of several species of livestock as well as poultry to prevent or control diseases, to stimulate growth and to improve feed efficiency & enhance egg production. In a similar manner probiotic are also introduced as growth promoters in poultry diets. The supplementation of probiotic in poultry diets as growth promoters are well documented.

2.1 PRODUCTION PERFORMANCE

2.1.1 Hen-day egg production

Tortuero and Fernandez (1995) studied the effect of probiotic *(Lactobacillus acidophilus* and *Lactobacillus casei)* at 2.3x10^8 cfu/kg of feed fed to Single Comb White Leghorns from 15 to 19 months of age, the egg production was significantly ($P<0.01$) increased. Similarly, Panda *et al*. (2006) observed significantly ($P<0.05$) improved hen day egg production in White Leghorn layers, when supplemented with probiotic (*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacteriumbifidum, and Asperigillusoryzae*) at 27 billion cfu/100 g during 65 to 76 weeks of age.

Nahashon *et al*. (1996) reported significant ($P<0.05$) improvement in hen day egg production in Dekalb XL Single Comb White Leghorn layers raised with Condensed cane molasses soluble (CCMS -1100 mg) containing *Lactobacillus* per kg^{-1} diet from 20 to 59 weeks of age.

Davis and Anderson (2002) noted that supplementation of probiotic ("Primalac") to, Hy-Line W-36 and Dekalb XL single comb white leghorn strains did not affect hen day egg production during 18-70 weeks of age. Similarly, supplementation of Bioplus2B (*Bacillus subtillis* and *Bacillus licheniformis*) at 0, 400, 1000 and 2000 per ton of feed in white leghorn Hy-line W-36 strain did not affect hen day egg production between 28-39 weeks of age (Mahdavi *et al*., 2005). Yousefi and Karkoodi (2007) reported that the per-cent hen day production was not affected by the commercial available dietary probiotic (Thepax) and yeast (*saccharomyces cerevisiae*) supplementation in Hy-Line W-36 hens during 63-75 weeks of age.

Panda *et al*. (2008) observed that there was a significant (P<0.05) improvement in egg production of White Leghorn layer breeders between 25-40 weeks of age when fed with commercial preparation containing *L.sporogenes* with 6000 million spores g^{-1} with respect to control group.

Sattar Bageri Dizaji and Rasoul Pirmohammadi (2009) reported that the egg production decrease in Hy-Line W-36 hens between 46-55 weeks fed with *Sacharomyce scerevisiae* ($5X10^{10}$ cfu/g) and Bioplus 2B ($3.2X10^{9}$ cfu/g).

Supplementation of commercial probiotic (Protexin$^{TM)}$ at the level of 0,250, 500, and 750 ppm to Hysex- Brown layer hybrids (Balevi *et al*., 2009) did not show any significant variation in per cent hen day egg production between 40-52 weeks of age.

Kalavathy Ramasamy *et al*. (2009) revealed that there is no improvement in hen-day egg production in *Lohmann* Brown layers fed on *Lactobaclilus* cultures during 20 to 68 weeks of age.

Moorthy *et al*. (2010) reported that diet supplemented with 0.1% probiotic and 0.1% turmeric powder significantly (P>0.05) decrease the per cent hen day egg production in White Leghorn layers during 21-52 weeks of age.

Yalcin *et al*. (2010) found that there was significant ($P<0.001$) improvement in percent hen day production in Hyline Brown laying hens of 22-38 weeks of age fed with dietary supplementation of yeast autolyse (*Sacharomyces cerevisiae*) at levels of 2, 3 and 4 g /kg.

Berrin (2011) reported that supplementation of probiotic and prebiotic to the diet of Japanese quail (*Coturnixcoturnix japonica*) during 18-30 weeks of age had positively affected the egg production.

Wei Fen Li *et al*. (2011) reported a significant increase in the per cent egg laying rate ($P<0.05$) increase in Shaoxing Ducks when supplemeted with *Bacillius subtilis* @ $1x10^8$ cfu/kg.

Supplementation with probiotic (*Pediococcus acidilactici)* @100 mg did not show any significant variation in per cent hen day egg production in Hyline brown hens during 23-46 weeks of age (Mikulski *et al*., 2012).

Zhang *et al*. (2012) investigated that there was a significant ($P<0.05$) improvement in egg production in *Lohmann* variety supplemented with four different probiotics (heat inactivated (*Lactobacillus salivarius*, *Clostridium butyricum*, *Bacillu ssubtilis*, and sodium butyrate) during 24- 32 weeks of age.

Abdelqader *et al*. (2013) noticed that significant ($P<0.001$) difference in per cent egg production in *Lohmann* white laying hens supplemented @ 1g/kg *Bacillus subtilis* ($2.3x10^8$ cfu/g) during 64 to 75 weeks of age.

Shalaei *et al*. (2014) studied the effects of antibiotic, organic acid, probiotic and prebiotic by adding to basal diet in Hy-line W-36 laying hens from 32 to 42 weeks of age and observed no significant ($P>0.05$) improvement in the egg production.

Sobczak*et al*. (2015) observed that the egg production was not improved in *Lohmann* Brown laying hens supplemented with a commercial probiotic preparation of (*Bscillussubtilis* ATCCPTA-6737 at 1×10^8) during 18-42 weeks of age.

Supplementation with probiotic *Lactobacillus acidophilus* at 0.1% and *Bacillus subtilis* at 0.05% during 16-36 weeks of age in old Hy-Line layer did not show significant improvement in per cent hen day egg production (Forte *et al*., 2016).

Upadhaya *et al*. (2016) observed that there was no significant difference in egg production in Hy-line Brown during 40-45 weeks of age when supplemented with 0.1% when *Bacillus subbtilis* challenged with *S.gallinarum* and 0.1% *Bacillus methylotrophicus* challenged *with S.gallinarum*.

Fathi *et al*. (2018) found that there was no significant difference among the treatment groups in egg production supplemented with probiotic at 0, 200, 400 ppm (*Bacillus subtilis*) in three different breeds (White Leghorn, Saudi black and Saudi brown) during 36-48 weeks of age.

Sheoran *et al*. (2018) reported that there was no significant difference in egg production among the treatment groups in White Leghorn layers supplemented with probiotics at (0.5g *Lactobacillus fermentum*, 1.0g *Bacillus spp*, 2.0g *Saccharomyces cerevisia*e) and prebiotic supplementation 0.5g, 1.0g, and 2.0g(mannonoligosachride of *saccharomyces* cell wall-47g, formic acid- 32g) during 22-38 weeks of age.

2.1.2 Feed intake

Tortuero and Fernandez 1995 found that there was no significant effect in feed intake of Single Comb White Leghorn laying hens from 15-19 months of age supplemented with *L.acidophilus* and *L.casei* at$2.3\text{x}10^8$ cfu /kg feed.

Nahashon *et al*. (1996) concluded that significant ($P<0.05$) increase in feed consumption in DeKalb XL Single Comb White Leg horn layers raised on condensed cane molasses solubles (CCMS-1100 mg) containing viable *Lactobacillus sp*s. Per kg diet from 20 to 59 weeks of age.

Panda *et al*. (2003) stated that feed intake was not influenced significantly by probiotic ("Probiolac" @100 or 200 mg/kg feed) when fed to White Leghorn layers from 25 to 72 weeks of age.

Yoruk *et al*. (2004) observed that there was no significant difference on feed intake in Hysex-Brown layers diets supplemented with probiotic (*Lactobacillus, Bifidobacterium, Streptococcus, and Enterococcus spp.* at 0.1 and 0.2%) during 54 -64 week of age.

Mahdavi *et al*. (2005) stated that there was no significant improvement of feed intake in Hy-Line, W-36 strain fed with probiotic (Bioplus2B) at 0, 400, 1000, and 2000g per ton of feed during 28-39 weeks of age.

Ezhilvalavan *et al*. (2006) found that supplementation of probiotic (*Lactobacillus acidophilu)* at 1.0 and 2.0g per kg feed fed to commercial layers has no significant difference in feed consumption.

Panda *et al*. (2006) noted that feed intake was not influenced by supplementation of probiotics in White Leghorn layers during 65 to 76 weeks of age with the concentration of 27 billion cfu/100g.

Yousefi and Karkoodi (2007) observed that there was no improvement of feed intake in Hy-Line W-36 hens by using probiotic (Thepax®) and yeast supplementation between 63-75 weeks of age. Similar results reported by Panda *et al*. (2008) in White

Leghorn layer breeders during 25-40 weeks of age fed with *Lactobacillus* cultures (100 mg/kg and 150 mg/kg)

Kalavathy Ramasamy *et al*. (2009) stated that no significant difference on feed intake in laying hens from 20 to 68 weeks of age supplemented with probiotic (*Lactobacillus)* culture with concentration of $1g/kg^{-1}$

Sattar Bageri Dizaji and Rasoul Pirmohammadi (2009) stated that numerical difference but not statistical, in Hy-Line W-36 hens aged 46-55 weeks fed with *Sacharomyces cerevisiae* ($5x10^{10}$ cfu/g) and Bioplus 2B ($3.2x10^{9}$ cfu/g).

Supplementation of probiotic at 900g per ton of feed in broiler chicken resulted in significant (P<0.05) higher feed consumption in comparison with control (Falaki *et al*., 2010).

Supplementation of probiotic 0.1% and 0.1% prebiotic in White Leghorn layers did not affect the feed consumption during 21-52 weeks of age. (Moorthy *et al*. 2010)

Yalcin *et al*. (2010) observed there was no difference in feed intake in Hyline Brown laying hens of 22-38 weeks of age fed with dietary supplementation of yeast autolyse (*Sacharomyces cerevisiae*) at 2, 3, and $4g/ kg^{-1}$.

Berrin (2011) reported that neither probiotic nor prebiotic supplementation was non-significant effect on feed consumption in Japanese quails (*Coturnixcoturnix japonica*) during 18-30 week of age.

Mikulski *et al*. (2012) reported that supplementation of probiotic (*Pediococcus acidilactici*) at 50 or 100 mg per kg in Hy-Line Brown hens during 23-46 weeks of age has no significant difference in feed intake.

In *Lohmann* variety layer birds supplemented with probiotic (heat inactivated *Lactobacillus salivarius*, *Clostridium butyricum*, *Bacillus subtilis,* and sodium butyrate) there was no improvement in feed intake during 24-32 weeks of age (Zhang *et al*., 2012).

Abdelqader *et al*. (2013) found no significant difference in feed intake in *Lohmann* white laying hens when fed basal diet supplemented with @ 1g/kg *Bacillus subtilis* (2.3x10^8 cfu/g) during 64-75 weeks of age.

Sobczak *et al*. (2015) reported that no significant difference of feed intake in *Lohmann* Brown laying hens supplemented with probiotic (*Bacillus subtilis* ATCCPTA-6737 at 1×10^8) during 18-42 weeks of age.

Fathi *et al*. (2018) reported that there was no significant difference in feed in take in three different breeds (White Leghorn, Saudi black and Saudi brown) during 36-48 weeks of age when fed with probiotic at 0, 200, 400 ppm (*Bacillus subtilis*)

Sheoran *et al.* (2018) found a significant (P<0.05) difference among treatments groups in feed intake during 16week feeding trial in White Leghorn layers during 22-38 weeks of age supplemented with probiotic at 0.5g *Lactobacillus fermentum*, 1.0g *Bacilludspp*, 2.0g *Saccharomyces cerevisiae* and 0.5, 1.0, and 2.0g prebiotic (mannonoligosacchride of *saccharomyces* cell all 47g, formic acid 32g) .

2.1.3 Feed conversion ratio

Tortuero and Fernandez (1995) noticed that no effect of *Lactobacillus acidophilus plus, Lactobacillus casei* supplementation (2.3x10^8cfu /kg feed) on feed efficiency in barley-based diets fed to laying hens.

Nahashon *et al*. (1996) observed significantly (P<0.05) better feed conversion (kg feed /kg egg) in DeKalb XL Single Comb White Leghorn layers raised on condensed cane molasses solubles containing viable *Lactobacillus* per kg diet from 20 to 59 weeks of age.

Panda *et al*. (2003) observed that feed efficiency was not influenced significantly by probiotic (Probiolac®) supplemented to the basal diets @100 or 200 mg/kg feed fed to White Leghorn layers from 25 to 72 weeks of age.

Supplementation of humate and probiotic at 0.1 % and 0.2% (*Lactobacillus, Bifidobacterium, Streptococcus, and Enterococcus spp.*) in Hisex Brown layers during 54-64 week of age resulted in improvement in feed efficiency than control (Yoruk *et al*., 2004).

Mahdavi *et al*. (2005) stated that there was no significant difference in feed conversion ratio in White Leghorn hens Hy-Line W-36 strain fed with Bio plus 2B at 0, 400, 1000 and 2000 g/ton of feed during 28-39 weeks of age.

Panda *et al*. (2006) noted that supplementation of probiotic (*Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Asperigillus oryzae)* with the concentration of 27 billion cfu/100g significantly (P<0.05) improved feed efficiency (kg feed/dozen eggs) in White Leghorn layers during 65 to 76 weeks of age.

Yousefi and Karkoodi (2007) observed that, feed conversion ratio (g/g) was not affected by 0.05% dietary probiotic (Thepax) and 0.15% yeast (*Saccharomyces cerevisiae*) supplementation in Hy-Line W-36 hens during 63-75 weeks of age.

Panda *et al*. (2008) reported that there was significant (P<0.05) improvement in feed efficiency of White Leghorn layer breeders of 25-40 weeks of age supplemented with commercial probiotic (*L.sporogenes* with 6000 million spores g^{-1}).

Yalcin *et al*. (2008) concluded that yeast culture (*Saccharomyces cerevisiae*) supplementation at 2g/kg to layer diets containing soybean meal and sunflower seed meal did not significantly affect feed efficiency (kg feed /kg egg and kg feed/ dozen eggs) in Lohmann Brown laying hens during 16 weeks of age.

Balevi *et al*. (2009) reported that supplementation of commercial probiotic (Protexin^{Tm}) was significantly (P<0.05) different in feed conversion ratio(kg food /kg egg) of old Hysex–Brown layer supplemented with probiotic at 0, 250, 500, or 750 ppm between 40-52 weeks old age.

Konca *et al*. (2009) observed that no significant difference in feed conversion ratio (feed/gain) when 10-weeks-old turkeys were fed with probiotic (*Saccharomyces cerevisiae* -strain SC 47) at @ 1 g per kg diet.

Sattar Bageri Dizaji and Rasoul Pirmohammadi (2009) observed significantl (P<0.05) decrease in feed conversion (g feed/g egg mass) in Hy-Line W-36 hens during 46- 55 weeks of age supplemented with *Sacharomyces Cerevisiae* (5x10^{10} cfu/g) and Bioplus 2B (3.2x10^{9} cfu/g).

Moorthy *et al*. (2010) reported that supplementation with probiotic and prebiotic did not affect the feed conversion ratio (kg feed /dozen eggs) in White Leghorn layers during 21-52 weeks of age.

Yalcin *et al*. (2010) observed that there was a significant (P<0.05) improvement in feed efficiency (kg feed/ kg egg) in Hyline Brown laying hens supplemented with yeast autolyse (*Sacharomyces cerevisiae*) at 2, 3 and 4 g kg−1 during 22-38 weeks of age.

Berrin (2011) reported that neither probiotic nor prebiotic supplementation was non-significant effect on feed efficiency (kg feed/ kg egg) Japanese quails (*Coturnixcoturnix japonica*) during 18-30 weeks of age.

Shivani Katoch *et al*. (2011) observed that the feed efficiency was significantly ($P<0.05$) increased with probiotic (*Lactobacillus sps*) supplemented in egg type chicken.

Wei Fen Li *et al*. (2011) reported that feed to egg ratio has no significant ($P>0.05$) increase in Shaoxing Ducks fed with *Bacilliussubtilis* at $1x10^8$ cfu/kg as dietary probiotic supplement.

Mikulski *et al*. (2012) reported a significant ($P<0.05$) difference with supplementation of probiotic (*Pediococcus acidilactici*) either at 50 mg or 100 mg per kg to Hy-Line Brown hens during 23-46 weeks of age for feed efficiency (kg feed/ kg egg)

Zhang *et al*. (2012) observed a significant ($P<0.05$) improvement in feed conversion ratio in Lohman variety when fed with probiotics (heat inactivated *Lactobacillus salivarius*, *Clostridium butyricum*, *Bacillus subtilis,* and sodium butyrate) during 24- 32 weeks of age.

Abdelqader *et al*. (2013) noticed significant($P<0.05$) difference in feed conversion (kg/kg) in *Lohmann* white laying hens supplemented @1g/kg *Bacillus subtilis* ($2.3x10^8$ cfu/g) during 64 to75 weeks of age.

Shalaei *et al*. (2014) reported that there was significant ($P<0.05$) difference in feed conversion ratio of Hy-line (W-36) laying hens from 32 to 42 weeks of age when supplemented with 150g per ton antibiotic (oxy tetracycline), 3kg per ton of organic acid mixtures, 50g per ton probiotic and 2kg per ton prebiotic.

Sobczak *et al*. (2015) noticed that no significant($P>0.05$) difference in feed conversion ratio in *Lohmann* Brown laying hens supplemented with probiotic (*Bacillus subtilis* ATCCPTA-6737 at 1×10^8) during 18 to 42 weeks of age.

Forte *et al*. (2016) observed that no significant (P>0.05) difference in FCR among the treatment groups when supplemented with *Lactobacillus acidophilus* at 0.1 and *Bacillus subtilis* at 0.05% in Hy-Line layer during 16-36 weeks of age.

Fathi *et al*. (2018) reported no significant difference among the treatment groups in FCR in three different breeds (White Leghorn, Saudi black and Saudi brown) during 36 - 48 weeks of age when supplemented with probiotic at 0, 200, 400 ppm (*Bacillus subtilis*)

Sheoran *et al*. (2018) reported that there was significant(P<0.05) difference in feed conversion ratio in White Leghorn layers of 22-23 weeks of age supplemented with probiotic of 0.5g *Lactobacillus fermentum*,1.0g *Bacillusspp*, 2.0g *Saccharomyces cerevisiae* and 0.5, 1.0, and 2.0g prebiotic (mannonoligosacchride of *saccharomyces* cell all 47g, formic acid 32g.

2.2 EGG WEIGHT

Tortuero and Fernandez (1995) reported significant (P<0.05) increase of egg weight in Single Comb White Leghorn laying hens from 15-19 months of age supplemented with *L.acidophilus and L.casei*.

Nahashon *et al.* (1996) found that no significant difference in egg weight of Dekalb XL single comb White Leghorn layers raised with condensed cane molasses soluble (CCMS -1100 mg) containing viable *Lactobacillus* per kg diet from 20 to 59 weeks of age. Similarly, supplementation of Bioplus2B (*Bacillus subtillis* and *Bacillus licheniformis*) at 0,400,1000 and 2000 per ton of feed in white leghorn Hy-line W-36 strain did not affect egg weight between 28-39 weeks of age (Mahdavi *et al*., 2005).

Davis and Anderson (2002) noted that supplementation with “Primalac®” to Single Comb White Leghorn Hy-Line W-36 and DeKalb XL laying hens had significantly ($P<0.002$) increase in egg weight during 18-70 weeks of age.

Panda *et al*. (2006) concluded that egg weight was not influenced by dietary supplementation of probiotic in White Leghorn layers during 65 to 76 weeks of age. Similarly, Yousefi and Karkoodi (2007) found that inclusion of probiotic (Thepax®) and yeast have no significant difference in egg weight of Hy-Line W-36 hens between 63-75 weeks of age.

Panda *et al*. (2008) reported that the egg weight was no significant improvement in White Leghorn layer breeders between 25-40 weeks of age supplemented with *Lactobacillus* cultures (100 mg/kg and 150 mg/kg).

Yalcin *et al*.(2008) concluded that yeast culture supplementation (*Saccharomyces cerevisiae*) at 2 g/kg to layer diets containing soybean meal and sunflower seed meal did not significantly affect but numerically increased the egg weight in Lohmann Brown laying hens during 16-37 week of age.

Sattar Bageri Dizaji and Rasoul Pirmohammadi (2009) observed that the supplementation with *Sacharomyces cerevisiae* ($5x10^{10}$ cfu/g) and Bioplus 2B ($3.2x10^{9}$ cfu/g) to Hy-Line W-36 hens at 46-55 weeks of age birds egg weight was significantly ($P>0.05$) decrease.

Balevi *et al*. (2009) found that supplementation with probiotic at 0, 250, 500, or 750 ppm levels in any significant variation in egg weight during 40-52 weeks of Hysex-brown layer hybrid birds.

Kalavathy Ramasamy *et al.* (2009) observed that the egg weight was significantly (P<0.05) heavier when compared with control in Lohmann Brown layers supplemented with *Lactobaclilus* cultures during 20 - 68 weeks of age

Yalcin *et al*. (2010) observed that there was significant (P<0.001) improvement in egg weight of Hyline Brown laying hens supplementation of yeast autolyse (*Sacharomyces cerevisiae*) at 2, 3 and 4 g kg−1 during 22-38 weeks of age.

Berrin (2011) reported that neither probiotic nor prebiotic supplementation to the diets had significant effect on egg weight in Japanese quails (*Coturnixcoturnix japonica*) during 18-30 weeks of age.

Wei Fen Li *et al.* (2011) observed that no significant improvement in egg weight in Shaoxing Ducks fed with probiotic supplement (*Bacillius subtilis)* at $1x10^8$cfu/kg.

Mikulski *et al*. (2012) reported that supplementation probiotic (*Pediococcus acidilactici)* at 100 mg per kg basal diet fed to Hy-Line Brown hens during 23-46 weeks of age has significantly (P<0.05) difference in egg weight .

Zhang *et al* .(2012) oserved that supplementation with probiotic (heat inactivated *Lactobacillus salivarius*, *Clostridium butyricum*, *Bacillus subtilis,* and sodium butyrate) in Lohman variety layer birds during 24-32 weeks of age has significantly (P,0.05) diiference in egg weight

Abdelqader *et al*. (2013) noticed significant(P<0.01) difference in egg weight of *Lohmann* white laying hens when supplemented with *Bacillus subtilis* ($2.3x10^8$ cfu/g) @ 1g/kg during 64 to 75 weeks of age.

Shalaei *et al.* (2014) investigate the effects of antibiotic, organic acid, probiotic and prebiotic in Hy-line (W-36) laying hens from 32 to 42 weeks of age supplemented with 150g per ton oxy tetracycline, 3kg per ton of organic acid mixtures, 50g per ton probiotic and 2kg per ton prebiotic to the basal diet showed significant ($P<0.05$) improvement in the egg weight.

Sobczak *et al*. (2015) observed that there was no significant difference in egg weight in *Lohmann* brown laying hens supplemented with a commercial probiotic (*Bacillus subtilis*ATCCPTA-6737 at 1×10^8) during the 18-42 week of age.

Forte *et al*. (2016) reported that there was no significant($P>0.05$) improvement of egg weight in Hy-Line layer supplemented with probiotic culture *Lactobacillus acidophilus* at 0.1% and *Bacillus subtilis* 0.05% during 16 -36weeks of age .

Sheoran *et al*. (2018) found that no significant improvement in egg weight in White Leghorn layers during 22-38 weeks of age, when supplemented with probiotic at 0.5g *Lactobacillus fermentum*, 1.0g *Bacilludspp*, 2.0g *Saccharomyces cerevisia*e and prebiotic supplementation 0.5, 1.0 and 2.0g (mannonoligosachride of *saccharomyces* cell wall-47g, formic acid- 32g).

2.3 INTERNAL EGG QUALITY PARAMETERS

2.3.1 Haugh unit

Tortuero and Fernandez (1995) observed that Haugh unit has a significant ($P<0.05$) improvement in Single Comb White Leghorn hens from 15 to 19 months of age when supplemented with *L.acidophilus* and *L.casei* at2.3×10^8 cfu /kg feed.

Nahashon *et al*. (1996) concluded that no significant difference in Haugh unit of eggs was observed in DeKalb XL Single Comb White Leghorn layers raised on condensed cane molasses solubles (CCMS -1100 mg) containing viable Lactobacillus per kg diet from 20 to 59 weeks of age.

Mahdavi *et al*. (2005) observed no significant difference in Haugh unit of eggs of White Leghorn hens Hy-Line, W-36 strain fed supplementation with Bio plus 2B @ 0,400, 1000, 2000g per ton of feed during 28-39 weeks of age.

Panda *et al*. (2008) reported that there was no improvement in Haugh unit of eggs of White leghorn layer breeders of 25 to 40 weeks of age fed with *Lactobacillus sporogenes* with 6000 million spores g^{-1}.

Yalcin *et al*. (2008) concluded that yeast culture (*Saccharomyces cerevisiae*) supplementation at 2g/kg to layer diets containing soybean meal and sunflower seed meal did not significantly affect Haugh unit in *Lohmann* Brown laying hens during 21-37 weeks of age.

Berrin (2011) reported that neither probiotic nor prebiotic supplementation to the diets had significant ($P>0.05$) effect on Haugh unit in Japanese quails (*Coturnix coturnix japonica*) during 18-30 weeks of age.

Wei Fen Li *et al*. (2011) reported that Haugh unit was significantly not affected in Shaoxing ducks supplemented with *Bacillius subtilis* at $1x10^8$cfu/kg as dietary probiotic.

Mikulski *et al*. (2012) reported that no significant difference of Haugh unit in Hy-Line Brown hens during 23-46 weeks of age supplemented with probiotic (*Pediococcus acidilactici)* @ 100 mg per kg basal diet.

Zhang *et al*. (2012) observed that the Haugh unit was significant($P<0.05$) difference in Lohman layer hens supplemented with heat inactivated probiotics (*Lactobacillus salivarius*, *Clostridium butyricum*, *Bacillus subtilis*, and sodium butyrate) during 24-32 week of age.

Sobczak *et al*. (2015) noticed a significant(P<0.05) difference Haugh unit among the treatment groups in Lohmann Brown laying hens supplemented with (*Bacillus subtilis* ATCCPTA-6737 at 1×10^8) during 18-42 weeks of age.

Fathi *et al*. (2018) found that no significant difference of Haugh unit in three different breeds (White Leghorn, Saudi black and Saudi brown) supplemented with probiotic at 0, 200, 400 ppm (*Bacillus subtilis*) during 36-48 weeks of age.

2.3.2 Albumen index

Supplementation of humate and probiotic at 0.1 % and 0.2% (*Lactobacillus, Bifidobacterium, Streptococcus, and Enterococcus spp.*) in Hisex Brown layers during 54-64 week of age there was no significant difference in yolk index (Yoruk *et al*., 2004).

Valavan *et al*. (2006) reported that neither chitin nor probiotic (*Lactobacillus acidophilus* at 0, 1 and 2 g per kg feed) supplementation to commercial layers had any significant difference in albumen index during 25 to 40 weeks of age.

Yalcin *et al*. (2008) concluded that yeast culture (*Saccharomyces cerevisiae*) supplementation at of 2 g/kg to layer diets containing soybean meal and sunflower seed meal did not significantly affect the albumen index in Lohmann Brown laying hens during21- 37 weeks of age.

In Japanese quails (*Coturnix coturnix japonica*) during 18-30 weeks of age neither probiotic nor prebiotic supplementation to the diets had significant effect on albumen index (Berrin, 2011).

2.3.3 Yolk index

Yoruk *et al*. (2004) observed that no significant difference in yolk index of Hysex-Brownlayers when supplemented with probiotic (*Lactobacillus*, *Bifidobacterium, Streptococcus, and Enterococcus spp.* at 0.1 and 0.2%) during 54-64 week of age.

Valavan *et al*. (2006) found that no significant difference in yolk index with supplementation of chitin and probiotics (*Lactobacillus acidophilus* at 0, 1 and 2g per kg feed) in commercial layers during 25 to 40 weeks of age.

Yalcin *et al*. (2008) concluded that supplementation of (*Saccharomyces cerevisiae*) yeast culture at 2 g/kg to layer diet did not significantly affect yolk index in *Lohmann* Brown laying hens during 21- 37 weeks of age.

Berrin (2011) reported that neither probiotic nor prebiotic supplementation to the diets had significantly affected yolk index in Japanese quails (*Coturnix coturnix japonica*) during 18-30 weeks of age.

Mikulski *et al*. (2012) reported that no significant difference in yolk index of eggs laid by Hy-Line Brown hens during 23-46 weeks of age upon feeding of probiotic (*Pediococcus acidilactici*) supplementation at 50 or 100 mg per kg to the basal diet.

2.4 EXTERNAL EGG QUALITY

2.4.1 Shell weight

Yousefi and Karkoodi (2007) noticed that significantly ($P<0.05$) increase in shell weight of Hy-Line W-36 hens supplemented with 0.05 and 0.15% dietary probiotic (Thepax) 0.05,0.1,& 0.15% yeast (*saccharomyces cerevisiae*) during 63-75 weeks of age.

Panda *et al*. (2008) reported that significant ($P<0.05$) improvement in shell weight of eggs of White Leghorn layer breeders of 25-40 weeks of age supplemented with probiotic (*L.sporogenes* with 6000 million spores g^{-1}).

Forte *et al*. (2016) reported that no significant ($P>0.05$) difference in shell weight in Hy-Line layer fed on probiotic (*Lactobacillus acidophilus*) at 0.1% and *Bacillus subtilis* at 0.05% during 16 -36 weeks of age.

Fathi *et al*. (2018) noticed significant($P<0.05$) difference among the treatment groups in shell weight supplemented with probiotic (*Bacillus subtilis*) in three different breeds (White Leghorn, Saudi black and Saudi brown) during 36 -48 weeks of age.

2.4.2 Shell thickness

Mahdavi *et al*. (2005) stated that the shell thickness has no significant difference in White Leghorn hens Hy-Line, W-36 strain fed with Bio plus 2B @ 0, 400, 1000, and 2000g per ton of feed during 28-39 weeks of age.

Panda *et al*. (2006) noted that supplementation of probiotic at 27 billion cfu/100g concentration was significantly ($P<0.05$) improved the shell thickness in White Leghorn layers during 65 to 76 weeks of age.

Yousefi and Karkoodi (2007) observed that significantly ($P<0.05$) increased shell thickness in Hy-Line W-36 hens supplemented with 0.05 and 0.15% dietary probiotic (Thepax) and 0.05,0.1 and 0.15% yeast (*Saccharomyces cerevisiae*) during 63-75 weeks of age.

Panda *et al*. (2008) reported that there was significant ($P<0.05$) improvement in shell thickness of eggs of White Leghorn layer breeders of 25-40 weeks of age supplemented with commercial probiotic (*L.sporogenes* with 6000 million spores g^{-1}).

Yalcin *et al*. (2008) concluded that yeast culture (*Saccharomyces cerevisiae*) supplementation at 2g/kg to layer diets did not significantly affect shell thickness in *Lohmann* Brown laying hens during 16 -37 weeks of age. Similarly, Wei Fen Li *et al*. (2011) reported that the shell thickness has no significant difference in Shaoxing ducks supplemented with (*Bacillius subtilis* at $1x10^8$ cfu/kg).

Supplementation of probiotic (*Pediococcus acidilactici*) at 50 or 100 mg during 23-46 weeks of age in Hy-Line Brown hens has resulted in significant ($P<0.05$) improvement in shell thickness (Mikulski *et al*., 2012) and similar results were reported by Zhang *et al*. (2012) in *Lohmann* variety with supplementation of probiotic (*Lactobacillus salivarius*, *Clostridium butyricum*, *Bacillus subtilis*, and sodium butyrate) during 24- 32 week of age.

Abdelqader *et al*. (2013) noticed significant ($P<0.05$) difference in egg shell thickness in *Lohmann* white laying hens fed with basal diet supplemented @ 1g/kg (*Bacillus subtilis* $2.3x10^8$ cfu/g) during 64 to 75 weeks of age.

Shalaei *et al*. (2014) investigated the effects of 150g/ton antibiotic, 3kg/ton organic acid, 50g/ton probiotic and 2kg/ton prebiotic in Hy-line W-36 laying hens during 32-42 weeks of age reported significant ($P<0.05$) difference in shell thickness.

Sobczak *et al*. (2015) observed that supplementation with (*Bacillus subtilis* ATCCPTA-6737 at 1×10^8) in *Lohmann* Brown laying hens during 26 weeks of age has significant ($P<0.05$) difference in shell thickness .

Fathi *et al*. (2018) noticed significant ($P<0.05$) difference in shell thickness of three different breeds (White Leghorn, Saudi black and Saudi brown) supplemented with probiotic at 0, 200, 400 ppm (*Bacillus Subtilis*) during 36 -48 weeks of age.

2.4.3 Shell percentage

Yousefi and Karkoodi (2007) reported that there was no significant difference in shell percentage when compared with control in Hy-Line W-36 hens during 63-75 weeks of age.

Mikulski *et al*. (2012) observed a significant difference in ($P<0.05$) shell percentage in Hy-Line Brown hens supplemented with (*Pediococcus acidilactici*) at 50 or 100 mg per during 23-46 weeks of age.

Shalaei *et al*. (2014) reported that no significant difference in egg shell percentage in Hy-line (W-36) laying hens supplemented with 150g/ton antibiotic, 3kg/ton organic acid, 50g/ton probiotic and 2kg/ton prebiotic during 32 to 42 weeks of age.

Sobczak *et al*.(2015) observed that shell percentage was significantly ($P<0.05$) different in *Lohmann* Brown laying hens supplemented with probiotic (*Bacillus subtilis* at 1×10^{8})during 18-42 weeks of age.

Forte *et al*. (2016) observed that there was no significant ($P>0.05$) difference in shell percentage among the treatment groups supplementated with probiotic *Lactobacillus acidophilus* at 0.1% and *Bacillus subtilis* at 0.05% in Hy-Line layer during 16-36 weeks of age.

2.4.4 Shell strength

Mahdavi *et al*. (2005) stated that shell strength has no significant difference in White Leghorn hens Hy-Line, W-36 strain supplemented with Bio plus 2B @ 0, 400, 1000, and 2000g per ton of feed during 28-39 weeks of age.

Panda *et al*. (2008) noted that supplementation of probiotic (*L.sporogenes* with 6000 million spores g^{-1}) significantly (P<0.05) improved the shell strength in White Leghorn layers during 65 to 76 weeks of age.

Supplementation of 150g per ton antibiotic (oxy tetracycline), 3kg per ton of organic acid mixtures, 50g per ton probiotic and 2kg per ton prebiotic has no improvement in shell strength in Hy-line W-36 during 32 -42 week of age (Shalaei *et al*., 2014).

Sobczak *et al.* (2015) reported that shell strength was significant(P<0.05) in *Lohmann* Brown laying hens supplemented with probiotic (*Bacillus subtilis* ATCCPTA-6737 at 1×10^8) during 18-42 weeks of age under environmentally controlled condition houses.

Upadhaya *et al*. (2016) observed that slight improvement in shell strength in Hy-line Brown birds at 40 weeks supplemented with 0.1% *Bacillus subbtilis* and *Bacillus methylotrophicus.*

Fathi *et al*. (2018) reported that shell strength has significant (P<0.05) difference in three different breeds (White Leghorn, Saudi black and Saudi brown) supplemented with probiotic at 0, 200, 400 ppm (*Bacillus* s*ubtilis*) during 36-48 weeks of age.

2.5 LIVEABILITY

Yoruk *et al*. (2004) observed that there was reduced mortality in Hysex-Brown layers diets supplemented with probiotic (*Lactobacillus, Bifidobacterium, Streptococcus, and Enterococcus spp.* at 0.1 and 0.2%) during 54 -64 week of age.

Pelicano *et al.* (2004) opined that the better viability (P<0.05) in broilers Supplemented with probiotic containing *Bacillus subtilis* @150g/ton or *Lactobacillus* *acidophilus, Lactobacillus casei*, *Streptococcus lactis*, *S.faecium*, *Bifidobacterium bifidum and Aspergillus oryzae* @ 1kg/ton (98.33%) compared to control group (96.19%).

Vincente *et al*. (2007) reported a significant (P<0.01) reduction in mortality (5.87%) compared to the control (6.72%) when probiotic FM- B11TM (10^{11} cfu of live *Lactobacillus* strains) was administered in drinking water at the rate of 10^6 cfu/ml.

The supplementation of probiotic (Protexin^{Tm}) at 0, 250, 500 and 750 ppm to Hi sex- Brown layer hybrids did not affect mortality between 40 -52 weeks of age (Balevi *et al.,* 2009). Similarly, panda *et al.* (2006) reported that mortality was not effected in White Leghorn chicks supplemented with probiotic *(Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum, and Asperigillus oryzae,*) with the concentration of 27 billion cfu/*100g* during 65 -76 weeks of age.

2.6 ECONOMICS

. Davis and Anderson (2002) noticed that feeding of direct fed microbial, probiotic supplement “Primalac” to Single Comb White leghorn, Hy-Line W-36 and DeKalb XL laying hens significantly (P<0.002) reduced the feed cost.

Sabiha *et al*. (2005) observed that the cost of production in broilers was lower in 0.025 and 0.05% probiotic (Yea-sacc1026, *Lactobacillus acidophilus, Sterptococcus faecium* etc.) supplemented groups at six and eight weeks of age, respectively.

Moorthy *et al*. (2010) reported that diets supplemented with probiotic and prebiotic supplements did not affect the return over feed cost per bird in White Leghorn layers aged 21-52 weeks of age.

CHAPTER – III

MATERIALS AND METHODS

An experiment entitled "**Effect of probiotic feed supplement in commercial layers chickens"** was planned to assess the effect of supplementation of probiotics to the laying hens on their performance during the peak production. The experiment was conducted at the Poultry Experimental Station, Livestock Farm Complex (LFC), Rajendranagar, Hyderabad. The laboratory analysis of the egg quality parameters was conducted at Department of Poultry science, College of Veterinary Science and - Directorate of Poultry Research, ICAR, Rajendranagar, Hyderabad. The experimental procedures and techniques adopted during the course of study are detailed in this chapter.

3.1 HOUSING AND MANAGEMENT

The experiment was conducted during August to December 2018. A total of 288 White Leghorn pullets (BV300) at the age of 16 weeks were procured from private agency. The birds were leg banded, weighed individually and housed in California type cages (16"Hx 12"Wx18"D) having provision of feeders and nipple watering system. The birds were given *ad lib* feed and water and raised under identical management conditions.

Before the commencement of the actual experiment, the cages, feeders and nipple drinking system were thoroughly cleaned, disinfected, and sprayed against external parasites. Other health precautions and sanitary measures were also taken throughout the study period. Diets were offered in separate feeder for different treatments with clear demarcation between replicates. Fluorescent lamp was placed for the lighting system to increase the lighting period to 16 h per day in order to increase feed intake and laying (Yasmeen *et al.,* 2008). Birds were adapted to respective treatment diet for a week before the commencement of the actual data collection.

3.2 EXPERIMENTAL DESIGN

The White Leghorn pullets (16weeks of age) were distributed randomly in to 8 different treatments with 6 replicates having 6 birds/replicate. Prior to experiment, the pre layer ration was fed to the birds till 21 weeks and layer ration from 22 weeks onwards.

3.3 EXPERIMENTAL DIETS

Three types of probiotic supplementation at graded levels of 0,100, 200 g/ton and Bacitracin methylene disalicylate (BMD) at 50g/ton feed fed to commercial layer chickens. The probiotic-1 (single strain i.e Competitor-BS) purchased from private agency. The probiotic-2 (Multi strain probiotics-Sporich-Total) and probiotic-3 (Multi strain probiotics - Prome-Max) are proprietary commercial probiotic products prepared by Sanzyme Biologics Private Limited, Hyderabad. The experimental layer diets were formulated (*isonitrogenous* and *isocaloric).* The Basal Diet (T1, Control) diet consisted of corn and soybean meal. In the remaining experimental diets Basal Diet + 4×10^{9} cfu/g of probiotic-1 contain (*Bacillus subtilis, Bacillus coagulants* and *Sacchromyces boulardii*), (T2) ; Basal Diet + 8×10^{9}cfu/g of probiotic- 1 (T3); Basal Diet + 4×10^{9}cfu/g of probiotic -2 contain (*Bacillus subtilis, Sacchromyces boulardii* and *Clostridium butryicum*) (T4) ; Basal Diet + 8×10^{9}cfu/g of probiotic- 2 (T5); Basal Diet + 4×10^{9}cfu/g of probiotic- 3 contain (*Bacillus sps Lactobacillus sps, Enterococcus sps, pediococcus acidilactici, sacchromyces boulardii* and *Clostridium butryicum*) (T6); Basal Diet + 8×10^{9}cfu/g of probiotic 3 (T7); Basal Diet +50g/ton AGP (BMD) (T8) in diet and evaluated for production performance The percent hen day egg production, feed intake, feed conversion ratio per dozen eggs, egg weight, egg quality parameters, liveability and relative economics were studied for a total of four laying periods of 28 days each from 22 to 37 week of age.

Table 1.Ingredient Composition of Basal Diets (in kgs) fed to the commercial White Leghorn layer birds from 22 to 37 week of age

Ingredients	T1	T2	T3	T4	T5	T6	T7	T8
Maize	56	56	56	56	56	56	56	56
Soya bean meal	23	23	23	23	23	23	23	23
DORB	8.63	8.62	8.61	8.62	8.61	8.62	8.61	8.58
Shell grit	10.2	10.2	10.2	10.2	10.2	10.2	10.2	10.2
DCP	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Vit.mix*	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Trace Mineral Mixture**	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline chloride (50%)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Toxin binder	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Sodium bicarbonate	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Probiotic	0.0	0.01	0.02	0.01	0.02	0.01	0.02	0.05
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

* Vitamin premix provided per kg diet: Vitamin A 200000 IU, Vitamin B2 25 mg, Vitamin D3 3000IU, Vitamin K 2mg. Riboflavin 25mg, Vitamin B1 1mg, Vitamin B6 2mg, Vitamin B12 40mg and Niacin 15mg.

** Trace mineral provided per kg diet: Manganese 120mg, Zinc 80mg, Iron 25mg, Copper 10mg, Iodine 1mg and Selenium 0.1mg.

Nutrient Composition								
CP (%)	16.97	16.97	16.96	16.97	16.96	16.97	16.96	16.94
ME(kcal/kg)	2620	2620	2618	2620	2618	2620	2618	2616
Calcium (%)	3.45	3.45	3.45	3.45	3.45	3.45	3.45	3.45
Available (%) Phosphorus	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Lysine (%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Methionine (%)	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38

3.4 PERFORMANCE STUDY DURING LAYER PHASE

3.4.1 Egg production

Data on egg production was recorded from 22 to 37 weeks for 4 laying periods of 28 days each. The percent hen day egg production was calculated for each treatment for each of the laying periods.

$$\text{Hen day egg production \%} = \frac{\text{No. of eggs laid on that day}}{\text{No. of live birds available on that day}} \times 100$$

3.4.2 Feed Intake

Feed consumption of each replicate was recorded at periodic intervals, on cumulative basis and the feed consumption /bird/day was arrived at the end.

3.4.3 Feed Conversion Ratio per dozen eggs

The feed conversion ratio (FCR) was calculated (feed intake /per dozen eggs) considering mortality, as and when it occurred to maintain accuracy in the data collection by weighing back the feed on the day of mortality in that particular group.

$$\text{FCR/ dozen eggs} = \frac{\text{Kg. of feed consumed}}{\text{Dozen eggs produced}}$$

3.5 EGG WEIGHT

The egg weight was recorded to nearest 0.1 gram accuracy in each of the replicate, the weight of all the eggs produced during the last three consecutive days of each laying period was recorded to calculate the mean egg weight of each treatment group.

3.6 INTERNAL EGG QUALITYPARAMETERS

Egg quality parameters were recorded at each laying period from the eggs laid by a bird consecutively for three days.one egg was collected from each replicate and utilised for measurement of egg quality traits.

3.6.1 Haugh Unit

Haugh unit was determined by the following formula: (Sing, R.A. 2006)

$HU = 100 \log (H – 1.7w^{0.37} + 7.57)$

Where H= height of the albumen (mm) and W=

egg weight (g)

3.6.2 Albumen Index

Albumen index was calculated after the measurement of height and diameter of thick albumen with the help of spherometer and vernier calipers respectively (Heiman and Carver, 1936).

$$\text{Albumen Index} = \frac{\text{Height of albumen}}{\text{Average width of albumen}}$$

3.6.3 Yolk Index

The yolk index which is an indirect measurement of the spherical shape of the yolk and strength of the yolk membrane, was calculated after the measurement of height and diameter of yolk with the help of spherometer and verniercalipers, respectively (Funk, 1948).

$$\text{Yolk Index} = \frac{\text{Yolk height}}{\text{Average width of yolk}}$$

3.7 EXTERNAL EGG QUALITY PARAMETERS

3.7.1 Shell weight

The shell weight (%) was recorded after air drying and the observations were measured by using digital balance (Shimadzu- ATX-224) nearest to 0.1 mg accuracy.

3.7.2 Shell thickness

From the dried shell, the thickness was measured using Mitutoya dial gauge meter (Model no.7301, Japan) to accuracy of 0.01 mm. The values were measured at three places viz., middle, narrow and broad ends and expressed in millimetres.

3.7.3 Egg shell percentage

It is calculated by dividing the shell weight with egg weight and then multiplied by 100

$$\text{Egg shell (\%)} = \frac{\text{Shell weight}}{\text{Egg weight}} \times 100$$

3.7.4 Shell strength

Shell breaking strength measured by shell force gauge (static compressor) expressed in Newton's/ kg^2, Universal testing machine was used to measure shell strength (model no KIC-1-100C) capacity 50 kg, speed-500mm/minute.

3.7.5. Egg density (g)

The egg density was measured by using digital density balance (LCGC – AS220/X) with 0.0001 g/cm^3 accuracy.

3.8 LIVEABILITY

The data on livability was calculated based on the mortalities recorded as and when they occurred.

3.9 RELATIVE ECONOMICS

The cost economics using three types of probiotic each at 100 & 200g/ton of feed different levels along with the Bacitracin methylene disalicylate (BMD) at 50g/ton and control diet were calculated in terms of returns over feed cost. For this purpose, the prevailing cost of feed ingredients and additives used were obtained from local market and cost of control and probiotic and BMD based diets were calculated for White Leghorn birds layer rations used in this study for the respective phases period-P1 (22-25wks), period-P2 (26-29wks), period-P3 (30-33wks) and period-P4 (34-37wks) of age. For calculating economics of overall period (22-37 wks) the cost of feeding during four phases was added. The prevailing sale price of egg (Rs. 3.75 per egg) was considered to calculate. The cost of probiotic was Rs.500/kg and BMD at 50g/ton cost also included.

4. STATISTICAL ANALYSIS

The data were analysed using General Linear Model procedure of statistical Package for social Sciences(SPSS) 15^{Th} version and comparison of means was done using Duccan’s multiple test (Duncan,1955) and significance was considered at $P<0.05$.

CHAPTER – IV

RESULTS

The results obtained from the study are presented in this chapter.

4.1 PRODUCTION PERFORMANCE OF COMMERCIAL LAYERS

The performance of the commercial White Leghorn layers (BV 300) which were fed with different diets having proprietary probiotic , commercial probiotic and antibiotic are compared to control group in terms of percent hen day egg production, feed consumption, feed efficiency per dozen eggs, egg quality parameters, livability, relative economics in White Leghorn layers during 22-37 weeks of age are presented below.

4.1.1Percent hen- day egg production

The data on percent hen day egg production as influenced by three types of probiotic at graded levels are shown in the Table 2, Figure 1. The per cent hen day egg production in White Leghorn layers were significantly ($P<0.05$) influenced during period-P1 (22-25wks), period-P2 (26-29wks), period-P3 (30-33wks), period-P4 (34-37wks) and over all period (22-37 wks) of age.

It is observed that in period-P1 (22-25wks) there was significant ($P<0.05$) difference in percent hen day egg production among different levels of probiotic fed groups. Highest percent hen day egg production was observed with probiotic-2 @ 100g per ton of feed (80.56%) followed by BMD @50g/ton of feed (77.38%), probiotic- 2 @ 200g/ton of feed & probiotic- 3 @ 200g /ton of feed were (75.71 and 75.71%,) while in period-P2(26-29wks), percent hen day egg production was higher in probiotic-2 @100g /ton of feed (95.01%) followed by probiotic-1 @100 & 200g/ton of feed (93.75,93.75%), probiotic-3 @100 & 200g per ton of feed (92.16, 93.05%), BMD @

50g/ton of feed (91.38%), probiotic-2 @200g/ton of feed were (90.09%) and lower in control (88.24%).

In the present study period-P3 (30-33wks) showed that there existed highly significant (P<0.05) difference in graded levels of probiotic fed group of probiotic-2 @100g/ton of feed (95.85%) followed by probiotic-3 100 & 200gper ton of feed (95.60 and 95.53%), probiotic-1 @100g/ton feed (94.16%), BMD @50g/ ton feed (93.97%) and probiotic-1@ 200g/ton feed (92.87%) compare with control (92.41%). During the period-P4 (34-37wks) significant (P<0.05) difference was observed among different treatments, highest percent hen day egg production was observed in probiotic-2 @100g/ton of feed (95.64%) followed by probiotic-3@100 & 200 g/ton of feed (94.18 and 94.35%), BMD@50g/ton of feed (94.18%) and probiotic-1 @200g/ton of feed (92.56%) compared with control (92.20%).

During the entire study period (22-37wks) a significant (P<0.05) difference in percent hen day egg production among different levels of probiotic fed groups was observed. The Highest percent hen day egg production in probiotic-2 @ 100g/ton of feed (91.77%) followed by probiotic-3 @ 200g/ton of feed (89.66%), BMD @ 50g/ton of feed (89.23%), probiotic-1@ 100 & 200g/ton of feed (88.75, 88.42%) & lower in probiotic-2 @200g/ton of feed (86.60%) and in control(86.76%).

4.1.2 Feed intake

The data on feed intake in commercial White Leghorn layers as influenced by different dietary treatments is presented in Table 3, Figure 2 .The feed consumption was significantly (P<0.05) different during first (22-25wks), second (26-29 wks), third (30-33 wks) and fourth (34-37 wks) periods and also overall period (22-37 wks) was also significantly (P<0.05) influenced by supplementation with probiotics and BMD. The least feed intake in control group (112.16g) compared to other treatment groups.

Table 2. Effect of dietary supplementation of probiotic at graded levels on percent hen day egg production in White Leghorn layers during 22-37 weeks of age

Treatment	g /ton	Age (weeks)				Mean 22-37 wks
		22-25(P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	74.21^{d}	88.24^{f}	92.41^{b}	92.20^{cd}	**86.76^{d}**
Probiotic -1	100	74.93^{cd}	93.75^{ab}	94.16^{ab}	92.16^{cd}	**88.75^{c}**
Probiotic -1	200	74.50^{cd}	93.75^{ab}	92.87^{b}	92.56^{c}	**88.42^{c}**
Probiotic -2	100	80.56^{a}	95.01^{a}	95.85^{a}	95.64^{a}	**91.77^{a}**
Probiotic -2	200	75.71^{c}	90.09^{e}	89.37^{c}	91.24^{d}	**86.60^{d}**
Probiotic -3	100	74.57^{cd}	92.16^{cd}	95.60^{a}	94.18^{b}	**89.13^{bc}**
Probiotic -3	200	75.71^{c}	93.05^{bc}	95.53^{a}	94.35^{b}	**89.66^{b}**
BMD	50	77.38^{b}	91.38^{de}	93.97^{ab}	94.18^{b}	**89.23^{bc}**
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.001	0.001	**0.001**
SEM		0.317	0.343	0.355	0.235	**0.241**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

Table 3. Effect of dietary supplementation of probiotic at graded levels on Feed intake (g/hen/day) in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25(P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	110.89^{bc}	111.15^{d}	109.87^{c}	116.83^{h}	**112.16^{e}**
Probiotic -1	100	108.54^{de}	116.86^{ab}	116.32^{a}	119.67^{g}	**115.36^{cd}**
Probiotic -1	200	107.24^{e}	116.50^{ab}	115.03^{ab}	121.17^{f}	**114.98^{d}**
Probiotic -2	100	109.48^{c}	115.44^{bc}	116.15^{a}	124.50^{e}	**116.43^{c}**
Probiotic -2	200	111.85^{ab}	117.74^{a}	113.93^{ab}	126.17^{d}	**117.45^{b}**
Probiotic -3	100	110.32^{c}	114.24^{c}	110.20^{c}	127.33^{c}	**115.55^{cd}**
Probiotic -3	200	112.92^{a}	117.50^{ab}	116.27^{a}	128.33^{b}	**118.79^{a}**
BMD	50	107.11^{e}	114.15c	113.37^{b}	129.83^{a}	**116.14^{c}**
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.001	0.001	**0.001**
SEM		0.326	0.378	0.448	0.631	**0.289**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

4.1.3 Feed conversion ratio per dozen eggs

The data on feed conversion ratio per dozen eggs (Kg of feed consumed for every /dozen eggs produced) is presented in the Table 4, Figure 3. The results revealed that, there exixted a significant (P<0.05) difference in feed conversion ratio per dozen eggs during period-P1 (22-25wks), period-P2 (26-29wks), period-P3 (30-33wks), period-P4 (34-37wks) of age and over all period (22-37wks) . During period-P1(22-25) the best feed conversion ratio per dozen eggs in commercial White Leghorn layers fed with supplementation of probiotic-2 @100g/ton of feed (1.631) followed by BMD @50gton of feed (1.661), probiotic-1 @100 & 200g/ton of feed (1.739, 1.728), probiotic-2 @ 200g/ton of feed (1.773) and poor in probiotic-3@ 200g/ton of feed (1.790) and control(1.794) were recorded.

Whereas best feed conversion ratio per dozen eggs was reported in commercial White Leghorn layers fed with supplementation of probiotic-2 @100g/ton of feed (1.458). The poor feed conversion ratio per dozen eggs (1.488) was reported in probiotic -2 @ 200g / ton of feed. It is also observed that the feed conversion ratio per dozen eggs was intermediate in other groups during period-P2 (26-29wks).

In the present experiment during period-P3 (30-33wks) in it is observed that there was significant (P<0.05) difference among different treatments fed with probiotic-3 @ 100g/ton of feed (1.384), probiotic-2@ 100g/ton of feed (1.455), probiotic-3 @ 200g/ton of feed (1.461)and BMD @ 50g/ton of feed (1.448) and probiotic-1@ 100 & 200g/ton of feed (1.482 & 1.486) and probiotic-2 @ 200g/ton of feed (1.530) compared with control (1.428).

The present experiment revealed a significant (P<0.05) difference during the period-P4 (34-37wks). The poor feed conversion ratio per dozen eggs was observed in commercial White Leghorn layers fed with probiotic supplemented and BMD compared with control group (1.519).

The overall period feed conversion ratio per dozen eggs was significantly (P<0.05) influenced with supplementation of probiotics and BMD in commercial White Leghorn layers between 22-37 weeks of age. The best feed conversion ratio per dozen eggs was recorded in probiotic-2 @ 100g/ton of feed (1.527) followed by probiotic-1 @ 100 & 200 g/ton of feed (1.569. 1.569), probiotic-3 @100g/ton of feed (1.568), BMD @ 50g/ton of feed(1.566), control & probiotic-3@ 200g/ton of feed (1.563 & 1,600) groups. Whereas the poor feed conversion ratio per dozen eggs was reported in probiotic-2 groups fed with 200g /ton of feed (1.633).

4.2 EGG WEIGHT

The data on egg weight (g) is presented in the Table 5, Figure 4. The egg weight was significantly (P<0.05) influenced by probiotic and BMD supplementation. The overall egg weight was significantly (P<0.05) higher (58.84g) in probiotic-1@100 g/ton of feed and followed by probiotic-3@ 200g/ton of feed (58.18g) and probiotic-2 @ 200g/ton of feed (58.12g). However, the probiotic-2 @ 200g/ton of feed has drastically increased the egg weight from 55.29 to 61.58g compared to other treatments during 22-37 weeks of age.

Table 4. Effect of dietary supplementation of probiotic at graded levels on Feed conversion ratio (kg feed consumed for dozen eggs) in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25(P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	1.794^{a}	1.512^{b}	1.428^{c}	1.519^{d}	**1.563^{c}**
Probiotic -1	100	1.739^{c}	1.496^{b}	1.482^{b}	1.560^{c}	**1.569^{c}**
Probiotic -1	200	1.728^{bc}	1.491^{bc}	1.486^{b}	1.571^{c}	**1.569^{c}**
Probiotic -2	100	1.631^{d}	1.458^{c}	1.455^{bc}	1.564^{c}	**1.527^{d}**
Probiotic -2	200	1.773^{ab}	1.569^{a}	1.530^{a}	1.661^{a}	**1.633^{a}**
Probiotic -3	100	1.776^{ab}	1.488^{bc}	1.384^{d}	1.624^{b}	**1.568^{c}**
Probiotic -3	200	1.790^{a}	1.515^{b}	1.461^{bc}	1.634^{b}	**1.600^{b}**
BMD	50	1.661^{d}	1.499^{b}	1.448^{bc}	1.656^{a}	**1.566^{c}**
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.001	0.001	**0.001**
SEM		0.009	0.006	0.007	0.007	**0.005**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

Table 5. Effect of dietary supplementation of probiotic at graded levels on Egg weight (g) in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25(P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	55.17^{bc}	55.65^{c}	57.12^{c}	60.07^{c}	**57.00^{d}**
Probiotic -1	100	57.00^{a}	58.44^{a}	59.83^{a}	60.12^{c}	**58.84^{a}**
Probiotic -1	200	56.32^{ab}	57.06^{b}	58.01^{bc}	60.97^{abc}	**58.09^{b}**
Probiotic -2	100	54.14^{cd}	57.78^{ab}	59.21^{ab}	58.43^{d}	**57.39^{cd}**
Probiotic -2	200	55.29^{bc}	55.71^{c}	59.91^{a}	61.58^{a}	**58.12^{b}**
Probiotic -3	100	55.77^{b}	57.72^{ab}	57.83^{c}	60.26^{bc}	**57.90^{bc}**
Probiotic -3	200	55.41^{b}	57.61^{ab}	58.34^{bc}	61.35^{ab}	**58.18^{b}**
BMD	50	53.75^{d}	57.61^{ab}	58.17^{bc}	61.42^{ab}	**57.74^{bc}**
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.001	0.001	**0.001**
SEM		0.194	0.179	0.195	0.192	**0.101**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

4.3 INTERNAL EGG QUALITY PARAMETERS

4.3.1Haugh Unit

Haugh unit (HU) was significantly (P<0.05) influenced during first (22-25wks), second (26-29 wks), third (30-33 wks) and fourth (34-37 wks) and also overall study period (22-37 wks) by supplementation with probiotics and BMD at graded levels. The higher haugh unit value in probiotic-1@ 200g/ton of feed (87.68) indicates that the quality of albumen was better and lower haugh unit value in probiotic-2@ 200g/ton of feed (84.95) as shown in the (Table 6).

4.3.2 Albumen index

The data on albumen index as influenced by different dietary treatments is presented in Table 7. Albumen index was significantly (P<0.05) influenced by supplementation of probiotic during fourth period (34-37 wks) of age. The higher albumen index value was noticed in BMD @ 50g/ton of feed (0.087) followed by probiotic-2&3 @ 100&200 g/ton of feed (0.084) and control (0.084) respectively. Whereas, the supplementation of probiotic at different graded levels was comparable with control during period-P1 (22-25wks), period-P2 (26-29wks), period-P3 (30-33wks) and over all period (22-37 wks).

4.3.3 Yolk Index

A significant (P<0.05) difference in yolk index among different levels of probiotic fed groups were observed in period-P1 (22-25wks), period-P2 (26-29wks), period-P4 (34-37wks) and over all period (22-37 wks) The overall period yolk index was better in the probiotic -2 @100g/ton of feed (0.467) and probiotic-3 @ 100 & 200g/ton of feed (0.473) followed by probiotic -1@ 200g/ton of feed (0.467). Where as was non-significant difference among the treatments groups were noticed in the yolk index in while higher during the third period 30-33 weeks (Table 8).

Table 6. Effect of dietary supplementation of probiotic at graded levels on Haugh unit in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25 (P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	87.61^{a}	78.50^{d}	88.78^{a}	86.95^{cd}	**85.46**^{cd}
Probiotic -1	100	84.89^{b}	84.92^{a}	89.72^{a}	87.67^{bcd}	**86.80**^{b}
Probiotic -1	200	87.56^{a}	84.65^{a}	90.28^{a}	88.26^{abc}	**87.68**^{a}
Probiotic -2	100	87.53^{a}	81.72^{c}	88.67^{a}	84.22^{e}	**85.53**^{cd}
Probiotic -2	200	83.97^{b}	79.28^{d}	87.05^{b}	89.51^{a}	**84.95**^{d}
Probiotic -3	100	87.77^{a}	80.98^{c}	86.84^{b}	84.43^{c}	**85.01**^{d}
Probiotic -3	200	86.41^{a}	85.20^{a}	86.56^{b}	86.34^{d}	**86.12**^{bc}
BMD	50	86.29^{a}	83.17^{b}	86.97^{b}	88.81^{ab}	**86.31**^{b}
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.001	0.001	**0.001**
SEM		0.248	0.374	0.261	0.312	**0.149**

Means with different superscripts in a column differ significantly (P<0.05),

BMD= Bacitracin methylene disalicylate

Table 7. Effect of dietary supplementation of probiotic at graded levels on Albumen index in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25(P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	0.085	0.083	0.083	0.084^{b}	**0.082**
Probiotic -1	100	0.085	0.083	0.083	0.083^{b}	**0.080**
Probiotic -1	200	0.086	0.084	0.083	0.083^{b}	**0.080**
Probiotic -2	100	0.086	0.085	0.085	0.084^{b}	**0.082**
Probiotic -2	200	0.084	0.084	0.084	0.083^{b}	**0.080**
Probiotic -3	100	0.083	0.084	0.085	0.083^{b}	**0.082**
Probiotic -3	200	0.084	0.084	0.084	0.084^{b}	**0.082**
BMD	50	0.085	0.085	0.083	0.087^{a}	**0.085**
N		6	6	6	6	**6**
P-Value		0.078	0.078	0.122	0.016	**0.251**
SEM		0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05),

BMD= Bacitracin methylene disalicylate

Table 8. Effect of dietary supplementation of probiotics at graded levels on Yolk index in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)	Mean

		22-25(P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	0.453[d]	0.455[c]	0.472	0.458[a]	**0.462[a]**
Probiotic -1	100	0.463[cd]	0.468[bc]	0.470	0.460[a]	**0.465[ab]**
Probiotic -1	200	0.487[ab]	0.467[bc]	0.465	0.452[ab]	**0.467[ab]**
Probiotic -2	100	0.490[ab]	0.492[a]	0.463	0.453[ab]	**0.473[a]**
Probiotic -2	200	0.472[bcd]	0.475[abc]	0.458	0.447[ab]	**0.463[ab]**
Probiotic -3	100	0.475[abc]	0.485[ab]	0.462	0.462[a]	**0.473[a]**
Probiotic -3	200	0.487[ab]	0.492[a]	0.462	0.440[b]	**0.473[a]**
BMD	50	0.493[a]	0.467[bc]	0.463	0.447[ab]	**0.465[ab]**
N		6	6	6	6	**6**
P-Value		0.001	0.002	0.669	0.036	**0.043**
SEM		0.008	0.003	0.002	0.002	**0.001**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

4.4 EXTERNAL EEGG QUALITY PARAMETERS

4.4.1 Egg Shell weight

The present experiment revealed that no statistically significant difference was observed statically between control and various experimental groups on egg shell weight. However, the values ranged between 5.581 to 5.712g (Table 9).

4.4.2 Shell thickness

The shell thickens was significant (P<0.05) difference in first and second period between 22-25 and 26-29 weeks of age where as third and fourth period between 30-33 and 34-37weeks of age there was no significant difference. However, during the overall period a significant (P<0.05) difference among the treatments were reported (Table 10).

Table 9. Effect of dietary supplementation of probiotic at graded levels on Egg shell weight (g) in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)	Mean

		22-25 (P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	5.438	5.473	5.778	6.161	**5.712**
Probiotic -1	100	5.333	5.622	5.841	5.969	**5.692**
Probiotic -1	200	5.210	5.433	5.624	6.091	**5.589**
Probiotic -2	100	5.257	5.472	5.743	6.053	**5.631**
Probiotic -2	200	5.163	5.411	5.807	6.122	**5.626**
Probiotic -3	100	5.121	5.534	5.635	6.032	**5.581**
Probiotic -3	200	5.415	5.336	5.682	6.172	**5.652**
BMD	50	5.114	5.608	5.834	6.116	**5.668**
N		6	6	6	6	**6**
P-Value		0.227	0.496	0.887	0.846	**0.804**
SEM		0.039	0.036	0.045	0.034	**0.022**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

Table 10. Effect of dietary supplementation of probiotic at graded levels on Egg shell thickness (mm) in White Leghorn layers during 22-37 weeks of age

Treatment	**g/ton**	**Age (weeks)**				**Mean**
		22-25 (P1)	**26-29(P2)**	**30-33(P3)**	**34-37(P4)**	
Control	0	0.406^{bc}	0.397^{b}	0.410	0.407	**0.404^{bc}**
Probiotic -1	100	0.396^{e}	0.396^{b}	0.397	0.400	**0.397^{d}**
Probiotic -1	200	0.401^{cde}	0.396^{b}	0.402	0.406	**0.401^{bc}**
Probiotic -2	100	0.412^{ab}	0.387^{c}	0.407	0.406	**0.404^{bc}**
Probiotic -2	200	0.394^{e}	0.386^{c}	0.405	0.410	**0.399^{cd}**
Probiotic -3	100	0.403^{cd}	0.407^{a}	0.404	0.404	**0.405^{a}**
Probiotic -3	200	0.415^{a}	0.398^{b}	0.405	0.407	**0.406^{a}**
BMD	50	0.402^{cde}	0.399^{b}	0.404	0.411	**0.404^{bc}**
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.061	0.425	**0.001**
SEM		0.001	0.001	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

4.4.3. Shell percentage

The data on shell percentage was evaluated in layer chicken as influenced by different dietary treatments with probiotic fed diets are presented in Table 11.During the first period between 22-25 wks of age the a significant (P<0.05) difference was reported for the shell percentage. However during the remaining periods no significant difference has reported among the treatments. During over all period (22-37wks) the supplementation of probiotics significantly (P<0.05) improved the shell percentage. The highest shell percentage was in the control (10.02) when compared with treatment groups.

4.4.4. Shell strength

The shell strength was significant (P<0.05) in supplementation with probiotics and BMD at different graded levels during the period wise and also in overall period. The shell strength has significantly (P<0.05) higher value in control group (21.15 N) compared with treatment groups (Table 12).

Table 11. Effect of dietary supplementation of probiotic at graded levels on egg shell percentage in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25 (P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	9.869^{a}	9.838	10.09	10.27	**10.02^{a}**
Probiotic -1	100	9.362^{bcd}	9.568	9.777	9.933	**9.658^{b}**
Probiotic -1	200	9.248^{cd}	9.542	9.681	10.02	**9.623^{b}**
Probiotic -2	100	9.712^{abc}	9.473	9.728	10.20	**9.775^{ab}**
Probiotic -2	200	9.343^{bcd}	9.723	9.686	9.983	**9.680^{b}**
Probiotic -3	100	9.187^{d}	9.593	9.763	10.03	**9.638^{b}**
Probiotic -3	200	9.805^{ab}	9.332	9.736	10.05	**9.732^{b}**
BMD	50	9.536^{abcd}	9.738	10.00	9.983	**9.818^{ab}**
N		6	6	6	6	**6**
P-Value		0.010	0.299	0.550	0.526	**0.019**
SEM		0.060	0.081	0.059	0.042	**0.035**

Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

Table 12. Effect of dietary supplementation of probiotic at graded levels on Egg shell strength (N*) in White Leghorn layers during 22-37 weeks of age

Treatment	g/ton	Age (weeks)				Mean
		22-25 (P1)	26-29(P2)	30-33(P3)	34-37(P4)	
Control	0	19.24^{c}	20.10^{abc}	19.55^{b}	25.71^{a}	**21.15^{a}**
Probiotic -1	100	20.22^{bc}	18.53^{de}	17.73^{c}	18.34^{d}	**18.71^{e}**
Probiotic -1	200	20.02^{bc}	20.50^{ab}	17.69^{c}	21.01^{c}	**19.81^{cd}**
Probiotic -2	100	19.50^{c}	19.05^{cde}	21.35^{a}	23.75^{b}	**20.91^{a}**
Probiotic -2	200	21.02^{b}	17.95^{e}	18.92^{b}	19.33^{d}	**19.30^{de}**
Probiotic -3	100	19.27^{c}	19.39^{bcd}	18.76^{bc}	22.84^{b}	**20.06^{bc}**
Probiotic -3	200	24.54^{a}	19.67^{abcd}	18.72^{bc}	19.57^{d}	**20.63^{ab}**
BMD	50	19.81^{bc}	20.83^{a}	21.68^{a}	21.33^{c}	**20.92^{a}**
N		6	6	6	6	**6**
P-Value		0.001	0.001	0.001	0.001	**0.001**
SEM		0.272	0.188	0.234	0.368	**0.140**

.Means with different superscripts in a column differ significantly (P<0.05)

BMD= Bacitracin methylene disalicylate

*Newton's

4.4.5 Density

The density (g/cm^3) of egg was non-significant in all the treatment groups during the period wise and also over all period (Table 13).

4.5 LIVEABILITY

Between the 22-37 weeks of age the mortality was within the limitation. During first (22-25wks) second (26-29wks) and fourth (34-37wks) period there was no mortality of birds but whereas in third period (30-33 wks) there was only one bird mortality was recorded in probiotic-1 (100g/ton) of feed (Table 14).

4.6 RELATIVE ECONOMICS IN WHITE LEGHORN LAYERS DURING 22-37 WEEKS OF AGE

The supplementation of probiotic at different graded levels influenced the Net profit per dozen eggs and it is highest in probiotic -2 @100 g/ton of feed (Rs.9.43) compared with control (Rs.8.67) and also with probiotic- 1 & 3 (100 & 200 g/ton) and BMD (50g/ton) of feed during the 22-37 weeks of age (Table 15).

Table 13. Effect of dietary supplementation of probiotic at graded levels on density (g/cm3) in White Leghorn layers during 22-37 weeks of age

Treatment	**g/ton**	**Age (weeks)**				**Mean**
		22-25 (P1)	**26-29(P2)**	**30-33(P3)**	**34-37(P4)**	
Control	0	1.094	1.087	1.089	1.090	**1.090**
Probiotic -1	100	1.086	1.088	1.086	1.089	**1.087**
Probiotic -1	200	1.087	1.087	1.086	1.089	**1.087**
Probiotic -2	100	1.086	1.086	1.086	1.088	**1.086**
Probiotic -2	200	1.083	1.083	1.086	1.089	**1.085**
Probiotic -3	100	1.083	1.083	1.087	1.089	**1.085**
Probiotic -3	200	1.086	1.083	1.086	1.089	**1.086**
BMD	50	1.084	1.085	1.089	1.088	**1.087**
N		6	6	6	6	**6**
P-Value		0.074	0.060	0.729	0.938	**0.180**
SEM		0.001	0.005	0.001	0.001	**0.001**

Means with different superscripts in a column differ significantly ($P<0.05$),

BMD= Bacitracin methylene disalicylate

Table 14. Effect of dietary supplementation of probiotic at graded levels on Liveability (%) in White Leghorn layers during 22-37 weeks of age

Treatment	**g/ton**	**Age (weeks)**				**Mean**
		22-25 (P1)	**26-29(P2)**	**30-33(P3)**	**34-37(P4)**	
Control	0	100	100	100	100	**100**
Probiotic -1	100	100	100	97	100	**99.25**
Probiotic -1	200	100	100	100	100	**100**
Probiotic -2	100	100	100	100	100	**100**
Probiotic -2	200	100	100	100	100	**100**
Probiotic -3	100	100	100	100	100	**100**
Probiotic -3	200	100	100	100	100	**100**
BMD	50	100	100	100	100	**100**

Table 15.Effect of dietary supplementation of probiotic at graded levels on Relative Economics in White Leghorn layers during 22-37 weeks of age

	Criterion	control	Probiotic-1 (100g/ton)	Probiotic-1 (200g/ton)	Probiotic-2 (100g/ton)	Probiotic-2 (200g/ton)	Probiotic-3 (100g/ton)	Probiotic-3 (200g/ton)	BMD 50g/ton
1	**Cost of feed per kg (Rs.)**	23.25	23.30	23.30	23.30	23.30	23.30	23.30	23.30
2	**Feed consumption/dozen eggs (kg)**	1.563	1.569	1.569	1.527	1.633	1.568	1.600	1.566
3	**Feed cost/dozen eggs (Rs.)**	36.33	36.55	36.55	35.57	38.97	36.53	37.28	36.48
4	**Selling price of dozen eggs(Rs.)**	45	45	45	45	45	45	45	45
5	**Net profit per dozen eggs(Rs.)**	8.67	8.45	8.45	9.43	6.03	8.47	7.72	8.52
6	**Net profit over control (Rs.)**		-0.22	-0.22	0.76	-2.64	-0.2	-0.95	-0.15

Selling price of egg Rs: 3.75, Avg. Probiotic cost Rs: 500/kg

CHAPTER V

DISCUSSION

The results obtained with regard to experimental diets, performance of layers during peak production fed on diets with different levels of probiotic in terms of percent hen day egg production, feed intake, feed efficiency per dozen eggs, egg quality parameters, relative economics and mortality are discussed in conjunction with the available literature.

5.1 PRODUCTION PERFORMANCE OF COMMERCIAL LAYERS

5.1.1 Hen-day egg production

In this experiment the dietary supplementation of graded levels of probiotics in White Leghorn (BV 300) layers had shown significant effect (P<0.05) on percent hen day egg production. The percent hen day egg production was significantly (P<0.05) higher in probiotic-1 (100g/ton) when compared with control and this trend has continued from 22-37 weeks. The above findings are in agreement with the findings of (Tortuero and Fernandez, 1995 ; Nahashon *et al*., 1996; Zhang *et al*., 2012; Yalcin *et al*., 2010 ; Panda *et al*., 2006; Abdelqader *et al*., 2013) who reported that percent hen day egg production was increased and improved in commercial layer birds and also with the findings of Berrin (2011) & Wei Fen Li *et al*. (2011) observed in Japanese quail and Shaoxing Ducks.

Contrary to the findings of the present study, Davis and Anderson 2002, Mahdavi *et al*., 2005,Yousefi and Karkoodi 2007, Balevi *et al*., 2009, KalavathyRamasamy *et al*., 2009, Mikulski *et al*., 2012, Shalaei *et al*., 2014, Sobczak *et al*., (2015), Forte *et al*., 2016, Upadhaya *et al*., 2016, Sheoran *et al*., 2018, Fathi *et al*., 2018 did not observe any significant variation in percent hen day egg production in commercial layer birds .

Different opinions were expressed by Sattar Bageri Dizaji and Rasoul Pirmohammadi 2009, Moorthy *et al*., 2010 who observed that significant ($P<0.05$) decrease in the per cent hen day egg production by supplementation with probiotic in White Leghorn layers.

5.1.2 Feed intake

In the experiment it is observed that the commercial layer birds fed with probiotic supplemented diet throughout the experimental period had consumed significantly ($P<0.05$) more in control compared with supplemented probiotic groups. The results were in accordance with the earlier reports of Nahashon *et al*. (1996), Falaki *et al*. (2010) and Sheoran *et al.* (2018) who found that significant ($P<0.05$) increase in feed consumption in White Leg horn layers.

Contrary to the above results Tortuero and Fernandez 1995, Panda *et al*., 2003, Yoruk *et al*., 2004, Mahdavi *et al*., 2005, Panda *et al*., 2006, Yousefi and Karkoodi 2007, Panda *et al*., 2008 , KalavathyRamasamy *et al*., 2009, Moorthy *et al*., 2010, Yalcin *et al*., 2010, Mikulski *et al*., 2012, Zhang *et al*., 2012, Abdelqader *et al*., 2013,Sobczak *et al*., 2015 and Fathi *et al*., 2018 reported that no significant difference of feed intake in commercial White Leghorn birds supplemented with probiotic and yeast culture.

Feed consumption in the treatment groups has increased when compared with control which might be due to the occurrence of naturally occurring beneficial bacteria in the intestinal tract of birds which may result in higher of feed consumption in probiotic supplemented group.

5.1.3 FEED CONVERSION RATIO PER DOZEN EGGS

A significant reduction ($P<0.05$) who observed in the feed conversion ratio per dozen eggs in probiotics -2 (100g/ton) supplemented group in White leghorn layers between 22-37 weeks of age.

Better feed efficiency observed in this study with supplementation of probiotics is in accordance with the earlier findings of Nahashon *et al*., 1996, Yoruk *et al*., 2004, Panda *et al*., 2006 & 2008, Balevi *et al*., 2009, Yalcin *et al*., 2010, ShivaniKatoch *et al*., 2011, Mikulski *et al*., 2012, Zhang *et al*., 2012 Abdelqader *et al*., 2013, Shalaei *et al*., 2014 and Sheoran *et al*., 2018 who observed that significant ($P<0.05$) improvement in feed conversion ratio in commercial layer birds fed with probiotic supplementation at different levels.

In contrast to the results of the present study Tortuero and Fernandez 1995, Panda *et al*., 2003, Yoruk *et al*., 2004, Mahdavi *et al*., 2005, Yousefi and Karkoodi 2007,Yalcin *et al*., 2008, Moorthy *et al*., 2010, , Sobczak *et al*., 2015, Forte *et al*., 2016 and Fathi *et al*., 2018) were reported no significant difference in feed conversion ratio per dozen eggs in White Leghorn layers supplemented with probiotics.

The effect of probiotic might be attributable to the probable production of natural antibiotic like acidophil in which it is active against pathogenic microbes like *E.Coli* and *Salmonella*. Further the probiotic not only check the growth of pathogenic microorganisms but also could improve the feed utilization with neutralization of toxins, apparently increased the absorption of nutrients and alteration of microbial metabolism.

5.2 EGG WEIGHT

The supplementation of probiotic 1,2,3 and BMD in White Leghorn layers showed significant ($P<0.05$) increase in egg weight. The results were in accordance with the findings of the Tortuero and Fernandez 1995 & Davis and Anderson 2002, Balevi *et al*., 2009, KalavathyRamasamy *et al*., 2009; Yalcin *et al*., 2010, Mikulski *et al*., 2012, Zhang *et al*., 2012, Abdelqader *et al*., 2013 and Shalaei *et al*., 2014.

Contrary to the above results (Nahashon *et al*., 1996, Mahdavi *et al*., 2005, Panda *et al*., 2006 &2008, Yousefi and Karkoodi 2007, Yalcin *et al*., 2008, SattarBageriDizaji and RasoulPirmohammadi 2009, Balevi *et al*., 2009, Sobczak *et al*., 2015, Forte *et al*., 2016 and Sheoran *et al*., 2018 who found that in the White Leghorn layer birds egg weight was non-significant in all treatment groups .

The improved egg weight observed in the present and earlier studies is probably due to the ability of probiotic cultures to perform well under stressful conditions, as egg weight increments in egg-type hens have been reported to be largely affected by environmental factors, although egg weight is a highly heritable trait in chickens. Furthermore, supplementation of probiotic in animal feed may improve the digestion and absorption of nutrients in the host.

The improvement in egg size was associated with the higher calcium and nitrogen retentions in the probiotic fed hens. Similar result was reported by Davis and Anderson 2002, in which hens supplemented with PrimaLac exhibited a higher percentage of extra-large eggs than control.

5.3 INTERNAL EGG QUALITY

5.3.1 Haugh Unit

Albumen quality evaluated as haugh unit score was significantly (P<0.05) influenced by supplemented probiotic and BMD during 22-37 weeks of age. The results were in accordance with the findings of Tortuero and Fernandez 1995, Zhang *et al*., 2012 and Sobczak *et al*., 2015.

Contrary to the observations several others, Nahashon *et al*.,1996, Mahdavi *et al*., 2005, Panda *et al*., 2008, Yalcin *et al*., 2008, Berrin 2011, Mikulski *et al*.,2012 and Fathi *et al*.,2018 reported no significant difference in Haugh unit score among the treatment groups.

5.3.2 Albumin index

The effect of dietary supplementation of probiotic at graded levels on albumin index in White leghorn layers during 22-33 weeks of age did not reveal any significant difference. However, there was a significant difference of albumen index during fourth period without following any specific trend. This might be due to biological variation. These findings were in accordance with Yoruk *et al*., 2004, Valavan *et al*., 2006, Yalcin *et al*., 2008 and Berrin 2011.

5.3.3 Yolk Index

The effect of yolk index was significantly (P<0.05) influenced during the first (22-25wks) second (25-29wks) and fourth period (33-37wks) and overall period (22-37 wks) However, during the third period (30-33wks) non-significant difference was recorded.

Different opinions were expressed by various workers Yoruk *et al*., 2004 Valavan *et al*., 2006, Yalcin *et al*., 2008, Berrin 2011 and Mikulski *et al*., 2012 who found that no significant difference in yolk index .

5.4 EXTERNAL EGG QUALITY

5.4.1 Shell weight

The effect of dietary supplementation of probiotic at graded levels on egg shell weight in White leghorn layers during 22-37 weeks of age revealed a non-significant difference. Similar findings were reported by Forte *et al*., 2016.

Contrary to the above results, Yousefi and Karkoodi 2007, Panda *et al*., 2008 and Fathi*et al*.,2018 reported significant ($P<0.05$) difference in shell weight among the treatment groups.

5.4.2 Shell thickness

A significant effect on egg shell thickness has observed during first ,second, and overall period of the study. However, the shell thickness was not affected during third and fourth periods. These results are in agreement with Panda *et al*., 2006, Yousefi and Karkoodi 2007, Panda *et al*., 2008, Mikulski *et al*., 2012, Zhang *et al*., 2012 Abdelqader *et al*.,2013, Shalaei *et al*., 2014, Sobczak *et al*.,2015 and Fathi *et al*.,2018.

Contrary to the findings of the present study Mahdavi *et al*., 2005 and Yalcin *et al*., 2008 showed that shell thickness was not significantly different among the treatment groups in layers birds.

This beneficial effect may be attributed to a favourable environment in the gastrointestinal tract resulting from the administration of probiotic to birds (Mohan *et al*., 1995; Panda *et al*., 2008; Mikulski *et al*., 2012). Probiotic bacteria increase the rate of fermentation and the production of short-chain fatty acids (SCFAs), which reduces the luminal pH (Scholz-Ahrens *et al*., 2007). Low luminal pH increases calcium solubility and absorption (Van den Heuvel *et al*., 1999). SCFAs stimulate intestinal epithelial cell proliferation and villus height (Garcia *et al*., 2007), which increases absorption efficiency (Scholz-Ahrens *et al*., 2007). As a result, more nutrients, including calcium, can be assimilated, thus improving eggshell quality

5.4.3. Shell percentage

The mean percentage of shell was significantly (P<0.05) influenced by three types of probiotic and BMD supplementation. However, differences in percentage of shell was recorded during the first period only. **The findings of the present study are in agreement with those of** Mikulski *et al*., 2012 and Sobczak *et al*., 2015.

Various workers Yousefi and Karkoodi (2007), Forte *et al*. (2016) Shalaei *et al*. (2014) reported contraory results to the present findings.

5.4.4. Shell strength

The effect of dietary supplementation of probiotic at graded levels on egg shell strength in White leghorn layers du ring 22-37 weeks of age did not follow a specific trend.

The above results are in agreement with the findings of Panda *et al*. 2008, Sobczak *et al*., 2015, Upadhaya *et al*., 2016 and Fathi *et al*., 2018 who reported a significant (P<0.05) improvement in shell strength in White Leghorn layers.

Shell breaking strength was significantly higher in the probiotic-fed groups. This could be attributed to the higher shell thickness, which might have created greater resistance resulting in higher breaking strength

Concomitant to the findings of the present study, Mahdavi *et al*., 2005 and Shalaei *et al*., 2014 **did not observe any difference in the** Shell strength.

5.4.5 Density

The density (g/cm^3) of egg was non-significant in all the treatment groups during the period wise and also in overall period.

5.5 LIVEABILITY

There was no mortality in layer birds. **Compared to the findings of the present study,** Panda *et al.,* 2006 and Balevi *et al.,* 2009 observed mortality was not effected in White Leghorn chicks supplemented with probiotic. However, Yoruk *et al*., 2004 and Vicente *et al*., 2007 reported a significant (P<0.01) reduction in mortality.

5.6 RELATIVE ECONOMICS

The supplementation of probiotic at different graded levels influenced the Net profit per dozen eggs which is highest in probiotic -2 @100 g/ton (Rs. 9.43) compared with control (Rs 8.67) and also with probiotic-1 & 3 (100 &200 g/ton)and BMD (50g/ton), during the 22-37 weeks of age

CHAPTER - VI

SUMMARY

In order to find out the feasibility of three types probiotic supplementation at graded levels of 0,100, 200 g/ton and Bacitracin methylene disalicylate (BMD) 50g/ton feeding probiotic supplementation to commercial layer chicken, the experiment was conducted during August to December 2018. A total of 288 commercial White Leghorn pullets (BV300) of 16 weeks age were procured then, leg banded and weighed individually. Prior to experiment, the pre layer ration was fed to the birds till 21 weeks and layer ration from 22 weeks onwards. Then, experimental layer diets were formulated (*iso nitrogenous* and *iso caloric)*. The Basal Diet (BD) (T1, Control) diet consisted of corn and soybean meal. The remaining experimental diets consisted of Basal Diet + 4×10^{9} cfu/g of probiotic-1 contain (*Bacillus subtilis, Bacillus coagulants* and *Sacchromyces boulardii*),(T2) ; Basal Diet + 8×10^{9}cfu/g of probiotic- 1 (T3); Basal Diet + 4×10^{9}cfu/g of probiotic-2 contain (*Bacillus subtilis, Sacchromyces boulardii* and *Clostridium butryicum*) (T4) ; Basal Diet + 8×10^{9}cfu/g of probiotic- 2 (T5); Basal Diet + 4×10^{9}cfu/g of probiotic-3 contain (*Bacillus sps. Lactobacillus sps. Enterococcus sps. pediococcus acidilactici, sacchromyces boulardii* and *Clostridium butryicum*) (T6); Basal Diet + 8×10^{9}cfu/g of probiotic 3 (T7); Basal Diet +50gAGP (BMD) (T8) in diet. The layers were allocated to each of the eight treatments in a replicated manner, six replicates/ treatment from 16 week of age (six birds/replicate) and evaluated for production performance from 22 to 37 week of age. The percent hen day egg production, feed intake, feed conversion ratio per dozen eggs, egg weight, egg quality parameters, livability and relative economics were studied for a total of four laying periods of 28 days each.

The per cent hen day egg production for four laying periods from 22 to 37 weeks of age was significantly (P<0.05) influenced by with or without supplementation of probiotic and BMD. The percent hen day egg production was significantly (P<0.05) reduced in control (86.76%) and higher in probiotic-2 @100g/ton (91.77%) followed by probiotic-3 @200g/ton (89.66%) of feed.

The mean feed intake was significantly (P<0.05) influenced by with or without supplementation of probiotic and BMD. The control group recorded significantly (P<0.05) lower feed intake (112.16g) and highest in probiotic-3 @ 200g/ton of feed (118.79g) and while the values in other treatment groups differed significantly from each other.

The feed conversion ratio per dozen eggs for four laying periods in White Leghorn layer birds significantly (P<0.05) influenced by with or without supplementation of probiotics and BMD to the basal diet. The better feed conversion ratio per dozen eggs was noticed in probiotic-2 @ 100g/ton (1.527) and poor in probiotic-2 & 3 @200 g/ton of feed (1.633 & 1.600).

The overall egg weight was significantly (P<0.05) higher in probiotic-1 @100g/ ton of feed (58.84g) and followed by probiotic-3 @ 200g/ton (58.18g) and probiotic-2 @200g/ton of feed (58.12g).

The internal egg quality parameters of Haugh unit and yolk index for overall period was significantly (P<0.05) influenced by with or without supplementation of probiotic and BMD. With respect to albumen index no significant (P>0.05) difference was observed among different treatment groups during 22-37 weeks of age.

In the external egg quality parameters of shell weight and density a non significant (P>0.05) difference was observed statically between control and various experimental groups. However, the egg shell thickness, shell percentage and shell strength were significantly ($P<0.05$) influenced by supplementation of probiotic and BMD. The mortality was within the limits during 22-37 weeks of age.

The supplementation of three types of probiotic at different graded levels influenced the Net profit per dozen eggs which is highest in probiotic -2 @100 g/ton of feed (Rs. 9.43) compared with control (Rs 8.67) during the 22-37 weeks of age.

CONCLUSION

Based on the findings of this study the following conclusions were drawn:

- The supplementation of probotic-2 @100g/ton of feed during 22-37 weeks of age to the commercial White Leghorn layers has influenced significantly ($P<0.05$) and more effective among all the dietary treatments in terms of major parameters such as percent hen day egg production and feed conversion ratio per dozen eggs.
- The supplementation of probiotics at graded levels influenced Net profit per dozen eggs and is highest in probotic-2 @100 g/ton of feed (Rs. 9.43) compared with control (Rs.8.67) during the 22-37 weeks of age.
- Mortality rate was within the limits & one and only mortality recorded in probotic-1 @ 100g/ton of feed and no specific disease was recorded in all the treatments.
- It can be concluded that, supplementation of multi strain probotic-2 @ 100g/ton of feed as an alternative to antibiotic (BMD) and has better performance compared to the single strain probiotic-1 @ 100g/ton of feed and control. Probotic-2 @ 100g/ton of feed has exhibited better production performance of commercial White Leghorn layer birds during the 22-37 weeks of age.

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Figure: 1 Effect of dietary supplementation of probiotic at graded levels on percent hen day egg production in White Leghorn layers during 22-37 weeks of age

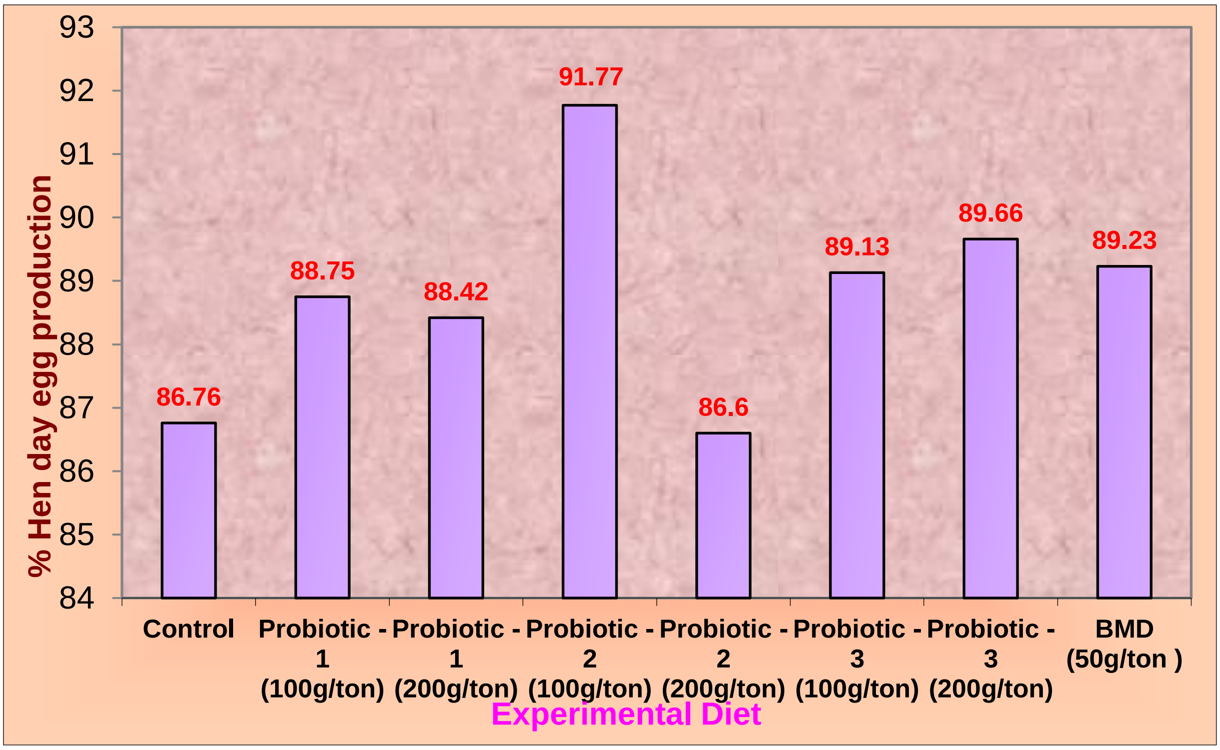

Figure: 4 Effect of dietary supplementation of probiotic at graded levels on Egg weight (g) in White Leghorn layers During 22-37 weeks of age

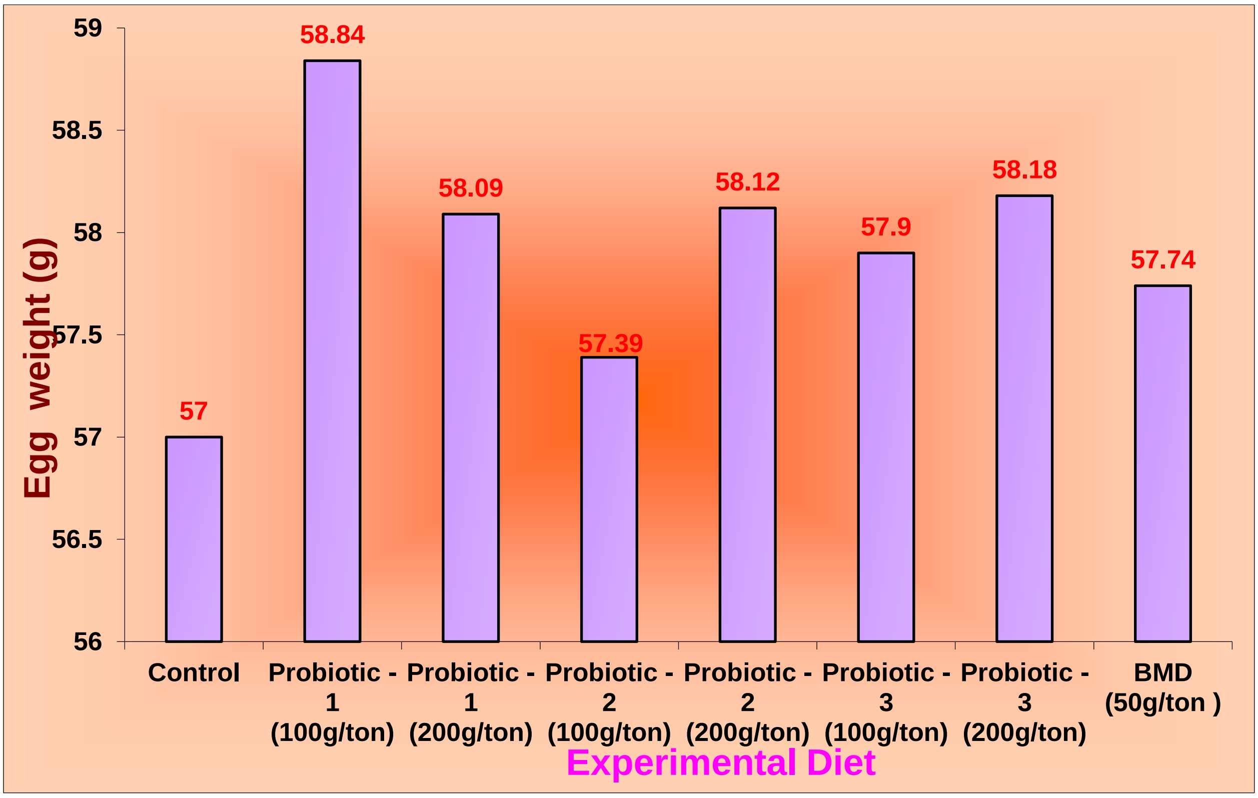

Figure: 2 Effect of dietary supplementation of probiotic at graded levels on Feed intake (g/hen/day) in White Leghorn layers during 22-37 weeks of age

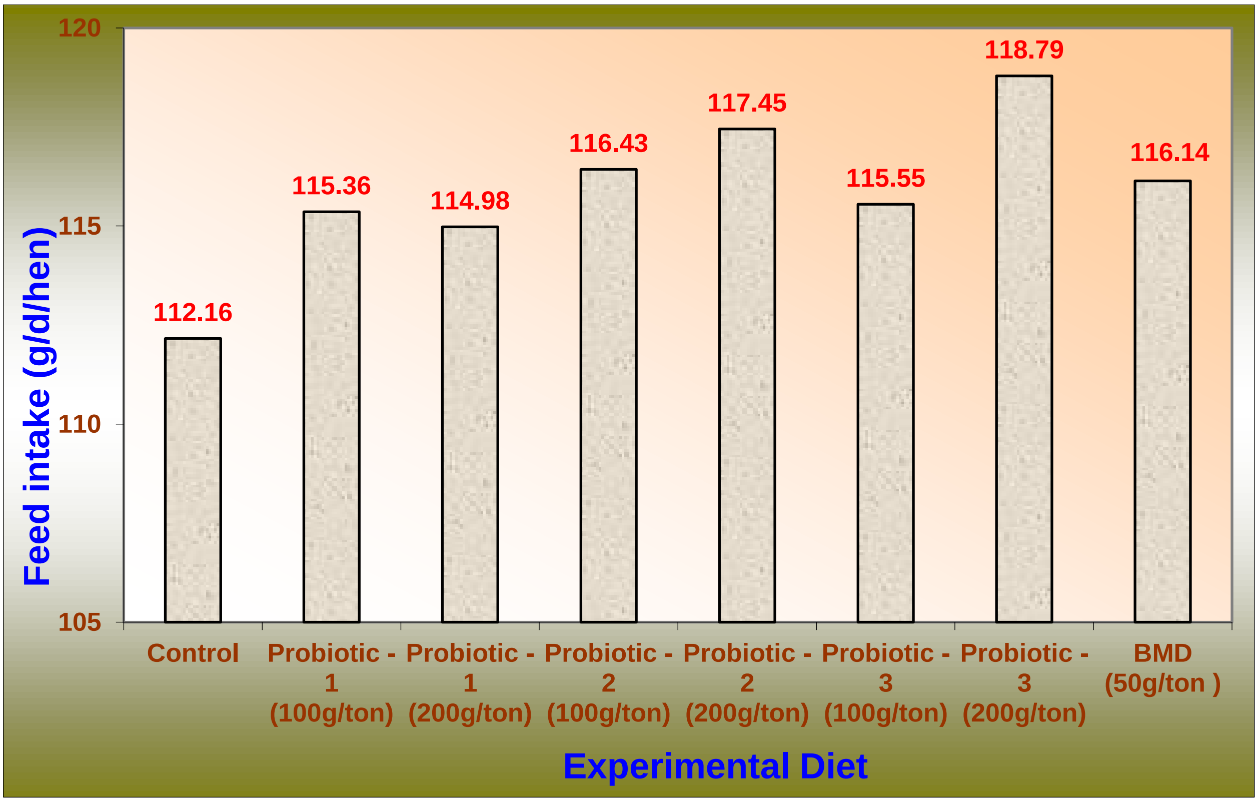

Figure: 3 Effect of dietary supplementation of probiotic at graded levels on Feed conversion ratio (kg feed consumed for dozen eggs) in White Leghorn layers during 22-37 weeks of age

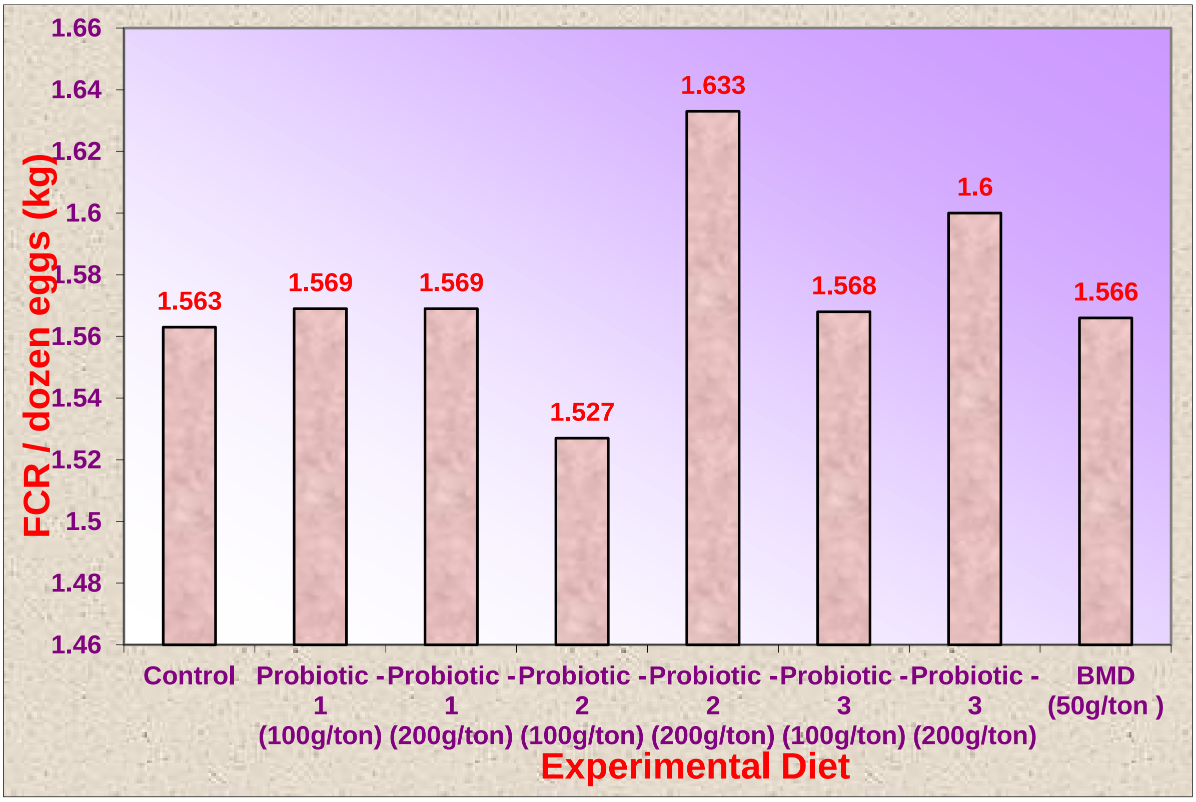

Figure: 5 Effect of dietary supplementation of probiotic at graded levels on Relative Economics in White Leghorn layers during 22-37 weeks of age