EFFECT OF DIETARY SUPPLEMENTATION OF CHILI (*Capsicum annuum*) LEAF POWDER ON PERFORMANCE OF RHODE ISLAND RED LAYING HENS

Thesis

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By

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Pantnagar *(Abhilasha)*
July, 2022 *Authoress*

CERTIFICATE-I

This is to certify that the thesis entitled **"EFFECT OF DIETARY SUPPLEMENTATION OF CHILI (*Capsicum annuum*) LEAF POWDER ON PERFORMANCE OF RHODE ISLAND RED LAYING HENS"** submitted in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** with major in **Animal Nutrition** of the College of Postgraduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of bona fide research carried out by **Ms. Abhilasha, Id. No. 48241**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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

Pantnagar
July, 2021

(B.C. Mondal)
Chairman
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CERTIFICATE-II

We, the undersigned, members of the Advisory Committee of **Ms. Abhilasha, Id. No. 48241**, a candidate for the degree of **Master of Veterinary Science** with major in **Animal Nutrition** agree that the thesis entitled **"EFFECT OF DIETARY SUPPLEMENTATION OF CHILI (*Capsicum annuum*) LEAF POWDER ON PERFORMANCE OF RHODE ISLAND RED LAYING HENS"** may be submitted in partial fulfillment of the requirements for the degree.

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Member

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LIST OF ABBREVIATION

%	:	Percentage, Per Cent
/	:	Per
@	:	At the rate of
±	:	Plus minus
≤	:	Greater than or equal to
≥	:	Smaller than or equal to
µl	:	Microlitre
ADF	:	Acid detergent fibre
ANOVA	:	Analysis of variance
BP	:	Boiling point
Ca	:	Calcium
CF	:	Crude fibre
Cm	:	Centimeter
CP	:	Crude protein
Dl	:	Decilitres
DM	:	Dry matter
EDTA	:	Ethylene diaminetetraacetic acid
EE	:	Ether extract
et al.	:	Ethically all
Fl	:	Femtolitre
FCR	:	Feed conversion ratio
Fig.	:	Figure
FTU	:	Phytase units

g or gm	:	Gram
Ha	:	Hectare
Hb	:	Haemoglobin
HDL	:	High density lipoprotein
IU	:	International units
Kcal	:	Kilocalories
kg	:	Kilogram
L	:	Litres
LDL	:	Low density lipoprotein
Mcal	:	Megacalories
Mg	:	Miligram
Min	:	Minutes
Ml	:	Mililiter
Mm	:	Milimeter
N	:	Nitrogen
NDF	:	Neutral detergent fibre
NFE	:	Nitrogen-free extract
Nm	:	Nanometer
NSP	:	Non starch polysaccharides
OM	:	Organic Matter
P	:	Phosphorus
PCV	:	Packed cell volume
Pg	:	Picogram
pH	:	Potential of hydrogen
ppm	:	Parts per million
RBCs	:	Red blood cells

Rpm	:	Revolutions per minute
SE	:	Standard error
SEm	:	Standard error of the mean
TEC	:	Total erythrocyte counts
TLC	:	Total leucocytes counts
v/v	:	Volume by volume
Viz.	:	Videlicet
WBC	:	White blood cells
Wk	:	Week
α	:	Alpha
β	:	Beta
Δ	:	Delta
ω	:	Omega
Rs.	:	Rupees

Introduction

Chapter1 INTRODUCTION

Ancestors of the modern chicken that were domesticated probably 7 to 8 thousand years ago in southeastern Asia belonged to members of the Gallus genus. Subsequently, they spread globally for meat and egg production **(Zaheer, 2015)**. Poultry production in India is acknowledged as a well-organized and scientifically based industry and it is one of the country's fastest-growing sectors, with an average annual growth rate of 6-8% in egg production and 10-12% in broiler chicken production. Poultry sector has developed high yielding layer (310-340 eggs/ annum) and broiler (2.4-2.6 kg at 6 weeks) varieties together with standardized package of practices on nutrition, housing, management and disease control in past 40 years **(Chatterjee and Rajkumar, 2015)**. The annual per capita availability also increased to 60 eggs and 2.5 kg of meat per year. However, it falls below Indian Council for Medical Research's recommended annual intake of 10.8 kg of poultry meat and 180 eggs per person per year. Poultry farming is one of the most important livestock sectors since it delivers the cheapest animal protein (eggs and meat) for human use in the shortest amount of time. India's poultry sector employs about 3% of the worldwide poultry population and ranks third in egg production and sixth in meat output globally **(DAHD, 2019)**.

According to India’s Ministry of Commerce and Industry/Agricultural and Processed Food Products Export Development Authority (APEDA), agricultural crop output has been increasing at a pace of 1.5 to 2% per year while production of egg and broiler chicken meat has been increasing at an annual rate of 8 to 10%. The sector's potential is attributed to a number of variables, including rising per capita income, an expanding urban population and dropping real poultry prices. Poultry meat is the fastest-growing segment of global meat demand and the poultry business in India is booming.

According to 20th Livestock census reported by the Department of Animal Husbandry & Dairying, the total poultry population has increased by 16.8%, backyard poultry has increased by 45.78% and commercial poultry has increased by 4.5% over the previous Census Furthermore, poultry production has expanded in recent years. According to Food and Agriculture Organization (FAO), roughly 130 million tons of chicken meat will be produced in 2020 to meet the need of a growing global population. Nowadays, the chicken industry is regarded as a rapidly expanding segment of the agricultural economy that produces protein-rich food at a low cost.

The cost of feed is commonly acknowledged to be the major cost of egg production. The rising feed prices have their direct impact on the composition of layer feed and are resulting in the increase in the use of alternative feed stuffs in laying hen nutrition. In order to lower feed costs, scientific understanding about using low-cost locally available agro-industrial by-products in chicken feed must be updated. As feed accounts for 60-70 percent of total production costs, remaining percent of total production cost depends upon management such as biosafety (medication and vaccination), housing etc. Maize is the popular cereal used in combination with protein meal like soybean meal which generally determines the cost of compounded feed. Fish meal, meat and bone meal, soybean meal, groundnut cake, and other traditional sources of vitamins and proteins used in poultry meals are getting more expensive in developing nations. Due to the rising cost of raw resources and the fierce competition with humans for the same foods, there is not an enough supply of these ingredients for feed. Therefore, in order to lower the cost of feed, the search for alternative feed sources has become necessary **(Swain *et al*., 2014)**. Any endeavor to minimize feed costs could result in a large reduction in total costs. In all poultry production systems, a wide variety of alternative feedstuffs are available for feeding. The mixtures of maize with other grains, particularly pearl millet, finger millet and sorghum could be tested to meet the energy demand. For development of poultry industries these combinations at 25-33 percent level should be encouraged **(Wiseman, 2006)**. For the optimum growth and profit margin in broiler chickens, soya bean meal was replaced with rapeseed meal and sunflower seed meal, as a protein source, each at a level of 10%. However, the use of unconventional feedstuffs for poultry production is limited due to their fibrousness and inability of birds to possess the cellulase enzyme that can digest the fibre **(Adebiyi *et al.,* 2010)**. Many natural additives have been studied and tested, such as skullcap extract **(An *et al.,* 2010)**, garlic and thyme **(Ghasemi *et al.,* 2010)**, mango skin and paprika extract **(Lokaewmanee *et al.,* 2011)**, broccoli stems and leaves **(Hu *et al.,* 2011)**, strawberry leaves **(Duru, 2013)**, black cumin **(Boka *et al.,* 2014)** and olive leaves **(Cayan and Erener, 2015)**

Chili leaves are one of the potent agro industrial wastes of chili farming after harvesting the chili which are not used for human consumption, do not adversely alter the laying hen performance and additionally the use of red chili as a potential natural colour pigment can improve egg-yolk colour **(Gurbuz *et al*., 2003)**. Therefore, chili leaves can be used as an alternate feed resource with lowered feed cost in chicken ration.

Chili belongs to the genus *Capsicum*, which is an associate of the Solanaceae family. Pepper (*Capsicum* spp.) is one of the oldest and most widely used crops. There are more than 200 species in the genus *Capsicum* and the fruits of each species vary in flavour and olfactory heat. Chili leaves are simple and are of different shapes and alternate, elliptical to lanceolate with smooth margins that are usually wrinkled. The fruits are many-seeded berries which may be long, cylindrical, ovoid, obtuse or oblong but with no sutures. *Capsicum annuum* fruits have a characteristic odour and pungent taste. The chemical composition of *Capsicum annuum* fruits include dry matter, total fat, protein, carbohydrates, dietary fibers, vitamin C, calories and energy **(Zaki *et al.*, 2013)**. The fruits of *Capsicum annuum* contains capsaicinoids, a kind of bioactive compound. Two main capsaicinoids, capsaicin and dihydrocapsaicin are accountable for 90% of the total sharp strong taste of pepper fruits.

The leaves contain alkaloids, tannins and flavonoids **(Marbut *et al.*, 2005)**, whereas the roots contain steroids, alkaloids, coumarins, glycosides and triterpenoids **(Al-Snafi, 2015)**. *Capsicum annuum* was used traditionally to cure toothache. Fruits are used to stimulate stomach's gastric activity and increase blood circulation. It is also a stimulant and a carminative and is utilized traditionally for neuralgia and rheumatism **(Sumner, 2000)**. Capsaicin and its derivatives were employed topically to cure chronic discomfort syndromes, musculoskeletal pain and diabetic complications **(Bianchi *et al.*, 2012)**. The butanol extract of *Capsicum annuum* fruit revealed a great antimicrobial activity compared to verified pathogens, though other extracts have displayed a relatively modest activity. *Capsicum frutescens* extracts are revealed to have antifungal properties. *Capsicum annuum* crude extracts are known to have antiviral effect against herpes simplex virus **(Hafiz *et al.*, 2017).** Inflammatory changes greatly enhance the analgesic effects of capsaicin. Capsaicin has a deep anticarcinomic effect on prostate cancer cells, causing the cell death of both androgen receptor-positive and negative prostate cancer cell lines lines **(Basith *et al.*, 2016).** Green chili is a potent memory booster and its primary mechanism of action appears to depend on (i) improving memory in exteroceptive models, (ii) retrieving information from memory gaps, (iii) improving radical scavenging and (iv) inhibiting the enzyme acetylcholinesterase **(Batiha *et al.*, 2020).** Capsicum plants also tend to possess antiobesity and anticoagulatory effects **(Batiha *et al.*, 2020).** It has been known that *Capsicum annuum* possesses anti-diabetic properties. These properties are regulated by variety of mechanism of action such as inhibition of alpha-glucosidase, alpha-amylase and antioxidant activities; the insulin mimicking ability, weight-regulating and hypolipidemic effects.

Capsicum fruits are high in keto-carotenoids (capsanthin) and spicy capsaicinoids. Capsaicin causes the peripheral ends of afferent neurons to emit substance P, neurokinin A, vasoactive intestinal polypeptide and calcitonin gene-related peptide. Chili is also high in vitamin A, vitamin C, vitamin E, vitamin D, vitamin B and carotenoid molecules. Ascorbic acids in capsicum are renowned for their antioxidant properties. Commercial chili processing generates a considerable number of various agricultural wastes, which are one of the most important and promising energy- and protein-rich animal feed sources. After harvesting the chili, the chili leaves are left. There have been a few studies on the impact of diets supplemented with chili leaf powder on production performance, feed conversion ratio and egg cholesterol in chicken. **(Lokaewmanee *et al*., 2019)**. Most often studied phytobiotics in broiler nutrition are spices and herbs like oregano, thyme, garlic, rosemary, sage, hot red pepper and black pepper. *Capsicum annuum* is the only plant that generates the alkaloid capsaicinoids, which are responsible for their spicy flavor. Because of biological factors, production and storage circumstances, the composition and amounts of various biological compounds in plants varies. Capsaicin makes up about 48% of the active ingredients in capsaicinoids (8-methyl-N-vanillyl-6-nonemide). Because of its neurotonic and antibacterial properties, this is an important alkaloid **(Tellez *et al*., 1993; Mcelroy *et al*., 1994; Fadile and Elife, 2005)** and its ability to reduce lipid peroxidation **(Oboh *et al*., 2006; Conforti *et al*., 2007)**. Capsaicinoids are alkaloids with neurological advantages that are essential in the pharmaceutical sector **(Hayman and Kam, 2009)**. Previous research on the phytochemical qualities of capsaicinoids has revealed a wide range of biochemical and pharmacological capabilities, including antioxidant, anti-inflammatory, antiallergenic and anti-carcinogenic effects **(Lee *et al*., 2005)**, which may help to reduce cancer risk **(Nishino *et al*., 2009)**. Capsicum is more effective than vitamin E in preventing lipid peroxidation **(Luqman and Razvi, 2006)**. Capsaicin can boost bile acid secretion, augment the activities of pancreatic and intestinal enzymes and increase body weight gain in broiler chickens **(Platel and Srinivasan, 2004)**, increase bile acid release **(Abdel Salam *et al*., 2005)** and increase body weight gain of broiler chickens **(Galib *et al*., 2011; Puvaca *et al*., 2014; 2015)**.

Egg yolk color is an important purchasing criteria for consumers **(Esfahani-Mashhour *et al*., 2009)** but the exact color desired varies among and within countries, with yellow to golden colors usually regarded as being the most appealing **(Baiao *et al*., 1999; Rowghani *et al*., 2006)**. Red and yellow pigments that change the colour of egg yolks are derived from carotenoids **(Baiao *et al*., 1999; Blount *et al*., 2000)**. Since birds can't produce these carotenoids, they must be obtained through foods like red pepper, pine, and corn meal **(Blount *et al*., 2000)**. The red pepper is a good egg yolk colorant and is widely cultivated around the world **(Şamlı *et al*., 2005; Barbero *et al*., 2008; Al-Kassie *et al*., 2011)**. The yellow carotenoids in the red pepper are much more abundant compared to the red pigments and they easily enter the yolk to intensify the yellow colour **(Hamilton *et al*., 1990; González *et al*., 1999)**. Feeding red pepper to poultry does not appear to have any negative effects on productivity parameters **(Furuse *et al*., 1994; Gurbuz *et al*., 2003; Rowghani *et al*., 2006)**.

To maintain optimal production, many unique natural substances such as probiotics, prebiotics, organic acids, plant extracts and essential oils have been used **(Fulton *et al*., 2002)**. Addition of plant extracts and essential oils obtained from plants into diets may play role in improving growth performance of animals and health status as well **(Denli *et al*., 2004)**.

There have been a limited number of studies on the effects of diets supplemented with chili leaf powder on the production performance, feed conversion ratio and egg quality parameters in poultry. Thus, a study is planned to assess the effects of dietary chili leaf powder in Rhode Island Red laying hens with following objectives.

Objectives

1. To study the effect of dietary supplementation of chili leaf powder on growth and nutrient utilization of laying hens.
2. To study the effect of dietary supplementation of chili leaf powder on egg quality.
3. To study the effect of dietary supplementation of chili leaf powder on the haemato-biochemical parameter of laying hens.
4. To study the effect of dietary supplementation of chili leaf powder on economics of laying chicken production.

Review of Literature

Chapter 2 REVIEW OF LITERATURE

The main goal of review literature is to learn about important research methodology, concepts and various experimental techniques to be used in research by gaining conception of pre-existing research and discussions related to a particular area of study or topic and presenting the knowledge obtained in the form of a written record. It revealed from the literature that there isn't much information available regarding the effect of feeding chili leaves in poultry. Current and past available literature related to feeding of chili leaves and other similar studies published globally have been discussed under following sub headings:

Chili is derived from the *Capsicum* genus, which is one of the most common and widely dispersed plants in the Solanaceae family. *Capsicum baccatum*, *Capsicum annuum*, *Capsicum pubescens*, *Capsicum frutescens* and *Capsicum chinense* are five domesticated species within the genus *Capsicum*. Chili peppers (*Capsicum annuum*) are widely grown and used for food and traditional medicine all over the world. Chili is a good source of vitamins and minerals, carotenoids and phenolic compounds such as capsaicinoids, luteolin and quercetin. Capsaicin (8-methyl-N-vanillyl-6-nonenamide), a bioactive chemical found in chili has been shown to enhance antioxidant capacity and anti-inflammatory effect, as well as pain alleviation and modification of lipid metabolism and the gut microbial population **(Lee *et al*., 2005; Prakash and Srinivasan, 2010)**. The ethanol extract of *Capsicum annuum* has a great antimicrobial activity against *Micrococcus* sp, *Bacillus*, *E. Coli*, *Pseudomonas* sp and *Citrobacter* sp **(Careaga *et al*., 2003).** The peptides extracted from chili pepper seeds inhibit the development of fungi and yeasts **(Oni *et al*., 2011)**. *Capsicum annuum* shows hypocholesterolemic properties in animal studies **(Srinivasan, 2005; Aizawa and Inakuma, 2009).**

Chili leaves are left after harvesting the chili. Chili leaves are good sources of Vitamins and Minerals such as vitamin A, vitamin C, vitamin B_1, B_2, Iron, calcium, Processing of chili pepper leaves yields into beta carotene (32,151.0 mg/100g), calcium (2,270.5 mg/100) g and iron (34.4 mg/100)g **(Abilgos-Ramos *et al*. 2012).** Feeding of chili leaves to laying hens has no negative effects on their performance and using red chili as a possible natural pigment could enhance the colour of the yolk in your eggs **(Gurbuz *et al*., 2003).**

2.1 Taxonomical classification of chili (*Capsicum annuum*)

Kingdom	-	Plantae
Phylum	-	Magnoliophyta
Class	-	Magnoliopsida
Order	-	Solanales
Family	-	Solanaceae
Genus	-	*Capsicum*
Species	-	*Annuum*

The following literature review has been presented in relation to supplementation of chili/ chili (*Capsicum annuum*) leaves in poultry.

2.2 Effect of chili leaves/chili supplementation on growth, nutrient intake and nutrient utilization in poultry:

Li *et al*. (2022) performed an experiment to examine the effects of capsaicin on growth performance, digestive enzyme activities and intestinal morphology of broiler chicks. For this experiment, two fifty-six, day-old male broiler chicks were randomly assigned into 4 dietary treatments with each treatment having 8 replications of 8 chicks each. Capsaicin was supplemented at level of 0, 2, 4 and 6 mg/kg respectively. The results revealed that growth performance, feed conversion ratio and digestive enzyme activities of intestinal contents were improved in supplemented groups.

Marić *et al*. (2021) conducted an experiment to determine the influence of chili pepper on broiler chicken performance**.** Within four replicates, three treatments with a total of 450 broiler chicken were formed. Chickens in the control treatment were fed a conventional feed mixture, while other treatment groups were fed the same diet with the supplementation of two levels of chili pepper 0.5% and 1.0%. When compared to a control treatment, the addition of 0.5 percent chili pepper resulted in the highest final body weight, followed by the addition of 1.0 percent with significant differences ($P<0.05$). Authors came to the conclusion that including chili pepper in broiler chicken diet had a good impact on production.

A study conducted by **Liu *et al*. (2021)** for determination of effectiveness of natural capsaicin extract as an alternative to the chlortetracycline on growth performance of chickens included a total of 168 one-day-old Arbor Acre male broiler chickens. Chicks were randomly allotted into 3 treatment groups, with 7 replicates per treatment and 8 broilers per pen. Dietary plan for three different treatment groups included a corn-soybean meal basal diet, a basal diet along with 75 mg/kg chlortetracycline and a basal diet along with 80 mg/kg natural capsaicin extract. Broilers chicks within natural capsaicin extract group showed higher average daily gain as compared to broilers chicks from the corn-soybean meal group at all stages ($P<0.05$). They also found that supplementation with natural capsaicin extract improved dietary nitrogen-corrected apparent metabolisable energy as compared to other treatment group ($P<0.05$). Organic matter and crude protein digestibility were found to be greater in the natural capsaicin extract diet than in the corn-soybean meal or chlortetracycline diets ($P<0.05$).

Arparjirasakul *et al*. (2018) conducted a study to investigate the effects of 2.5 ppm capsaicin extract on growth performance and intestinal morphology of broiler chicks. In this experiment, a total of 192 chicks were evenly divided into 2 treatment groups with each treatment having 3 replications of 16 chicks each. The results suggested that supplementation with capsaicin extract did not significantly affect body weight, feed intake, feed conversion ratio and intestinal morphology of broiler chicks.

Islam *et al*. (2018) in their experiment included a total of 450, day old broiler chicks that were randomly allotted to six dietary treatments each with three replicates of 25 chicks/replicate. The control group (T_0) was subjected to the commercial broiler chicks diets whereas T_1, T_2, T_3, T_4 and T_5 groups received the commercial broiler chicks diets with supplementation of 0.50%, 0.75% red chili, 0.50%, 0.75% garlic and 0.50% mixture of red chili and garlic respectively. Feed intake, digestibility and growth performance of commercial broiler chicks were all evaluated. Authors further resulted that the T_4 group had considerably higher feed intake and body weight growth than the other groups ($P<0.05$). The feed conversion ratio in the T_3 group was considerably lower than in the other groups ($P<0.05$).

Tripathi *et al*. (2017) conducted a 5-weeks feeding trial on growing Japanese quail (*Cortunix japonica*) ranging from 2 to 6 weeks age. Treatment groups T_1, T_2 and T_3 were supplemented with 0.5 percent ajwain, 0.5 percent hot red pepper and 0.25 percent black pepper, respectively in basal diet. Each group was divided into two replicates of 15

Japanese quails each. The findings showed that hot red pepper increased body weight (P<0.05). The hot red pepper supplemented group had a higher feed conversion ratio, while the black pepper supplemented group had the highest performance index. Based on the findings, it was concluded that hot red and black pepper may be added into the diet of Japanese quail broiler as a viable herbal feed additive to improve weight increase and feed efficiency ratio.

Ali *et al.* (2016) studied the effect of capsaicin on growth Performance, intestinal digestive enzymes activity and nutrients utilization of Pekin ducks. In this experiment, 120, fourteen days old Pekin ducks were randomly assigned into four treatment groups of 30 ducks. Ducks were fed with four different diets incorporated with different level of capsaicin *i.e.*, 0, 50, 100 and 150 ppm capsaicin, respectively. The results demonstrated a considerable increase in body weight and feed conversion in ducks, especially at the high level (150 ppm), followed by 100 ppm capsaicin. Furthermore, supplementing capsaicin at various dosages improved nutrient utilization in Pekin ducks, particularly ether extract and nitrogen free extract utilization. The results revealed a considerable increase in the activity of amylase, lipase and trypsin enzymes throughout the small intestine in treatment groups supplemented with capsaicin. They concluded that capsaicin supplementation of 100 to 150 ppm was considered satisfactory for improving growth performance parameters, nutrient utilisation in ducks.

Abo *et al.* (2016) performed a 49-days experiment to study the effect of *Capsicum annuum* on growth performance and feed conversion ratio of broiler chickens. 1g/ kg of chili powder were supplemented into basal diet of chickens. At the end of the trial, they found that body weight and feed conversion ratio were significantly (P <0.05) improved in groups of chickens supplemented with *Capsicum annuum.*

Bravo *et al.* (2014) in their study included a total number of 210, 1-day-old Ross 308 male broiler chickens in order to investigate the effects of a supplementary mixture of 5% carvacrol, 3% cinnamaldehyde and 2% capsicum on dietary energy utilization and growth performance. Chickens ranging from 0 to 21 d of age were fed two diets *ad lib* that included a maize-based diet serving as control diet and the test diet with 100 g/t of supplementary plant extracts. It was observed that feeding the mixture of carvacrol, cinnamaldehyde and capsicum apart from improving the feed efficiency by 9.8% (P =0.055) also increased weight gain by 14.5% (P=0.009).

El-Deek *et al*. (2012) performed an experiment to study the effect of feeding corn-soy diets with or without two levels of hot pepper (1.5 and 3 g/kg diet) as a non-antibiotic growth promoter or Oxytetracycline (0.1 g/kg) as an antibiotic growth promoter on growth performance, included hubbard broiler chicks that were randomly allotted into 4 equal groups each of six replicates of 6 birds each. Hot pepper supplementation showed significant positive effect on body weight gain; however, Oxytetracycline had no effect on body weight gain when compared to the control group. In comparison to the control and Oxytetracycline, hot pepper increased feed consumption.

Al-Kassie *et al*. (2011) conducted an experiment on day old broiler chicks (n=250) to evaluate the performance of broilers chicks fed with hot red pepper (*Capsicum annuum*). For six weeks, 5 levels of hot red pepper @ 0.00%, 0.25 percent, 0.50 percent, 0.75 percent and 1 percent were included into the basal diet. Addition of hot red pepper @ 1%, 0.75% and 0.5% improved body weight gain along with the conversion ratio. Authors further concluded that including hot red pepper as a feed additive @ 1%, 0.5% and 0.75% improves broiler chicks' overall performance.

Atapattu and Belpagodagamage (2011) performed an experiment to study the effect of Chili (*Capsicum annuum*) powder on growth performance of broiler chicks. For this research, one hundred broiler chicks were evenly divided into four groups and were provided with four different diets containing 0, 1, 3, 5% of chili powder respectively. At the end of the experiment, they found that chicks fed diet containing 5% chili powder significantly ($P<0.05$) gained highest live weight when compared to control group. However, feed conversion ratio was non significantly improved.

Since, there is limited information regarding effect of chili leaf powder supplementation on layer bird’s growth performance, nutrient intake, and utilization so research work is aimed to do in this direction.

2.3 Effect of chili leaves/chili supplementation on egg quality parameters:

Liu *et al*. (2021) studied the influence of capsaicin supplementation on egg quality parameters in laying ducks. In this experiment, three hundred and eighty-eight, 58-week-old laying ducks were allocated into two treatments at random. A control group of ducks was fed a basal diet, whereas the other groups were provided a diet containing 150 mg/kg capsaicin for the period of eight weeks. The results revealed that dietary supplementation with capsaicin increased egg production and egg weight in laying ducks but had no influence on daily egg mass. According to the findings, dietary capsaicin administration boosted egg production performance, most likely via activating the calcium regulatory system and increasing redox state.

Saleh *et al*. (2021) performed an experiment to investigate the effect of natural paprika powder on egg quality parameters and yolk fatty acid content in laying hens. A total of 240 Bovans laying hens were randomly assigned to four treatments and fed diet supplemented with a control diet, a control diet with 4 kg/ton paprika, a control diet with 150 g carmoisine/ton for 12 weeks. The feed-conversion ratio and egg-production rate were significantly improved (P<0.05) when paprika powder was added to the diet, while egg weight and mass were not significantly altered. By adding paprika, the degrees of egg yolk and egg white were raised (P<0.05). These results indicated that supplementing laying hens' diets with natural paprika powder can improve egg production performance and egg yolk fatty-acid content in laying hens.

Filik *et al*. (2020) conducted a study in Japanese quail chickens to investigate the effect of supplementation of varying degrees of dietary hot pepper (*Capsicum annuum*) waste on egg production and quality. A total of 192, Japanese quail layers aged seven weeks were randomized into four treatment groups with equal mean weights. Quail chickens were fed diet supplemented with 0, 1, 2, or 4 grams of hot pepper waste powder per kg of feed. The results revealed that supplementing the layer diet with hot pepper waste powder had a substantial impact on average egg weight and total egg production (P<05). At the end of the experiment, they concluded that hot pepper waste powder can be added into quail layer diets because of its beneficial influence on egg quality since it is a cost-effective and simple agricultural by-product generated from red pepper paste industrial waste.

Lokaewmanee (2019) performed a study to determine effect of chili leaf powder on laying hens’ performance, egg quality and cholesterol levels in egg yolks. The laying hens were provided a control (no chili leaf powder) diet and experimental diet supplemented with chili leaf powder at 1, 2 or 3%. The results revealed that supplementing with chili leaf powder at 0, 1, 2 and 3% did not significantly affect egg yield, egg weight or cholesterol levels (P>0.05), also egg quality, breaking strength, shell thickness, yolk colour, yolk percentage, shell percentage, albumen % and haugh unit did not change significantly among groups (P>0.05).

Abou-Elkhair *et al*. (2018) evaluated the effects of supplementing laying hen diet with phytogenic additives on laying performance, egg quality and egg lipid peroxidation. Two hundred laying hens were divided randomly into to 4 dietary treatments and fed control diet (without additive), fennel seeds (5g/kg), black cumin seeds (5g/kg) and hot red pepper (5g/kg). For 8 weeks, each of the 4 diets was fed to five replicates of ten hens. Authors stated that supplementing diet with black cumin or red pepper may help to create a low-cholesterol diet and improve egg's antioxidant capacity.

2.4 Effect of chili leaves/chili supplementation on egg yolk color:

Lokaewmanee (2019) carried out a study to determine the effect of chili leaf on egg yolk color in laying hens. One hundred and twenty laying hens at 61 weeks of age were evenly assigned into 4 treatments. Experimental diets were incorporated with chili leaf powder at 1, 2 or 3%. The results of the effect of incorporation with chili leaf powder revealed that yolk color, lightness of yolk color, redness of yolk color and yellowness of yolk color were not significantly affected among the treatment groups ($P>0.05$).

Sözcü (2019) conducted a study to evaluate the effects of red pepper powder (*Capsicum annuum*) on egg yolk pigmentation of laying hens. One hundred sixty laying hens at 78 weeks of age were evenly assigned into four dietary treatment groups. Red pepper powder included at a level of 0%, 0.5%, 1.0% and 1.5% in the experimental diets. The findings of this experiment suggested that the darkest yolk colour (with a value of 13.2) was found in the group supplemented with 1.5% red pepper powder using Roche colour fan. According to these findings, red pepper powder can be used as a natural source for yolk pigmentation.

Abou-Elkhair *et al*. (2018) performed an 8- weeks feeding experiment to investigate the effects of supplementation of red pepper (5 g/kg) on yolk colour pigmentation of two hundred laying hens. The results suggested that the egg yolk color score enhanced by the supplementation of hot red pepper in diet of laying hens when compared with control.

Rossi *et al*. (2015) performed a 16-weeks feeding trial to study the effects of sweet green pepper on egg yolk pigmentation of laying hens. In this experiment one hundred forty-four HyLine laying hens were randomly divided into 4 treatments groups. Sweet green pepper was supplemented to basal diet of laying hens at different levels at 0, 75 ppm, 125ppm and 225 ppm. The results showed that the addition of sweet green pepper to the diets increased yolk color using a Roche fan.

Li *et al.* (2012) conducted two experiments to study the effects of red pepper (*Capsicum frutescens*) powder on egg yolk color of laying hens. In Experiment 1, thirty weeks old, Hyline Brown laying hens (n=210) were fed one of seven diets containing 0.3, 0.6, 1.2, 2.0, 4.8 or 9.6 ppm red pepper pigment or 0.3 ppm carophyll red for fourteen days. In Experiment 2, thirty weeks old, Hyline Brown laying hens (n=180) housed as similar to those in Experiment 1, were fed basal diet along with treatments including 0.8% red pepper supplementation to the basal diet.On 14th day, scores for egg color increased linearly with the level of red pepper pigment in the diet. When compared to the control group in 2nd experiment, supplementation with all of the red pepper powder treatments increased ($P<0.05$) the yolk color score as compared to control. Both red pepper powder and pigment were found to be excellent feed additives for increasing egg yolk colour in laying hens, according to the findings.

Vicente *et al.* (2007) investigated the impact of capsaicin derived from chili pepper on the color of egg yolks. Dekalb hens were fed dietary capsaicin at two distinct dosages (18 and 36 ppm) for 28 days. On day 20 of the experiment, eggs were collected and the color of the yolks was measured using a chroma meter. In comparison to the control group, both doses of dietary capsaicin significantly increased the deposition of red pigment on egg yolks. According to the findings of this study, Natural capsaicin, isolated from paprika elevated red pigmentation of the egg yolk.

Gurbuz *et al.* (2003) supplemented twelve diets based on white corn and wheat grain by various amounts of red pepper and artificial colouring pigments to 96-layer hens for 13 weeks from the start of the laying circle. They observed that the diet (wheat + yellow corn) with 3.0 and 4.0 percent red chili addition gave the highest colour pigmentation, while the diet (white corn + no wheat) with no chili or artificial pigments gave the lowest colour pigmentation. They reported that by increasing red chili content in yellow corn and wheat-based diets also resulted in increased reddish colour pigmentation of egg yolks. The findings revealed that optimum egg-yolk colour pigmentation can be achieved by combining artificial and natural colour pigments in appropriate proportions. They concluded that using red chili as a potential natural colour pigment resulted in an optimal egg yolk colour.

González *et al.* (1999) performed an experiment to compare the deposition of carotenoids in egg yolk due to different capsaicin levels in the chickens' diet. In this experiment, laying hens were randomly allocated into six groups and fed control diet (yellow corn basal diet) while the other five treatments fed along with red carotenoids in test group were Oleoresin (commercially available unsaponified extract of red chili), Biored (commercially available saponified extract of red chili) and rest three fed supplemented with red chili with different levels of capsaicin at 0.76 mg/kg, 12.26 mg/kg and 35.26 mg/kg diet. In all five groups, total level of carotenoids was 20-25 mg/kg diet. There was no statistical difference in total carotenoids in egg yolks (30±0.34 mg/kg) of groups supplemented with red pepper diets containing three levels of capsaicin. However, the carotenoid content of commercially prepared egg yolks was lower (saponified, 26±86 mg/kg; unsaponified, 24±85 mg/kg). According to the researcher oleoresin was an ineffective pigment whereas capsaicin had a good effect on the deposition of carotenoids in egg yolk without influencing productivity parameters.

2.5 Effect of supplementation of chili leaves/chili on Hematological parameters of poultry

Liu *et al*. (2021) performed an experiment for determination of effectiveness of natural capsaicin extract as an alternative to the chlortetracycline on blood parameters of broiler chickens included a total of 168 one-day-old Arbor Acre male broiler chickens. Chicks were randomly allotted into 3 treatment groups, with 7 replicates per treatment and 8 broilers per pen. Dietary plan for three different treatment groups included a corn-soybean meal basal diet, a basal diet along with 75 mg/kg chlortetracycline and a basal diet along with 80 mg/kg natural capsaicin extract. In this study, they observed that broilers chicks fed natural capsaicin extract along with basal diet had significantly reduced serum urea-nitrogen concentration, low-density lipoprotein cholesterol and total cholesterol content ($P<0.05$) when compared to the Corn Soyabean meal group.

Saleh *et al*. (2021) randomly allotted Bovans laying hens (n=240) in a completely randomized design in four treatments according to four experimental diets: a control diet (no colorants), a control diet + 4 kg/ton paprika, a control diet + 150 g carmoisine/ton and a control diet + combination of 4 kg/ton paprika plus 150 g carmoisine/ton, fed from 42 to 54 weeks of age. It was found that paprika and carmoisine alone and in combination improved ($p<0.05$) blood lipid profile in treated hens.

Reda *et al*. (2020) conducted a study on 1-week old growing quails (n=240). Quails were divided into five equal groups, each with 48 birds (4 replicates of 12 birds each). First group was fed a basal diet without red pepper oil (0 g/kg diet). Diet containing red pepper oil were given to the second, third, fourth and fifth groups at the rate of 0.4, 0.8, 1.2 and 1.6 g/kg diet, respectively for five weeks. The results suggested that plasma globulin levels were significantly lower (P= 0.0102) in birds fed with red pepper oil (0.4 and 1.2 g/kg) but the albumin/globulin ratio was significantly higher (P=0.0009). Incorporating red pepper oil (0.8 g/kg diet) into quail diets improved plasma lipid profile (P<0.05).

Puvača *et al.* (2019) conducted a study to evaluate the effect of different level of chili pepper on serum cholesterol and triglycerides concentration of broiler chickens. They formed three experiments with the total of 450 broiler chickens, within four replicates. In control treatment, chickens were fed with mixture based on corn flour and soybean meal of standard quality and composition. The experimental treatments were fed the same combination as the control group, but with the supplementation of two levels of chili pepper, 0.5g/100gm and 1.0g/100gm. Lowest levels of triglycerides, total cholesterol, low density lipoprotein and non-high-density lipoprotein were found in broilers treated with chlli with statistically significant (P<0.05) differences compared to a control treatment. Chili pepper treatments had significantly (P<0.05) highest concentration of high-density lipoprotein. They came upto conclusion that supplementation of chili pepper in diet of broiler chicken has positive impact on improvement of chicken blood lipid profile.

Tripathi *et al.* (2017) conducted a 5-weeks feeding trial in a completely randomized block design on growing Japanese quail ranging from 2 to 6 weeks of age. Treatment groups T1, T2 and T3 were supplemented with 0.5 percent ajwain, 0.5 percent hot red pepper and 0.25 percent black pepper in feed, respectively, with the exception of the control group, which received no herbal feed additives. Each group was divided into two replicates of 15 Japanese quails in each. The percentage of blood haemoglobin was identical in all treatment groups, according to the findings. By supplementing hot red and black pepper, the serum lipid profile in terms of cholesterol, low density lipoprotein cholesterol and triglycerides decreased significantly (P<0.05), whereas high density cholesterol increased significantly (P<0.05). Dietary treatment with red and black pepper significantly increased serum total protein (P<0.05).

Ali *et al.* (2016) evaluated the effect of using graded levels of capsaicin on serum metabolites of Pekin ducks. 14days old Pekin ducks (n=120) were divided into four groups of 30 ducks randomly. The first group was fed on the basal diet (control), while groups 2, 3 and 4 were given the basal diet supplemented with 50, 100 and 150 ppm capsaicin, respectively. Serum level of total proteins, globulin, high density lipoprotein, triiodothyronine and thyroxin were significantly increased for ducks fed on diets contained either 100 or 150ppm capsaicin. The concentrations of total lipid, cholesterol, triglycerides, low density lipoprotein, glucose and malondialdehyde, which is the primary stable by-product of lipid peroxidation, were reduced.

2.6 Effect of supplementation of chili leaves/chili on antioxidant capacity and immune status of poultry

Liu *et al*. (2021) studied the effects of natural capsaicin extract on antioxidant capacity of broiler chickens. They found that in comparison to broiler chicks fed corn-soybean meal diets, broiler chicks fed diet supplemented with natural capsaicin extract at 80 mg/kg had higher serum total antioxidant capacity, glutathione peroxidase, superoxide dismutase and lower levels of interleukin-1 and tumor necrosis factor ($P<0.05$). The natural capsaicin extract group also had significantly higher liver catalase activity than the corn-soybean meal group ($P<0.05$). It was concluded that broilers' antioxidant status and immunological function could be improved by adding 80 mg/kg of natural capsaicin extract to their diets.

Peregrine *et al*. (2021) in order to determine antioxidant activity of *Capsicum frutescens* powder on local chickens fed either with cotton seed cake or sunflower seed diets for 37 days, randomly assigned birds into four dietary treatments (0, 1.1, 2.2 and 4.4%) of *Capsicum frutescens* powder. The antioxidant activity scores evaluated on the basis of 1,1-diphenyl-2-picryl-hydrazyls (DPPH) radical scavenging activity at concentrations 100, 200, 400 and 500 µg/ml were found to be 17.1, 20.8, 29.8 and 33.3%, respectively. Authors revealed that the antioxidant activity of *Capsicum frutescens* is greater than that of oregano and tocopherol which are commonly used in chicken diets.

Reda *et al*. (2020) conducted a study on 1-week old growing quails (n=240). Quails were divided into five equal groups, each with 48 birds (4 replicates of 12 birds each). First group was fed a basal diet without red pepper oil (0 g/kg diet). Diet containing red pepper oil were given to the second, third, fourth and fifth groups at the rate of 0.4, 0.8, 1.2, and 1.6 g/kg diet, respectively for five weeks. They found that Glutathione and catalase activity were significantly higher in the groups fed diets supplemented with red pepper oil (0.8 g/kg).

Ali *et al*. (2016) evaluated the effect of using graded levels of capsaicinon oxidative responses of 14-day old Pekin ducks and found that antioxidant enzymes was significantly increased for ducks fed on diets containing either 100 or 150ppm capsaicin.

2.7 Effect of supplementation of chili leaves/chili on carcass parameters of chicken

Liu *et al*. (2021) conducted an experiment to determine the effects of natural capsaicin extract as an alternative on meat quality of broiler chickens. One hundred sixty-eight, day old broiler chicks were divided into 3 groups and fed with corn-soybean meal basal diet, a basal diet along with 75 mg/kg chlortetracycline and a basal diet with 80 mg/kg natural capsaicin extract. The findings showed that broilers fed diets containing natural capsaicin extract had lighter breast muscles than those fed diets containing corn-soybean meal and had higher pH values than broilers fed diets containing corn-soybean meal ($P< 0.05$). However, Supplementing with natural capsaicin extract had little impact on drip loss.

Islam *et al*. (2018) conducted a study to investigate the effect of dietary supplementation of spice herbs red chili (*Capsicum annum*) and garlic (*Allium sativum*) on carcass quality of broiler chickens. The broiler chicks were randomly assigned to six different treatment groups 1, 2, 3, 4, 5 and 6 were fed with basal diet supplemented with 0%, 0.50%, 0.75% red chili, 0.50%, 0.75% garlic and 0.50% mixture of red chili and garlic respectively. The values of the breast, back, liver, gizzard and heart did not significantly change ($P<0.05$). The dressing percentages in the first group was substantially higher than those in the other groups ($P<0.05$). The fourth group greatly outweighed the other groups in terms of the relative weight of the thigh and wing. On the other hand, the spleen and proventriculus considerably enlarged in the third group ($P<0.05$).

El-Deek *et al*. (2012) conducted an experiment to determine the effect of feeding corn-soy based diets with or without hot pepper at 1.5 and 3 g/kg diet as a non-antibiotic growth promoter or Oxytetracycline at 0.1 g/kg as an antibiotic growth promoter on carcass and organs criteria and meat quality of broiler chickens, were allotted into four equal groups. Significant reduction in dressing percentage was due to Oxytetracycline supplementation. Significant reduction in abdominal fat percentage was attributed to either hot pepper at 1.5 g/kg or Oxytetracycline. Furthermore, in 45-day-old chicks, 1.5 and 3 g hot pepper /kg diets significantly reduced gizzard percentage and plasma triglyceride.

Supplementation with 3 g hot pepper /kg diet resulted in a significant loss in flavour, colour and acceptability of breast meat, whereas 1.5 g hot pepper or Oxytetracycline at 0.1 g/kg diet resulted in a significant increase in taste, flavour, tenderness, colour and acceptability of thigh meat. It was eventually concluded that hot pepper at 1.5 g/kg could be used as an alternative antibiotic growth promoter for broilers without any sort of negative impact on carcass and organs grades.

2.8 Effect of supplementation of chili leaves/chili as effective alternative to antibiotics in poultry feed

Abd El-Hack (2022) studied the impact of phytobiotic (hot red pepper) in broilers chicks and found it as a potential antibiotic substitute and defined its influence on productivity of broiler and layer birds.

Reda *et al*. (2020) found that caecal bacterial population, *Salmonella spp, Coliform and E.coli* was reduced (P<0.05) in groups treated with red pepper oil (0.8, 1.2 and 1.6 g/kg). It was concluded that red pepper oil (0.8 g/kg) supplementation improves the health of Japanese quail by decreasing intestinal pathogens.

Arparjirasakul *et al*. (2018) investigated the effectiveness of combination between capsaicin extract and liquid methionine on antimicrobial efficacy against *Escherichia coli* in broiler chicks. In this experiment, chicks (n=192) were evenly allotted into two treatment groups, each with three replications of 16 chicks. The results suggested that liquid methionine and capsaicin extract combination supplementation decreased *Escherichia coli* count in the gut of broiler chicks.

Hossain and Howlader (2016) evaluated the effect of chili powder as a safe alternative to antibiotics for broiler chicks. Broiler chicks (n=480) were allotted in eight groups and given antibiotics @ of 1g/L (positive control), 0g antibiotic (negative control), and 3, 2.5, 2, 1.5, 1 and 0.5 percent chili powder. SP ratios of 2.0 percent, 2.5 percent and 3.0 percent chilli powder for Gumboro and Newcastle disease were significantly (P<0.05) higher than antibiotics. *E. coli* numbers were considerably lower in all treatments apart from the control (P<0.05).

Aziz. (2010) investigated the impact of *Capsicum annuum* on isolation and rate of shedding of bacteria "*Salmonella paratyphoid"* in broiler chickens. The results demonstrated that a diet supplemented with *Capsicum annuum* at 2% and 1% was beneficial in fighting against *Salmonella typhimurium* infection by reducing faecal shedding, isolation rate, *Salmonella typhimurium* bacterial count and fatality rate.

Vicente *et al*. (2007) investigated the impact of chili pepper derived capsaicin on protective impact on *Salmonella enteritidis* experimental infection. For 28 days, Dekalb hens were fed dietary capsaicin at two different doses (36 ppm and 18ppm). It was suggested that natural capsaicin, isolated from *Capsicum annuum* seeds and fed to laying hens @ 36 ppm, had a prophylactic against experimentally induced *Salmonella enteritidis* infection.

Materials and Methods

Chapter 3 MATERIALS AND METHODS

The present experiment was conducted to evaluate the dietary supplementation of chili (*Capsicun annuum*) leaf powder on performance of Rhode Island Red laying hens. The experiment was conducted at Instructional Poultry Farm, Nagla, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. The experiment was carried out on Rhode Island Red layering hens of 28 weeks of age for a period of 12 weeks.

The details of the experimental procedures, experimental design and analytical techniques used during the experiment are briefly described in the following chapter.

3.1 Preparation of Chili leaf powder

Chili (*Capsicun annuum*) leaves were obtained from plants in the local area of Jaspur, Udham Singh Nagar, Uttarakhand. After being collected, the leaves were first shade dried for 3-4 days before being sun-dried on a clean concrete floor. The leaves were then placed in a hot air oven at 70°C until its constant weight was achieved. The required amount of chilli leaves were then finely processed to powder and stored in sealed polythene bags in a cold, dry place.

3.2 Experimental birds

The experiment was conducted on 72 Rhode Island Red laying hens of 28 weeks of age at Instructional Poultry Farm of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. All birds were individually weighed and then randomly assigned into four treatment groups with three replicates of 6 birds each in such a way that the average body weight of all the treatment groups was almost similar.

All of the treatment groups were provided the same management practices throughout the entire experiment.

3.3 Housing and Management of experimental chicken

The feeding trial was performed at Instructional Poultry Farm, Nagla, Pantnagar. The birds were housed in a deep litter system with good management standards for the feeding trial. The birds were individually weighed and leg banded for identification before being assigned to each treatment group. Optimal light, temperature and ventilation were maintained for 24 hours during the experiment. Throughout the experiment, the hens were given *ad libitum* feed and fresh water, as well as adequate ventilation and no dust in the surroundings. All of the birds had access to clean, fresh drinking water in adequate troughs during the trial. In replicates, individual bird body weight and residual feed were recorded fortnightly and at the end each week respectively. The management conditions were same for different treatment groups.

3.4 Experimental design

The planned study used a Completely Randomized Design (CRD).

3.5 Experimental treatments

Treatment Group	No. of layer chickens/ Replicate			Description of treatment
	R_1	R_2	R_3	
T_1 (18 birds)	6	6	6	Basal diet (control)
T_2 (18 birds)	6	6	6	Incorporation of 0.5% chili leaf powder in basal diet
T_3 (18 birds)	6	6	6	Incorporation of 1.5% chili leaf powder in basal diet
T_4 (18 birds)	6	6	6	Incorporation of 2.5% chili leaf powder in basal diet

Feeding experiment of 12 weeks duration was performed on Rhode Island Red laying hens. 72 birds of similar age and body weight were divided into four treatment groups at random, with each treatment group comprising three replicates of six birds. Feed consumption, body weight gain, and the impact of dietary interventions on nutrient retention, egg production, and egg quality were monitored.

Four treatments included:

(T_1): Basal diet

(T_2): Incorporation of 0.5% chili leaf powder in basal diet

(T_3): Incorporation of 1.5% chili leaf powder in basal diet

(T_4): Incorporation of 2.5% chili leaf powder in basal diet

3.6 Experimental diet

Standard laying hen basal diets were prepared by combining ingredients to meet the birds' nutritional requirements, as recommended by **BIS (2007).** The proximate composition of the experiment feed was determined as per the method of **AOAC (2003).** The ingredients in standard laying bird basal feeds are shown in the table below:

Table 3.1: Ingredients in experimental Rhode Island Red laying hens' basal diets

Ingredients	Percent
Yellow Maize	57
Deoiled Rice bran	6.5
Rice polish	4.5
Groundnut cake- Solvent extracted	09
Soyabean meal	18
Marble powder	03
Dicalcium Phosphate	01
DL- methionine	0.15
Choline Chloride	0.10
Mineral mixture	0.10
Common salt	0.40
Hepatocare	0.10
Vitamin Premix	0.10
Toxin binder	0.05
Total	100

3.7 Feed analysis

Proximate analysis of different feeds used in the experimental feeding trial was conducted for determining the nutrient composition such as, Dry Matter, Crude Protein, Crude Fiber, Ether Extract, Total Ash and Nitrogen free extract. Proximate analysis was performed at Animal Nutrition Department, C.V.A.Sc., Pantnagar as per **AOAC (2003).**

3.8 Metabolic trial

A 7-day metabolic trial was performed at the end of the feeding experiment to determine nutrient utilization by the laying hens. The hens were moved to metabolic cages during the adaptation as well as excreta collection period. Two hens were chosen at random from each replicate group to assess nutrient utilization. During this period of 7 days, 4 days were considered as adaptation period succeeded by excreta collection period of 3 days. Throughout the collection period the laying hens were provided a weighed amount of feed, i.e., 140 grams, every day at 8:30 a.m. and the residue left was weighed the next morning at same time. Concurrently, faecal trays covered with polythene sheets were placed for collection of excreta. Voided excreta were collected and weighed separately daily. The excreta were dried for a period of 48 h in a hot-air oven at 70°C to estimate dry matter. The dried faeces were then ground into a fine powder and stored for further analysis of the proximate principles **(AOAC, 2003).** A part of fresh excreta samples were maintained in 5% sulphuric acid (V/V) for nitrogen assessment. A representative sample of feed was also subjected to chemical analysis. Nutrient utilization was calculated using the following formula:

$$\text{Nutrient Utilization (\%)} = \frac{\text{Nutrient intake through feed} - \text{Nutrient loss in excreta}}{\text{Nutrient intake through feed}} \times 100$$

3.9 Experimental schedule

In the experiment, the effects of dietary interventions on production performance, egg quality parameters, haematobiochemical parameters and economics of commercial laying hens were explored. The feed offered daily was weighed and documented. Blood samples for haematological parameters and serum biochemical profile were taken at the end of the experimental trial, i.e., at the 12th week of the experimental feeding trial. At the end of the experimental feeding trial, a metabolic trial was performed. Egg quality parameters were examined after 12 weeks of feeding trial.

3.10 Production parameters

3.10.1 Feed intake

The feed was given to the various treatment groups every morning at 8:00 a.m., and a record was kept. In each replicate, residual feed was weighed and recorded on a weekly basis. During the experimental feeding period, the feed intake in separate groups was calculated on a weekly basis by subtracting the weight of left-over feed from the weight of total feed delivered in each week.

3.10.2 Egg production

Egg production in all the replicates of each treatment group was monitored daily for a period of 12 weeks. Egg collection was made twice a day in the morning and in the evening in all the replicates of each treatment group. Data on daily egg production was recorded and grouped into three periods: Period I (28th – 32nd weeks), Period II (32nd – 36th weeks) and Period III (36th -40th weeks). Weekly average egg production in each treatment group was also documented. The following formula was used to calculate the egg production % for each treatment group:

$$\text{Egg Production (\%)} = \frac{\text{Total no. of egg produced per replicate}}{\text{Total no. of hen per replicate } \times \text{Total no. of days}} \times 100$$

3.10.3 Recording of weight

Weight of the layer birds in all the replicates of each treatment group was recorded at fortnightly intervals.

3.10.4 Body weight gain

Body weight of individual laying hens from each treatment group was measured at the beginning of the feeding trial and then every fifteen days until the end of the experiment. The average body weight gain of each treatment group was computed fortnightly based on these data.

3.10.5 Feed conversion ratio

The feed conversion ratio (FCR) is the proportion of feed eaten by hens to every dozen or kilograms of eggs produced. The higher the ratio, the less efficiently the feed is turned into eggs. FCR was calculated using the following formula:

$$\text{Feed conversion ratio/dozen eggs } = \frac{\text{Feed consumed in kg}}{\text{Dozen eggs}}$$

$$\text{Feed conversion ratio/kg eggs } = \frac{\text{Feed consumed in kg}}{\text{kg of egg mass}}$$

3.11 Egg quality traits

To measure the impact of dietary treatments on egg quality, internal and external egg quality parameters were analysed at the end of the feeding study. Two eggs from each replicate were used to examine egg quality.

3.11.1 Egg weight

Weight of selected individual eggs was noted in grams on an electronic weighing balance.

3.11.2 Egg width and length

A Vernier calliper was used to measure the length and width of the egg.

3.11.3 Shape index

The shape index was calculated as the ratio of the maximum length to maximum width breadth of the eggs in percentage. A Vernier calliper was used to determine the length and width of the egg.

Shape index (%) = (Width of egg in mm ÷ Length of egg in mm) × 100

3.11.4 Shell weight

The eggs were delicately shattered with a scalpel after being weighed and the egg contents were retrieved and preserved in a clear glass plate. The separated egg shell was dried for 72 hours at 60^{o}C in the oven. On an electronic weighing balance, the dry shell weight was then measured in grams. The percentile ratio of shell weight and egg weight was used to get the shell weight percentage.

3.11.5 Shell thickness

The egg shell thickness (including the shell membrane) was measured using a Micrometer Screw Gauge at three separate locations on the eggs: the air cell, the equator and the sharp end. It was determined by taking the average of three measurements and expressed in millimetres.

3.11.6 Albumen height

The contents of the egg were placed to a clear glass plate on a flat surface after the shell was separated, where they remained immovable. Then, the height of thick albumen was measured at three distinct points/sites by using a Spherometer and their average was calculated. The height of the albumen was measured in millimetres.

3.11.7 Yolk weight

After separating the yolk from the albumen, the weight was determined in grams by using an electronic balance. The yolk weight percentage was obtained by dividing the yolk weight by the overall egg weight and multiplying by 100.

3.11.8 Albumen weight

Subtracting the shell and yolk weights from the total weight of the egg yielded the albumen weight. The proportion of albumen weight was also calculated.

Albumen weight = Egg weight – (Shell weight + Yolk weight)

3.11.9 Haugh unit

The Haugh unit is a measurement of egg protein quality based on the height of the egg white (albumen). Raymond Haugh developed this technique in 1937. The following formula was used to calculate the Haugh unit:

$$\text{Haugh unit} = 100 \log (H + 7.57 - 1.7\, W^{0.37})$$

Where,

H = Albumen height in mm; W = Egg weight in gram

3.11.10 Yolk index

The yolk index, which is defined as the ratio of yolk height to yolk diameter, is a measurement of the egg's freshness. The highest height of the yolk and the average yolk diameter were measured at numerous points to calculate the yolk index. **(Card and Nesheim, 1972).**

Yolk index (%) = (Height of yolk in mm ÷ Width of yolk in mm) × 100

3.11.11 Yolk colour

Roche yolk colour fan strip was used to assess the yolk colour. The standard colour scale ranged from 1 (very light) to 15 (intensive yellow-orange) was used to measure the yolk color. The yolk colour was compared to the 15 bands of the Roche yolk colour fan strip to determine the yolk colour **(Galobart *et al.*, 1982).**

3.11.12 Egg cholesterol

To estimate the egg yolk cholesterol, the egg yolk was separated from the albumen and then the yolk lipids were isolated using the technique **(Folch *et al*. 1957)**. 15 ml of chloroform and methanol mixture (2:1 v/v) were added to one gram of separated egg yolk in a centrifuge tube, vortexed for 2 to 3 minutes, then centrifuged at 2500 rpm for 15 minutes. The chloroform layer was filtered using fibre glass filter paper into a graduated centrifuge tube and the volume was preserved for cholesterol content measurement. Cholesterol content of extracted yolk was evaluated using a commercial kit

(Erba diagnostic kit) and a colorimetric approach (wavelength 560 nm) as described by **Zaltkis *et al*. (1953).**

Concentration of egg yolk cholesterol was measured in mg/dl.

Pipette into tubes marked	Blank	Standard	Test
Working Reagent	1000µl	1000 µl	1000 µl
Distilled water	20 µl	-	-
Standard	-	20 µl	-
Test	-	-	20 µl

Incubate for 10 min. at 37^{o} C.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 200 mg/dl

3.11.13 Egg triglycerides

Egg triglycerides were determined using an Erba diagnostic kit and the GPO-Trinder, End point assay method in a spectrophotometer at 505 nm wavelength, as described by **McGowan *et al*. (1983)** and **Fossati (1969).** The concentration was measured in milligrams per litre.

Pipette into tubes marked	Blank	Standard	Test
Reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test	-	-	10µl

Incubate for 10 min. at 37^{o} C.

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 200 mg/dl

3.12 Analysis of feed and excreta samples

Excreta samples were taken during the metabolic trial, as well as feed samples utilised in all four dietary treatment groups were collected, and proximate analysis was done using the standard principles **(AOAC, 2003)**.

3.12.1 Dry matter estimation

20 g of fresh feed samples and 50 g excreta was transferred in a pre-weighed petri dish for the estimate of dry matter of feed and excreta samples, respectively. After that, the petri dishes were dried in a hot air oven at 80°C for 48 hours or until totally dried. The samples were then removed from the oven and placed in a desiccator for a few minutes before being weighed using an electronic weighing scale. The following formula was used to compute the percentage of dry matter based on the observations made during the procedure:

$$\text{Dry matter (\%)} = \frac{\text{Weight of dry sample g}}{\text{Weight of fresh sample (g)}} \times 100$$

3.12.2 Crude protein estimation

For crude protein estimation, the nitrogen content of feed and faeces samples was evaluated. The nitrogen content was calculated by using the Kjeldahl method. 2 g of feed sample was taken in a Kjeldahl digestion flask for feed analysis, while 20 g of fresh faeces sample preserved in 1.25 percent H_2SO_4 for nitrogen estimation of faeces. The Kjeldahl flask holding samples was then filled with three to five grams of digestion mixture of K_2SO_4 and $CuSO_4$ in 9: 1 and 25 ml of concentrated sulphuric acid. The materials were digested on a digestion bench until a clear bluish/greenish liquid resulted. The digested samples were then transferred entirely into a 100 ml volumetric flask after cooling, with multiple washing of digestion flask with distilled water to bring the volume up to the desired level. In a Kjeldahl distillation assembly, a 25 ml aliquot of the digested mixture was distilled with an excess of 40 percent NaOH solution, and the released ammonia was captured in a 20 ml of 2 percent boric acid indicator solution containing methyl red and bromocresol green in a ratio of 0.2 ml: 0.1 ml solution (5 ml mixed indicator solution in one litre of 4 percent boric acid solution). In the same way, a reagent blank was digested and distilled, then titrated against N/10 H_2SO_4. The percentage of nitrogen in the sample was calculated using the following formula:

$$\text{Nitrogen (\%)} = \frac{T_{\text{difference}} \times NH_2SO_4 \times 0.014 \times V}{\text{Aliquot} \times W} \times 100$$

Where,

$T_{difference}$ = Sample titre - Blank titre

N_{H2SO4} = Normality of H_2SO_4

V = Volume made up (ml)

W = Weight of the sample (g)

Crude protein present in the sample was calculated by multiplying the nitrogen percent with factor 6.25.

3.12.3 Ether extract

The ether extract of the samples was calculated using the Soxhlet extraction procedure. A 2.5 grams dried and powdered sample was weighed and put in a dry thimble for this purpose. A cotton plug was delicately inserted within the thimble and the thimble was fitted into an ether extraction assembly. Petroleum ether (B.P 40 – 60ºC) was used as the solvent in an oil flask, which was refluxed for 8 hours and then evaporated and the fat/ether extract was weighed after the oil flask was completely dried in a hot air oven at 60ºC. The % ether extract in the dried sample was determined using the formula:

$$\text{Ether extract (\%) DM basis } = \frac{b - c}{\text{a}} \times 100$$

Where,

a = weight of sample on DM basis (g)

b = weight of oil flask after extraction (g)

c = weight of empty oil flask (g)

3.12.4 Crude fibre estimation

For the determination of crude fibre, moisture and fat-free sample recovered after ether extraction were transferred from thimbles to a spoutless beaker with a 1 litre capacity. After that, each beaker received 200 ml of 1.25 percent H_2SO_4, which was refluxed for 30 minutes on a hot plate before being filtered through muslin cloth. To eliminate the acid from the residue, it was rinsed many times with hot water and filtered through muslin cloth with a suction pump. The leftover sample on the muslin cloth was transferred to the spout-less beakers once more and 200 ml sodium hydroxide (NaOH) solution at a concentration of 1.25 percent was added to each beaker. After the boiling process began, it was refluxed for 30 minutes before being filtered through muslin cloth and rinsed with hot water 5-6 times to remove all of the alkali solution. After acid and alkali treatment, the entire residue was transferred to a clean, dry silica crucible and maintained in a hot air oven at 100°C for 24 hours. After being held in desiccators, it was weighed. In a Muffle furnace, the residue was then burned for 2 hours at 600°C. The ash-filled silica crucibles were transported to a desiccator, cooled and weighed again after 12 hours. Weight loss during ignition was considered as the weight of crude fibre:

$$\text{Crude fibre (\%) DM basis} = \frac{b - c}{\text{a}} \times 100$$

Where,

a = weight of sample on DM basis (g)

b = weight of silica crucible plus residue before ignition (g)

c = weight of silica crucible plus residue after ignition (g)

3.12.5 Total ash

Five grams of dried and ground samples were placed in a pre-weighed silica crucible to determine ash content. The samples in the crucibles were burned until there was no more smoke coming out of the charred pile of samples. The silica crucibles containing the charred mass of samples were then placed in the Muffle furnace and ignited at 600°C for 2 hours using a metal tong. The ash-carrying silica crucibles were carried to the desiccator, cooled and weighed after 12 hours. The total ash percentage was calculated using the following formula:

$$\text{Total ash (\%) DM basis} = \frac{b - c}{\text{a}} \times 100$$

Where,

a = Crucible weight with ash (g)

b = Empty crucible weight (g)

c = weight of dry sample taken (g)

3.12.6 Determination of organic matter

Organic matter on dry matter basis in the feed and faeces samples was calculated by subtracting the total ash percent from 100.

Organic matter = 100 – Total ash %

3.12.7 Determination of nitrogen-free extract (NFE)

On a DM basis, the nitrogen-free extract content was estimated by subtracting the sum total of crude protein %, crude fibre%, ether extract% and total ash% from 100.

NFE (%) on DM basis = 100 – (CP% + EE% + CF% + Ash%)

3.13 Blood collection for analysis of haemato- biochemical analysis

For the collection of blood samples, six birds were chosen at random from each treatment group. At the end of the feeding trial, blood was collected. 3 ml of blood was drawn from the wing vein under aseptic conditions using a sterile syringe and needle. Half of this blood was forwarded into an EDTA vial for haematological parameter analysis and the remaining blood was poured into a serum vial (without anticoagulant) for serum separation to carry out analysis of biochemical parameters. These serum vials were allowed to stand in a slanting posture at room temperature for three to four hours before being centrifuged for 10-15 minutes at 3000 rpm. The serum was then taken in an Eppendorf tube and maintained at −20°C in a deep freezer with the date and sample number marked on the tube for biochemical parameter analysis.

3.14 Haematological parameters

3.14.1 Haemoglobin

The haemoglobin concentration (g/dl) was determined using Sahli's haemoglobinometer with the acid haematin technique, as described by **Sharma and Singh (2000)**. The brown colour was matched to a standard colour and the haemoglobin concentration was estimated.

3.14.2 Packed cell volume (PCV)

Micro- haematocrit method was used for determining PCV as reported by **Sharma and Singh (2000).** Blood containing an anticoagulant was injected into micro capillaries and one end of the capillary tube was sealed with wax to determine PCV. After that, blood-filled capillaries were centrifuged for 30 minutes at 10,000 rpm. PCV was calculated and expressed as a percentage using a Citro Cap Micro-hematocrit tube reader.

3.14.3 Total erythrocyte count (TEC)

The red blood cells were counted using Neubauer's chamber as described by **Jain (1986).** The blood samples containing anticoagulant were diluted using Natt and Herrick's diluting solution **(Campbell, 1994)**. Well mixed blood was drawn into a diluting pipette until it reached the 1.0 mark. The dilution fluid of Natt and Herrick was sucked up to the 101 mark. The mixture was allowed to settle in the pipette for 1-2 minutes. The central principal square of Neubauer's chamber was charged and RBCs were counted (four corner squares and one central square within the large primary square).

Total erythrocyte counts (TEC) were measured in million per microlitre.

3.14.4 Total leukocyte count (TLC)

Neubauer's counting chamber was used to perform the total leukocyte count (TLC) **(Jain, 1986).** Natt and Herrick's solution **(Campbell, 1994)** was employed as a diluting fluid. The number of total leukocytes was expressed in thousands per microlitre.

3.14.5 Mean corpuscular volume

Mean corpuscular volume (MCV) was calculated by using following formula and was expressed in femtolitre (fl).

$$\mathrm{MCV} = \frac{\mathrm{PVC\%}}{\mathrm{TEC}(10^6/\mu\mathrm{l})}$$

Where,

PCV= Packed Cell Volume

TEC= Total Erythrocyte Count

3.14.6 Mean corpuscular haemoglobin (MCH)

The mean corpuscular haemoglobin (MCH) was determined by using the following formula and expressed in picogram (pg).

$$\mathrm{MCH} = \frac{\mathrm{Haemoglobin}(\mathrm{g/dl})}{\mathrm{TEC}(10^6/\mu\mathrm{l})}$$

Where,

TEC = Total Erythrocyte Count

3.14.7 Mean corpuscular haemoglobin concentration (MCHC)

The following formula was used to compute the mean corpuscular haemoglobin concentration (MCHC), which was expressed in g/dl.

$$\mathrm{MCHC} = \frac{\mathrm{Haemoglobin}(\mathrm{g/dl})}{\mathrm{PCV}(\%)}$$

Where,

PCV= Packed Cell Volume

3.15 Serum Biochemical Parameter

Serum glucose, serum cholesterol, serum albumin, serum total protein serum globulin, serum phosphorus and serum calcium were among the serum biochemical parameters examined during the experiment. Erba diagnostic kit was used to estimate these parameters.

3.15.1 Serum glucose

The serum glucose was estimated spectrophtometrically with an Erba diagnostic kit by using the enzymatic GOD-POD (glucose oxidase –peroxidase) method at a wavelength of 505 nm against a blank reagent **(Sacks, 1998)**. The enzyme converts glucose to gluconic acid and hydrogen peroxide in this mechanism. The hydrogen peroxide produced combines with 4-aminoantipyrine and phenol to form the coloured Quinoneimine dye in the presence of the enzyme peroxidase. The glucose concentration in the sample can be measured as it is directly proportional to the absorbance of coloured dye measured at 505 nm. Serum glucose concentration was expressed in mg/dl.

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test	-	-	10 µl

Incubate for 15 min. at 37^{o} C.

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 100 mg/dl

3.15.2 Serum cholesterol

The serum cholesterol was determined using a spectrophotometric technique at 505 nm using an Erba diagnostic kit and the Enzymatic CHOD-PAP (cholesterol oxidase-phenol + amino phenazone) method **(Tietz, 1998)**. In the presence of the enzyme cholesterol esterase, the cholesterol esters in the serum were hydrolyzed to free cholesterol and free fatty acids. Cholesterol oxidase converts cholesterol to cholest-4-en-3one and hydrogen peroxide in the presence of oxygen. The hydrogen peroxide produced combines with 4-aminoantipyrine and phenol to form the red coloured Quinoneimine dye in the presence of the enzyme peroxidase that can be measured spectrophotometrically. The amount of quinoneimine generated is proportional to the amount of cholesterol in the blood. The standard cholesterol levels were 200 mg/dl. The concentration of serum cholesterol was measured in mg/dl.

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working Reagent	1000µl	1000 µl	1000 µl
Distilled water	20 µl	-	-
Standard	-	20 µl	-

Incubate for 10 min. at 37^o C.

$$\text{Cholesterol (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 200 mg/dl

3.15.3 Serum triglycerides (GPO-Trinder method)

Spectrophotometric measurements of serum triglycerides were performed using an Erba diagnostic kit based on Wako's method at 505 nm wavelengths and the modifications by **McGowan et al. (1983)**, **Fossati and Prencipe (1969)**. Lipoprotein lipase catalyses the hydrolysis of triglycerides to glycerol and fatty acid. Glycerol is then phosphorylated in an ATP-dependent process catalysed by glycerophosphate. The generated glycerophosphate is oxidised to dihydroxyacetone and H_2O_2 in a glycerophosphate oxidase-catalyzed process. H_2O_2 reacts with 4- aminoantipyrine (4-AAP) and 4- chlorophenol under the catalytic action of peroxidase to form a coloured quinoneimine complex that can be detected spectrophotometrically. Serum triglycerides concentration was expressed in mg/dl.

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-

Incubate for 10 min. at 37^o C.

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 200 mg/dl

3.15.4 Serum glutamate pyruvate transaminase

Erba diagnostic kit was used to determine serum glutamate pyruvate transaminase (SGPT), often known as ALT (alanine aminotransferase). Alanine transaminase (ALT) catalyses the transamination of L-alanine and 2- oxoglutarate to generate pyruvate and L-glutamate. Lactate dehydrogenase (LDH) oxidises nicotinamide adenine dinucleotide NADH to NAD while converting pyruvate to L-lactate. Absorbance taken at 340 nm was directly proportional to ALT activity, which was expressed in units per litre (U/L).

Pipette into tubes marked	**Volumes**
Working Reagent	1000 µl
Test	100 µl

Mix well and aspirate.

$$\mathrm{IU/L} = \frac{(\Delta\,\mathrm{A/\,min.})\ \times\ \mathrm{T.V.}\times\ 10^{3}}{\mathrm{S.V.}\times\ \mathrm{Absorptivity}\ \times\ \mathrm{P}}$$

Where,

T.V. = Total reaction volume in µL

S.V. = Sample volume in µL

Absorptivity = absorptivity of NADH at 340 nm

= 6.22

P = cuvette light path = 1cm

Activity of ALT at 37^{o} C (IU/L) = (ΔA/min) × Factor (1768)

3.15.5 Serum SGOT

SGOT (serum glutamate oxaloactetate transaminase) also known as AST (aspartate aminotransferase) was measured by the IFCC method using an Erba diagnostic kit **(Tietz, 1998)**. Aspartate aminotransferase (AST) catalyses the transamination of L- aspartate and 2- oxoglutarate to oxaloacetate and L- glutamate in this reaction. The enzyme malate dehydrogenase (MDH) then converts oxaloacetate to malate with simultaneous oxidation of NADH. Absorbance was taken at 340 nm, which was directly proportional to AST activity and quantified in units per litre.

Pipette into tubes marked	**Volumes**
Working Reagent	1000 µl
Test	100 µl

Mix well and aspirate.

$$IU/L = \frac{(\Delta\ A/\ min.)\ \times\ T.V.\times\ 10^3}{S.V.\times\ Absorptivity\ \times\ P}$$

Where,

T.V. = Total reaction volume in µL

S.V. = Sample volume in µL

Absorptivity = absorptivity of NADH at 340 nm

= 6.22

P = cuvette light path = 1cm

Activity of ALT at 37^{o} C (IU/L) = (ΔA/min) × Factor (1768)

3.15.6 Serum total protein

Using the Erba diagnostic kit at 540 nm wavelength, the biuret method was utilised to estimate total protein content in serum **(Johnson *et al.*, 1999)**. Protein peptide bonds react with cupric ion in alkaline solution to generate a coloured chelate (Protein + Cu_2+ Cu-protein complex), which has a wavelength of 578 nm. The absorbance of final is directly related to the total protein content in the sample. The total protein concentration in serum was measured in g/dl.

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Reagent	1000µl	1000 µl	1000 µl
Distilled water	20 µl	-	-
Standard	-	20 µl	-
Test	-	-	20 µl

Incubate for 10 min. at 37^{o} C.

$$\text{Total Protein (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 6.0 (g/dl)

3.15.7 Serum albumin

The concentration of serum albumin was determined using the bromocresol green end point test method with the help of Erba diagnostic kit **(Johnson *et al.*, 1999)**. The absorbance was taken at a wavelength of 630nm. The colour intensity of the complex was exactly related to the albumin concentration in the serum sample.

The serum albumin concentration was measured in g/dl.

Pipette into tubes marked	Blank	Sample Blank
Saline	1000µl	1000 µl
Sample	-	20 µl

After the sample has been added, the final absorbance values should be taken within 90 seconds. Compare the absorbance of the sample blank to that of the saline blank at 630nm. Then subtract the sample blank's absorbance from the test's absorbance.

3.15.8 Serum globulin

To serum albumin content was subtracted from serum total protein content to get serum globulin content. Concentration of Serum globulin was expressed in g/dl.

3.15.9 Serum Calcium

Using the Erba diagnostic kit and the O-cresolpthalein complexone technique, serum calcium (Ca) was determined spectrophotometrically **(Baginski, 1973)**. In an alkaline solution, calcium reacts with O-cresolphalein complexone to form a purple-colored complex with an absorbance proportionate to the calcium level. At a wavelength of 578 nm, the absorbance of the test and reference samples was measured against a blank.

Pipette into tubes marked	Blank	Standard	Test
Reagent	1000µl	1000 µl	1000 µl
Distilled water	10 µl	-	-
Standard	-	10 µl	-
Test	-	-	10µl

$$\text{Calcium (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times \text{Concentration of Standard (mg/dl)}$$

Concentration of Standard= 10.0 mg/dl

3.15.10 Serum Phosphorus

A standard approach was used to determine the content of phosphorus in the blood by using an Erba diagnostic kit **(Morrin and Prox, 1973)**. Phosphorus in serum interacts with ammonium molybdate to form the phosphomolybdate complex, which is then reduced to a blue-colored complex under acidic circumstances. On a spectrophotometer, the absorbance of the standard and test against the blank was measured at a wavelength of 340 nm.

3.16 Economics of egg production

The feed cost was determined by using the purchase price of all of the materials used in making the ration. Data from daily observations was used to compute the average dozen egg production. The cost of total feed consumed divided by dozen egg production has been used to calculate the economics of dozen egg production in each treatment group. The average feed price per dozens of eggs was calculable by multiplying the quantity of feed consumed (in kilo) over the time by the cost of per kg feed.

$$\text{Feed cost/dozen egg production (Rs.)} = \frac{\text{Feed consumed in kg} \times \text{ Feed cost (Rs./kg)}}{\text{Dozen egg production}}$$

3.17 Statistical methods

The experimental data generated from this study was analysed statistically using the method analysis of variance (ANOVA) generated from the general linear model process SPSS programme **(Snedecor and Cochran, 1994)**. Duncan's Multiple range test was made to assess the variations between treatment means.

Plate 3.1 Shade drying of Chili leaves

Plate 3.2 Birds in metabolic cages during metabolic trial

Plate 3.3 Digestion of samples for Crude protein estimation

Plate 3.4 Digested samples in volumetric flasks for estimation of Crude protein

Plate 3.5 Crude fibre estimation of samples by treatment with acid and alkali

Plate 3.6 Estimation of albumen height using dial test indicator

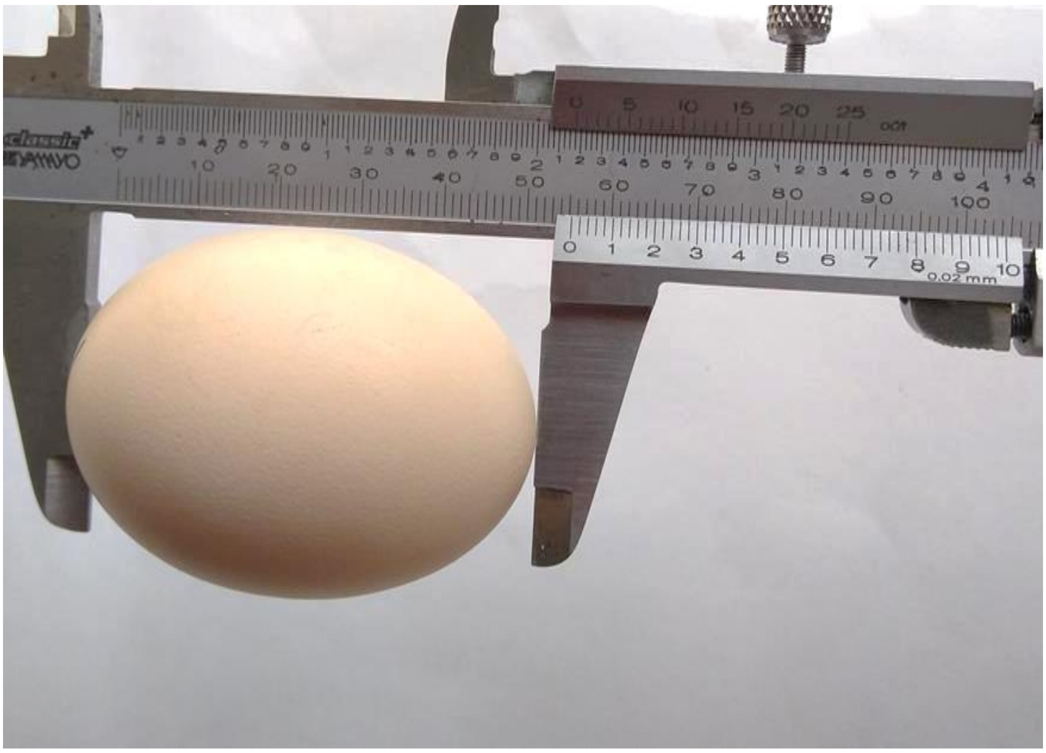

Plate 3.7 Estimation of egg length using vernier calliper

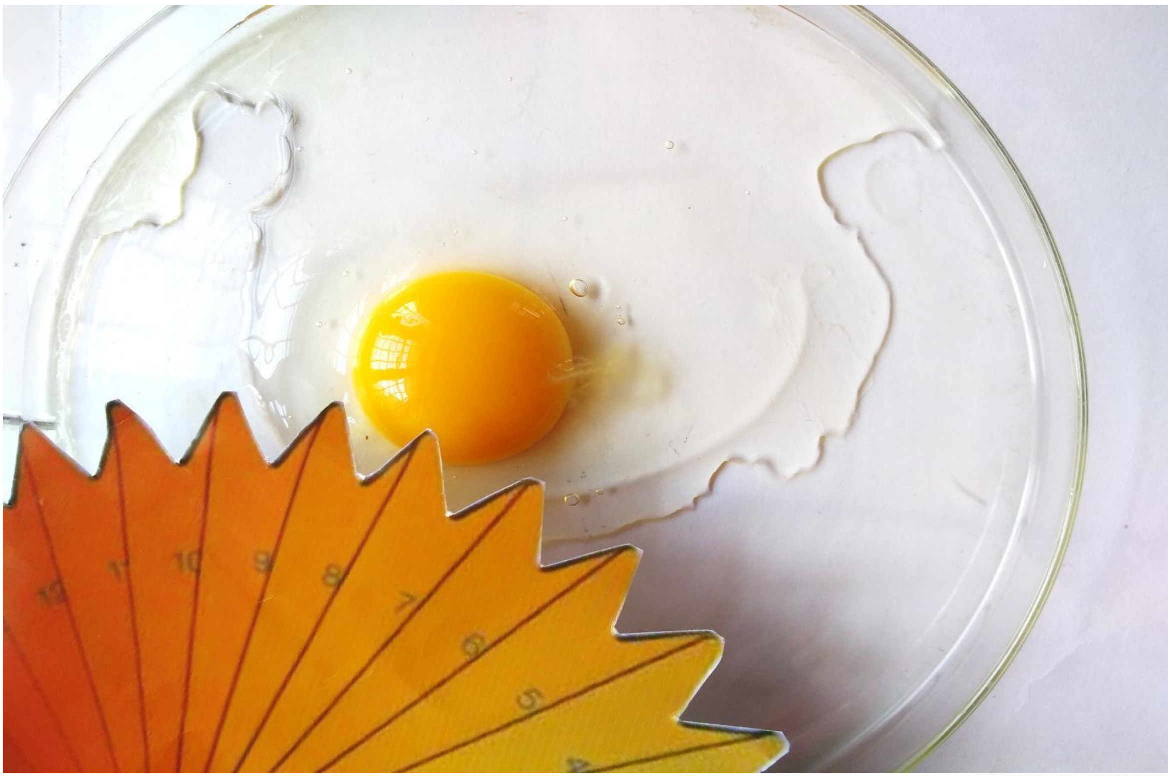

Plate 3.8 Estimation of yolk colour score using Roche yolk colour fan strip

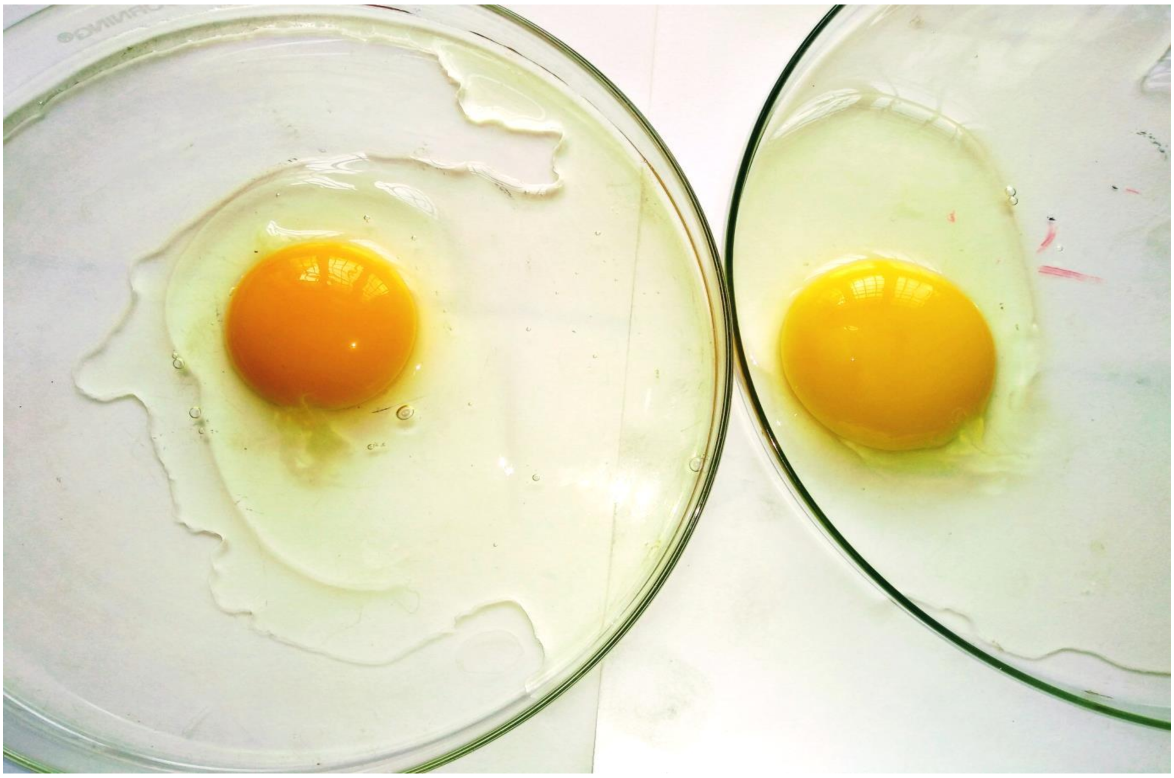

Plate 3.9 Comparison in egg yolk colours between treatment (T_4) and control group

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

The present experiment was conducted to evaluate the effect of dietary supplementation of chili (*Capsicum annuum*) leaf powder on growth performance, nutrient utilization, haemato-biochemical parameters and economics of commercial laying hens' production. This chapter presents and discusses the outcomes of the current feeding trial. Four treatments were designated as follows: T_1 (Basal diet), T_2 (Basal diet + 0.5% chili leaf powder), T_3 (Basal diet + 1.5% chili leaf powder), T_4 (Basal diet + 2.5% chili leaf powder).

4.1 Chemical composition of chili (*Capsicum annuum*) leaf powder

The chemical composition of chili (*Capsicum annuum*) leaf powder has been presented in table 4.1.

Table 4.1: Chemical composition of chili (*Capsicum annuum*) leaf powder (on% dry matter basis)

Nutrients	Chili Leaf
Crude Protein	6.78
Crude Fibre	14.52
Ether extract	7.89
Ash	5.15
Nitrogen free extract	65.66

Proximate analysis showed that chili leaf contained 6.78% crude protein, 7.89% ether extract, 14.52% crude fibre, 5.15% total ash and 65.66 % nitrogen free extract on dry matter basis.

4.2 Chemical composition of experimental diets

A basal diet for Rhode Island Red laying hens was formulated by combining ingredients to meet the hen's nutritional requirements, as recommended by BIS (2007). The chemical composition of experimental diets provided to laying hens in different treatment groups have been presented in Table 4.2. The proximate analysis of the meal was determined using standard methods **(AOAC, 2003)**. The crude protein (CP) content of diets on a dry matter basis fed to laying hens of different treatment groups T_1, T_2, T_3 and T_4 was 17.56, 17.50, 17.40 and 17.29 percent, respectively.

Whereas ether extract of different treatment groups T_1, T_2, T_3 and T_4 was 3.80, 3.79, 3.81 and 3.72 percent, respectively.

The crude fibre content of different treatments T_1, T_2, T_3 and T_4 was 4.19, 4.28, 4.45 and 4.35 percent and total ash content of different treatments T_1, T_2, T_3 and T_4 was 9.72, 9.13, 8.89 and 8.62 percent respectively on dry matter basis.

The nitrogen free extract (NFE) of diets given to laying hens of different treatment groups T_1, T_2, T_3 and T_4 was 64.73, 65.30, 65.45 and 66.02 percent, respectively on dry matter basis.

Table 4.2: Chemical composition of diet (% dry matter basis) provided to different treatment groups

Nutrients	Treatments/Groups			
	T_1	T_2	T_3	T_4
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder
Crude protein	17.56	17.50	17.40	17.29
Ether extract	3.8	3.79	3.81	3.72
Crude Fibre	4.19	4.28	4.45	4.35
Total Ash	9.72	9.13	8.89	8.62
Nitrogen Free Extract	64.73	65.30	65.45	66.02

4.3 Production Parameters

Table 4.3, 4.4, and 4.5 demonstrated the production performance of different treatment groups T_1, T_2, T_3 and T_4 in terms of average weekly feed intake, average fortnightly body weight gain and average weekly egg production. The performance of the production line was investigated further by dividing the 12-week trial into three periods., period-1 (28-32 weeks), period-2 (32-36 weeks), period-3 (36-40 weeks) and overall period (28-40 weeks) and are presented in Table 4.6, 4.7, 4.8 and 4.9 respectively.

4.3.1 Average weekly feed intake

Weekly average feed intake of laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder in different treatment groups T_1, T_2, T_3, and T_4 have been presented in Table 4.3.

Average feed intake in different treatment groups T_1, T_2, T_3 and T_4 recorded at the end of 1st week of feeding trial was found to be 120.67±0.66, 119.95±0.63, 120.29±0.15

and 120.17±0.29 g, respectively. No significant difference was found in the average feed intake among the different treatment groups during the 1st week of feeding trial.

During 2nd week of the experimental feeding trial, average values of feed intake in different treatment groups T_1, T_2, T_3 and T_4 was 121.84±0.47, 120.16±0.27, 120.83±0.34 and 120.15±0.04 g, respectively. As per the findings, feed intake significantly ($P<0.05$) reduced in treatment group T_2 (120.16±0.27) and T_4 (120.15±0.04) as compare to T_1 (121.84±0.47). No significant difference was observed between T_1 and T_3 group. The feed intake was also similar among T_2, T_3 and T_4 groups and no significant difference was seen among them.

During 3rd week average feed intake of laying hens of different treatment groups T_1, T_2, T_3 and T_4 was found to be 120.06±0.46, 120.24±0.73, 120.11±0.26 and 120.15±0.44 g, respectively. However, during the 4th week average feed intake was 120.74±0.54, 119.70±0.28, 119.54±0.37 and 119.41±0.60 g, respectively was non significantly changed.

During 5th and 6th, 7th week average feed intake of laying hens of different treatment groups T_1, T_2, T_3 and T_4 was found to be 119.90±0.75, 120.42±0.57, 119.76±0.70, 119.23±0.38 and during 6th week, 120.24±0.73, 120.12±0.40, 119.80±0.32, 119.60±0.34 and during 7th week, 120.29±0.78, 120.08±0.49, 119.70±0.24, 119.41±0.39. The results on average feed intake revealed no significant difference among the treatment groups during 5th and 6th, 7th week.

Average feed intake during 8th, 9th and 10th week in different treatment groups were recorded to be 120.14±0.30, 120.21±0.41, 119.75±0.34, 119.42±0.23; 120.23±0.67, 120.13±0.54, 119.75±0.58, 119.48±0.20 and 120.22±0.28, 120.14±0.55, 119.73±0.61, 119.44±0.13) g, respectively and was not significantly affected among the treatment groups by adding chili leaf powder to basal diet of laying hens.

During 11th and 12th week average feed intake of laying hens of different treatment groups T_1, T_2, T_3 and T_4 was found to be 120.20±0.28, 120.16±0.61, 119.75±0.24 and 119.44±0.14; 120.21±0.43, 120.14±0.82, 119.74±0.34 and 119.45±0.20 g, respectively. The average feed intake during 11th and 12th week differed significantly ($P<0.05$) among various treatment groups. Feed intake was significantly ($P<0.05$) lower in T_4 group, as compared to the T_1, T_2 and T_3 groups, whereas, feed intake was statistically similar between T_2 and T_3 groups. No significant difference was observed among T_1, T_2 and T_3 groups.

Overall average feed intake per day during the experimental feeding trial in all treatments fed diet supplemented with chili (*Capsicum annuum*) leaf powder was found to be significant. Overall average feed intake per day in treatments groups T_1, T_2, T_3 and T_4 was 120.40±0.18, 120.15±0.27, 119.97±0.12 and 119.45±0.08 g, respectively. Out of which overall average feed intake per day was significantly (P<0.05) reduced in treatment group T_4 as compared to T_1, T_2 and T_3 groups. However, overall feed intake in groups T_2 and T_3 was statistically similar. No significant difference was observed among T_1, T_2 and T_3 groups.

The present findings are in agreement with **Lokaewmanee (2019),** who reported that supplementing laying hens with dietary chili leaf powder at a concentration of 3% reduced feed intake (P<0.05) when compared to a control and dietary chili leaf powder at a concentration of 1% which are corroborated with the present study. **Li *et al*. (2022)** showed that dietary supplementation with 4 mg/kg capsaicin decreased the feed intake.

Table 4.3: Average weekly feed intake (g) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Period (week)	Treatments/Groups				P-Value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
1	120.67±0.66	119.95±0.63	120.29±0.15	120.17±0.29	0.76
2*	121.84^{b}±0.47	120.16^{a}±0.27	120.83^{ab}±0.34	120.15^{a}±0.04	0.02
3	120.06±0.46	120.24±0.73	120.11±0.26	120.15±0.44	0.99
4	120.74±0.54	119.70±0.28	119.54±0.37	119.41±0.60	0.24
5	119.90±0.75	120.42±0.57	119.76±0.70	119.23±0.38	0.62
6	120.24±0.73	120.12±0.40	119.80±0.32	119.60±0.34	0.65
7	120.29±0.78	120.08±0.49	119.70±0.24	119.41±0.39	0.59
8	120.14±0.30	120.21±0.41	119.75±0.34	119.42±0.23	0.53
9	120.23±0.67	120.13±0.54	119.75±0.58	119.48±0.20	0.38
10	120.22±0.28	120.14±0.55	119.73±0.61	119.44±0.13	0.23
11*	120.20^{b}±0.28	120.16^{b}±0.61	119.75^{b}±0.24	119.44^{a}±0.14	0.52
12*	120.21^{b}±0.43	120.14^{b}±0.82	119.74^{ab}±0.34	119.45^{a}±0.20	0.01
Overall*	120.40^{b}±0.18	120.15^{ab}±0.27	119.97^{ab}±0.12	119.45^{a}±0.08	0.03

$^{a,\ b}$ Mean values bearing different superscripts within a row differ significantly from each other, *P<0.05

In contrary, **Liu *et al*. (2021)** found no significance effect on daily feed intake in broiler chickens supplemented with capsaicin. **Arparjirasakul *et al* (2018)** noted a non-significant effect in broilers fed diet supplemented with 2.5 ppm capsaicin. **Abou-Elkhair *et al*. (2018)** also noted that feed intake between the groups of laying hens were similar when fed with red chili powder. **Atapattu *et al*. (2011)** also found that feed intake of broiler chickens was not affected by dietary supplementation of chili powder. While, **Shahverdi *et al.* (2013)** found significant increase in feed intake in broiler chickens supplemented with red pepper.

4.3.2 Average body weight gain

Table 4.4 shows the fortnightly average body weight gain (g) of Rhode Island Red laying hens fed a diet containing chili (*Capsicum annuum*) leaf powder over a period of 28-40 weeks.

Table 4.4: Average fortnightly body weight (g) of Rhode Island Red laying hens during 28-40 weeks period fed diet incorporated with chili (*Capsicum annuum*) leaf powder

Period (fortnight)	Treatments/Groups				P - value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
0	1543.89±3.09	1538.74±2.93	1538.37±3.41	1540.43±2.58	0.41
1	1645.56±6.12	1653.33±6.67	1629.93±2.54	1579.18±3.29	0.59
2	1706.49±4.98	1649.93±5.78	1650.43±3.38	1640.97±3.14	0.98
3	1756.40±4.89	1673.89±3.09	1649.27±5.50	1660.23±6.81	0.98
4	1821.37±5.30	1718.33±5.36	1681.22±2.74	1693.53±14.30	0.25
5	1819.70±6.14	1754.94±11.18	1736.27±2.28	1727.67±14.08	0.43
6	1858.45±3.82	1835.00±7.83	1819.37±1.89	1820.23±9.76	0.90
Total body weight gain(gm)	314.56±5.76	296.26±8.98	281.00±5.17	279.80±9.46	0.70

Average body weight at first day of experimental trial in the laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder in different treatment groups T_1, T_2, T_3 and T_4 was 1543.89±3.09, 1538.74±2.93, 1538.37±3.41 and 1540.43±2.58 g, respectively. After 15 days, body weight in different treatment groups T_1, T_2, T_3 and T_4

was 1645.56±6.12, 1653.33±6.67, 1629.93±2.54and 1579.18±3.29g, respectively, whereas after 30 days of the experimental feeding trial, the body weight was found to be 1706.49±4.98, 1649.93±5.78, 1650.43±3.38 and 1640.97±3.14 g in treatment groups T_1, T_2, T_3 and T_4 respectively. According to the findings, there was no significant difference in average body weight among treatment groups after 15 and 30 days of the feeding trial. After 45 days, 60 days, 75 days and 90 days of experimental feeding trial, the average body weight in different treatment groups T_1, T_2, T_3 and T_4 did not change significantly. The average body weight after 45 days was 1756.40±4.89, 1673.89±3.09, 1649.27±5.50 and 1660.23±6.81 g whereas after 60 days, the body weight was 1821.37±5.30, 1718.33±5.36, 1681.22±2.74 and 1693.53±14.30 g, after 75 days of experimental feeding trial, the body weight was 1819.70±6.14, 1754.94±11.18, 1736.27±2.28 and 1727.67±14.08 g and after 90 days, the body weight was 1858.45±3.82, 1835.00±7.83, 1819.37±1.89 and 1820.23±9.76 g in different treatment groups T_1, T_2, T_3 and T_4 respectively. The average body weight gain in different treatment groups was non-significantly changed during the feeding period of 12 weeks.

Weight gain during the experimental period among the various treatment groups T_1, T_2, T_3 and T_4 fed diet supplemented with chili (*Capsicum annuum*) leaf powder was also found to be statistically similar. Weight gain during the entire experimental period in different treatment groups T_1, T_2, T_3 and T_4 was 314.56±5.76, 296.26±8.98, 281.00±5.17and 279.80±9.46 g, respectively.

The results of present study are line with **Abou-Elkhair *et al*. (2018)** who reported that body weight between the groups of laying hens were statistically similar when fed with red chili powder. **Arparjirasakul *et al* (2018)** noted a non-significant effect in broilers fed diet supplemented with 2.5 ppm capsaicin. While, **Atapattu *et al*. (2011)** found that the birds fed 5% chili powder gave higher weight gain compared to control group. **Abo *et al*. (2016)** also reported that broiler chicks fed the diet supplemented with *Capsicum annuum* was significantly gained the higher body weight.

4.3.3 Average weekly egg production

Table 4.5 shows the average weekly egg production (percentage) of Rhode Island Red laying hens fed a diet supplemented with chili (*Capsicum annuum*) leaf powder.

Table 4.5: Average weekly egg production (%) of Rhode Island Red laying hens fed a diet supplemented with chili (*Capsicum annuum*) leaf powder

Period (week)	Treatments/Groups				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
1	60.32±0.79	60.32±2.10	60.32±0.79	61.11±0.00	3.12
2	59.52±1.37	60.32±1.59	60.32±1.59	61.11±2.10	1.86
3*	59.52^{b}±1.37	61.11^{ab}±0.78	61.11^{ab}±0.00	61.90^{a}±0.79	2.61
4	57.94±0.79	58.73±0.79	58.73±1.37	59.52±0.79	2.17
5	60.32±0.79	59.52±1.37	61.11±1.59	61.90±1.37	3.02
6	60.32±2.10	61.11±2.10	61.90±2.75	63.49±2.10	3.84
7	61.90±1.37	62.70±1.59	63.49±0.79	65.08±1.59	4.45
8	61.90±1.37	62.70±0.79	62.70±0.79	64.29±1.37	4.45
9	65.08±1.59	65.87±2.10	66.67±1.37	67.46±1.59	3.52
10	66.67±1.37	67.46±2.10	67.46±2.10	69.05±1.37	3.78
11	66.66±2.75	67.46±3.46	68.25±3.46	68.25±2.86	3.63
12	69.84±2.10	70.63±1.59	71.43±2.38	72.22±2.10	4.59
Overall egg Production	62.50±1.06	63.16±0.22	63.62±1.13	64.62±0.22	0.94

[a,b] Mean values bearing different superscripts in a row differ significantly, *P<0.05

Egg production during the 1st week of experimental feeding trial in the laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder was 60.32±0.79, 60.32±2.10, 60.32±0.79 and 61.11±0.00 percent among various treatment groups T_1, T_2, T_3 and T_4, respectively while during the 2nd week, it was 59.52±1.37, 60.32±1.59, 60.32±1.59 and 61.11±2.10 percent, respectively. According to the findings, there was no significant difference in egg production across the various treatment groups T_1, T_2, T_3 and T_4 during the first and second week of the experimental feeding period.

Further, throughout the third week of the experimental feeding trial, average egg production during the 3rd week in treatment groups T_1, T_2, T_3 and T_4 was 59.52±1.37, 61.11±0.78, 61.11±0.00 and 61.90±0.79 percent respectively. The average egg production of laying hens of group T_4 (61.90±0.79) was significantly ($P<0.05$) higher than the treatment groups T_1, T_2 and T_3. Whereas, average egg production in groups T_2 and T_3 was non- significantly different from each other. No significant difference was observed among T_1, T_2 and T_3 groups. While during 4th week, average egg production showed a similar, non- significant influence among the various treatment groups. It was 57.94±0.79, 58.73±0.79, 58.73±1.37 and 59.52±0.79 percent among treatment groups T_1, T_2, T_3 and T_4, respectively.

Egg production percent among different treatment groups T_1, T_2, T_3 and T_4 was statistically similar during the 5th week and it was 60.32±0.79, 59.52±1.37, 61.11±1.59 and 61.90±1.37 percent respectively.

Egg production during 6th week was found to be 60.32±2.10, 61.11±2.10, 61.90±2.75 and 63.49±2.10 percent whereas during 7th week it was 61.90±1.37, 62.70±1.59, 63.49±0.79 and 65.08±1.59 percent in different treatment groups T_1, T_2, T_3 and T_4, respectively. Average weekly egg production during 8th week of experimental feeding trial was 61.90±1.37, 62.70±0.79, 62.70±0.79 and 64.29±1.37 percent, while during the 9th week, it was 65.08±1.59, 65.87±2.10, 66.67±1.37 and 67.46±1.59 percent in treatment groups T_1, T_2, T_3 and T_4, respectively. Although, egg production percent in all the treatment groups T_2, T_3 and T_4 was slightly higher than the control group, T_1 during 6th, 7th, 8th and 9th week of the experimental feeding trial, but the difference was statistically, non- significant.

The average weekly egg production during 10th week was 66.67±1.37, 67.46±2.10, 67.46±2.10 and 69.05±1.37 percent, while during 11th week, average egg production was 66.66±2.75, 67.46±3.46, 68.25±3.46 and 68.25±2.86 percent in treatment groups T_1, T_2, T_3 and T_4 respectively. No significant difference was found in egg production during 10th and 11th week of the experimental feeding trial.

Similar, non- significant results were obtained during 12th week also. The egg production during 12th week was 69.84±2.10, 70.63±1.59, 71.43±2.38 and 72.22±2.10 percent respectively.

Overall weekly egg production in the treatment groups T_1, T_2, T_3 and T_4 fed diet supplemented with chili (*Capsicum annuum*) leaf powder was 62.50±1.06, 63.16±0.22, 63.62±1.13 and 64.62±0.22 percent, respectively. Hence, supplementation of chili (*Capsicum annuum*) leaf powder had overall non- significant effect on egg production percent of laying hens among various treatment groups.

A non-significant effect on egg production of laying hens on dietary supplementation of chili leaf powder was reported by **Lokaewmanee (2019). Liu *et al*. (2021)** also found non-significant effect on egg production in laying hens supplemented with capsaicin which is corroborated with present study. **Rossi *et al*. (2015)** noted that Egg production was not significantly affected by the addition of chili powder to the diets of laying hens. In contrast, **Saleh *et al.* (2021)** who reported that egg production of laying hens supplemented with paprika powder was significantly improved. **Filik *et al*. (2020)** found significant increase in egg production of Japanese quail among all the hot pepper (*Capsicum annuum*) waste supplemented groups. **Abou-Elkhair *et al*. (2018)** noted that dietary inclusion red pepper in diet of laying hens improved egg production compared with control.

4.3.3.1 Period 1 (28-32 weeks)

The average egg production, feed intake and feed conversion ratio (FCR) in Rhode Island Red laying hens during period 1 (28-32 weeks) of experimental feeding trial are presented in Table 4.6 and Figure 1a, 1b and 1c.

Table 4.6: Average egg production, feed intake and feed conversion ratio in Rhode Island Red laying hens during period 1 (28-32 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Period (28-32 weeks)	**Treatments/ Groups**				**P-value**
	T_1	**T_2**	**T_3**	**T_4**	
	Basal diet (control)	**Basal diet+0.5% chili leaf powder**	**Basal diet+1.5% chili leaf powder**	**Basal diet+2.5% chili leaf powder**	
Feed intake (g)	120.83±0.37	120.01±0.12	120.19±0.27	119.97±0.19	1.14
Egg production (%)	59.33±0.50	60.12±0.50	60.12±0.50	60.91±0.50	0.96
FCR (kg feed/ dozen egg)	2.45±3.65	2.39±0.01	2.37±0.02	2.37±0.03	0.96
FCR (kg feed/ kg egg mass)	3.65±0.02	3.60±0.02	3.52±0.02	3.52±0.04	0.69

The average egg production of laying hens in several treatment groups, including T_1, T_2, T_3 and T_4 did not differ significantly over this period due to a diet supplemented with chilli (*Capsicum annuum*) leaf powder.

The average egg production percent during period 1 was 59.33±0.50, 60.12±0.50, 60.12±0.50 and 60.91±0.50 percent in laying hens of treatment groups T_1, T_2, T_3 and T_4, respectively. Although numerically, highest but statistically similar egg production was observed in T_4 group (60.91±0.50 percent), in which 2.5% chili powder was added in basal diet.

The average daily feed intake also did not differ significantly in different treatment groups and were found to be 120.83±0.37, 120.01±0.12, 120.19±0.27 and 119.97±0.19 g/day in treatment groups T_1, T_2, T_3 and T_4, respectively. However, maximum feed intake was recorded in T_1 (120.83±0.37) group.

The average feed conversion ratio (kg feed/ dozen eggs) in treatment groups T_1, T_2, T_3 and T_4 was 2.45±3.65, 2.39±0.01, 2.37±0.02 and 2.37±0.03, respectively with no statistically significant difference. Feed conversion ratio (kg feed/kg eggs) among various treatment groups was also statistically equivalent among the various treatment groups. The feed conversion ratio (kg feed/kg eggs) was 3.65±0.02, 3.60±0.02, 3.52±0.02 and 3.52±0.04 in T_1, T_2, T_3 and T_4 groups, respectively.

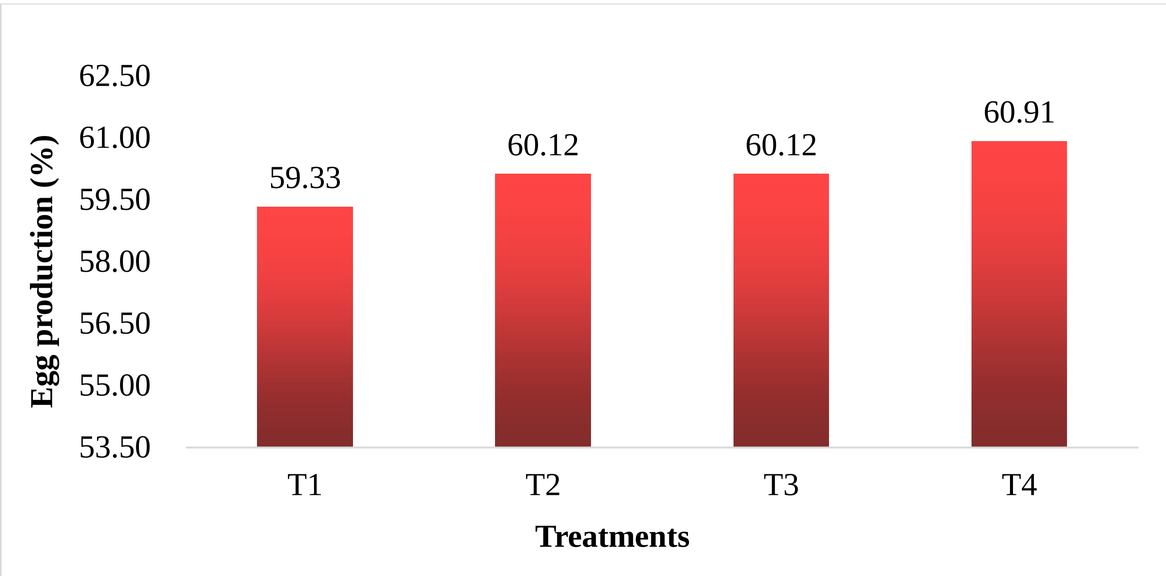

Fig. 1a: Average egg production of Rhode Island Red laying hens during period 1 (28-32 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

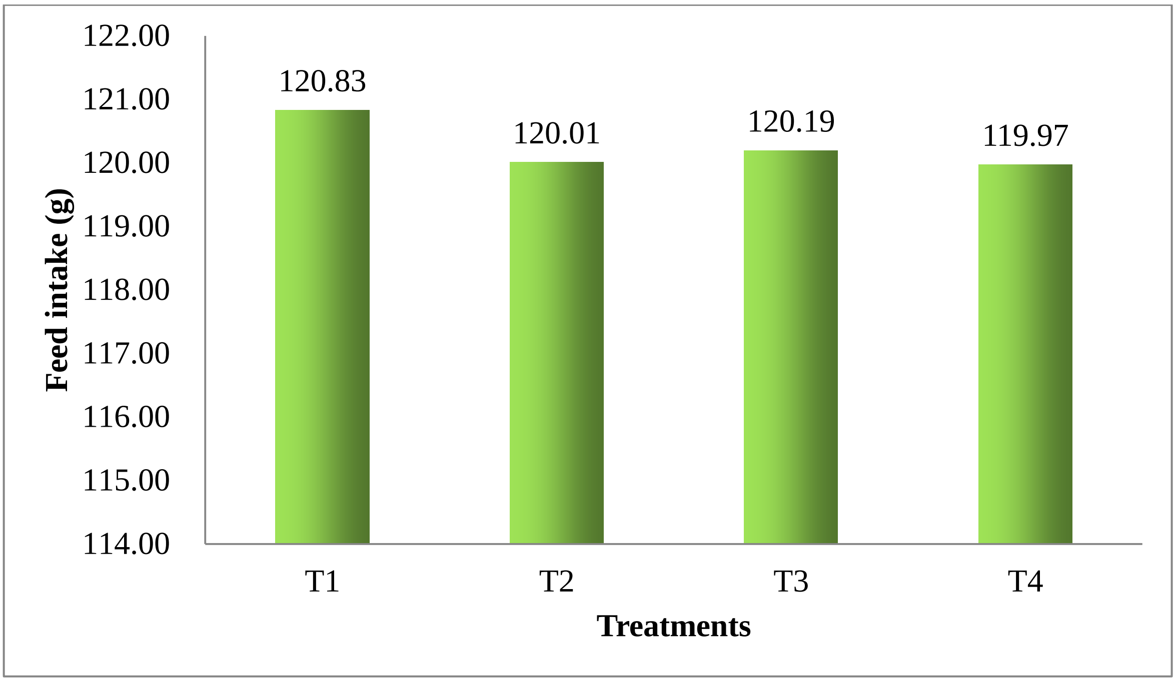

Fig. 1b: Average feed intake of Rhode Island Red laying hens during period 1 (28-32 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

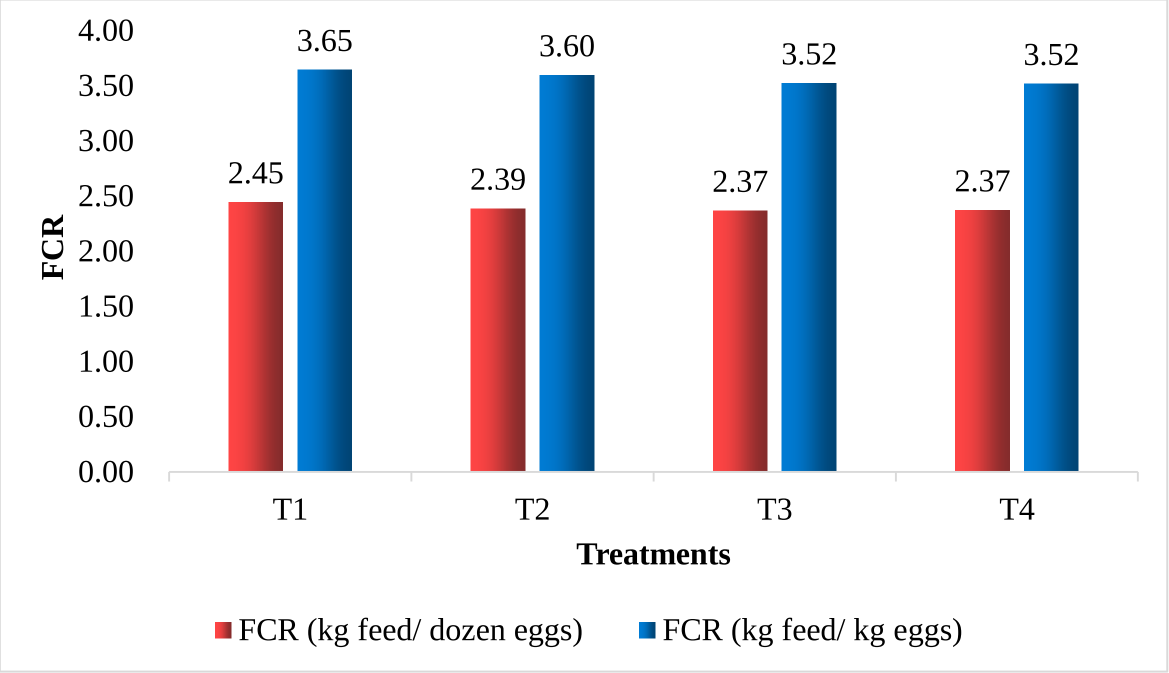

Fig. 1c: Average FCR (kg feed/ dozen eggs and kg feed/ kg eggs) of Rhode Island Red laying hens during period 1 (28-32 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.3.3.2 Period 2 (32-36 weeks)

The average egg production, feed intake and feed conversion ratio in Rhode Island Red laying hen during period 2 **(**32-36 weeks of age) of experimental feeding trial are presented in Table 4.7 and Figure 2a, 2b and 2c.

Table 4.7: Average production performance of Rhode Island Red laying hens during period 2 (32-36 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Period (32-36 weeks)	Treatments/Groups				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Feed intake (g)	119.81±0.30	120.07±0.46	119.90±0.31	119.55±0.18	1.08
Egg production (%)	61.11±0.46	61.51±0.76	62.30±0.51	63.69±0.68	0.98
FCR (kg feed/ dozen egg)	2.35±0.23	2.34±0.35	2.31±0.21	2.25±0.23	0.98
FCR (kg feed/ egg mass)	3.54±0.3	3.53±0.5	3.44±0.3	3.34±0.3	0.68

There was no significant difference in average egg production between treatment groups over the 32–36 weeks period. The average egg production during this phase was 61.11±0.46, 61.51±0.76, 62.30±0.51 and 63.69±0.68 percent in laying hens of treatment groups T_1, T_2, T_3 and T_4, respectively.

The average feed intake, feed conversion ratio (kg feed/dozen eggs), and feed conversion ratio (kg feed/kg eggs) were also not significantly different across the T_1, T_2, T_3 and T_4 treatment groups. The average feed intake (g/day) was 119.81±0.30, 120.07±0.46, 119.90±0.31 and 119.55±0.18, during this period in treatment groups T_1, T_2, T_3 and T_4, respectively. Although the differences were non- significant, but, numerically, highest feed intake during this period also was found in T_2 group (120.07±0.46 g/day). Feed conversion ratio (kg feed/dozen eggs) was 2.35±0.23, 2.34±0.35, 2.31±0.21, 2.25±0.23 in treatment groups T_1, T_2, T_3 and T_4 respectively. Feed conversion ratio (kg feed/kg eggs) among various treatment groups T_1, T_2, T_3 and T_4 was 3.54±0.30, 3.53±0.50, 3.44±0.30and 3.34±0.30, respectively. However, differences were non-significant among various treatment groups, but, numerically, better feed conversion ratio was seen in T_4 (3.34±0.3) group.

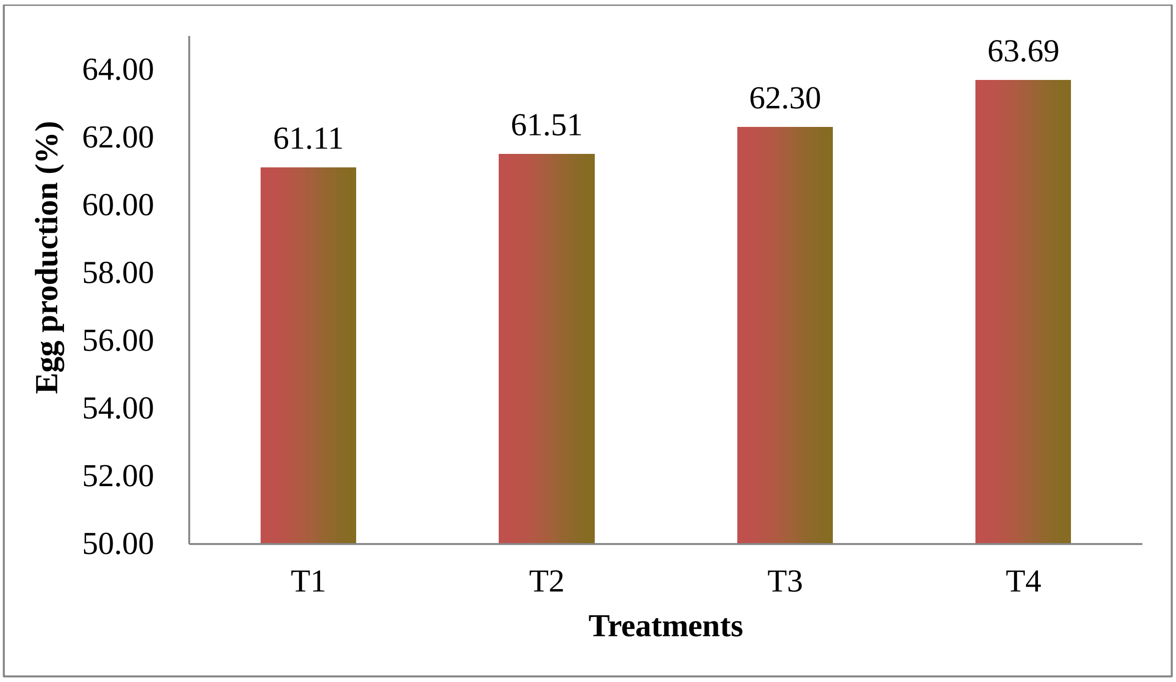

Fig. 2a: Average egg production of Rhode Island Red laying hens during period 2 (32-36 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

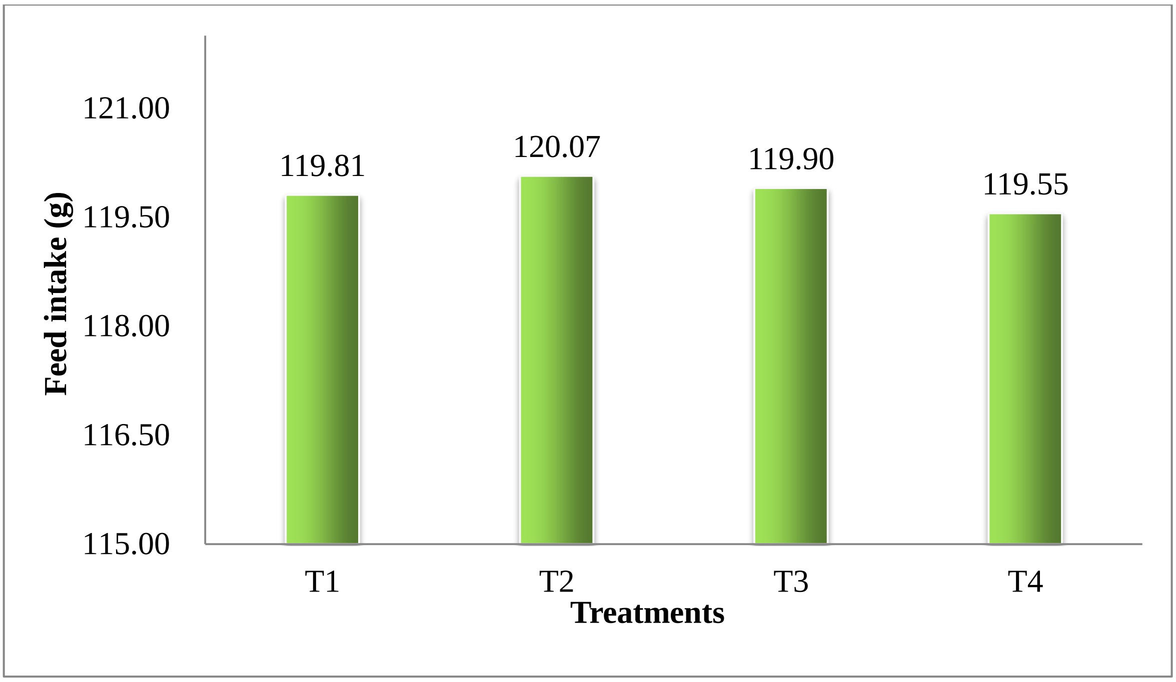

Fig. 2b: Average feed intake of Rhode Island Red laying hens during period 2 (32-36 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

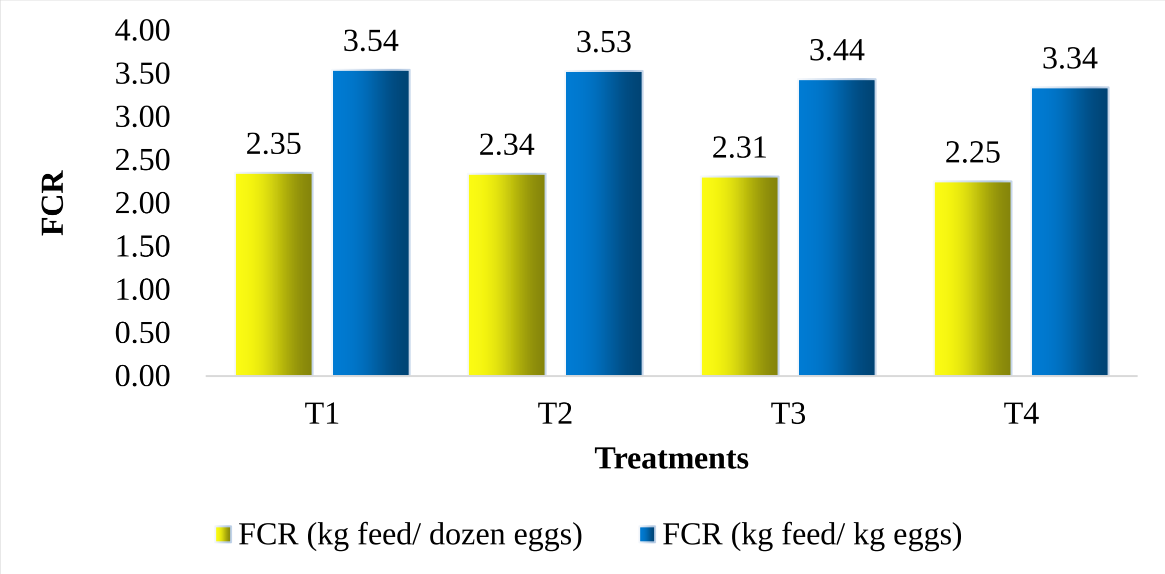

Fig. 2c: Average FCR (kg feed/ dozen eggs and kg feed/ kg eggs) of Rhode Island Red laying hens during period 2 (32-36 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.3.3.3 Period 3 (36-40 weeks)

The average egg production, feed intake and feed conversion ratio in Rhode Island Red layers during period 3 (36-40 weeks) of feeding trial are presented in Table 4.8 and Figure 3a, 3b and 3c.

Table 4.8: Average production performance of Rhode Island Red laying hens during period 3 (36-40 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Period (36-40 weeks)	Treatments/Groups ±				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Feed intake (g)*	120.55^{b}±0.30	120.36^{b}±0.39	119.83^{ab}±0.26	118.83^{a}±0.23	0.03
Egg production (%)	67.06±1.00	67.86±1.00	68.45±1.04	69.25±1.04	0.98
FCR (kg/dozen egg)	2.16±0.29	2.13±0.28	2.10±0.32	2.06±0.34	0.98
FCR (kg feed/kg egg mass)	3.25±0.40	3.21±0.40	3.13±0.50	3.06±0.50	0.89

[a, b] Mean values bearing different superscripts in a row differ significantly, *P≤0.05.

The average egg production during period 3 (36-40 weeks)was 67.06±1.00, 67.86±1.00, 68.45±1.04 and 69.25±1.04 percent in laying hens of treatment groups T_1, T_2, T_3 and T_4, respectively. The above findings suggested that there was no any significant difference in average egg production among various treatments fed diet supplemented with chili (*Capsicum annuum*) leaf powder. It was also found that, numerically, average egg production in the treatment group T_4 was higher than control group.

The average feed intake due to diet supplemented with chili (*Capsicum annuum*) leaf powder in treatment groups T_1, T_2, T_3 and T_4 groups was 120.55±0.30, 120.36±0.39, 119.83±0.26 and 118.83±0.23 g/day, respectively. As per above findings, it was found that average feed intake of laying hen of group T_4 was significantly ($P<0.05$) reduced than treatment groups T_1, T_2 and T_3. However, no significant difference was observed between groups T_2 and T_3. Average feed intake was also similar among the groups T_1, T_2 and T_3.

Feed conversion ratio (kg feed/dozen eggs), and feed conversion ratio (kg feed/kg eggs) due to supplemented with chili (*Capsicum annuum*) leaf powder were also not significantly different across the T_1, T_2, T_3 and T_4 treatment groups. Feed conversion ratio (kg feed/dozen eggs) in treatment groups T_1, T_2, T_3 and T_4 was 2.16±0.29, 2.13±0.28, 2.10±0.32 and 2.06±0.34 whereas average feed conversion ratio (kg feed/kg eggs) was 3.25±0.40, 3.21±0.40, 3.13±0.50 and 3.06±0.50, respectively.

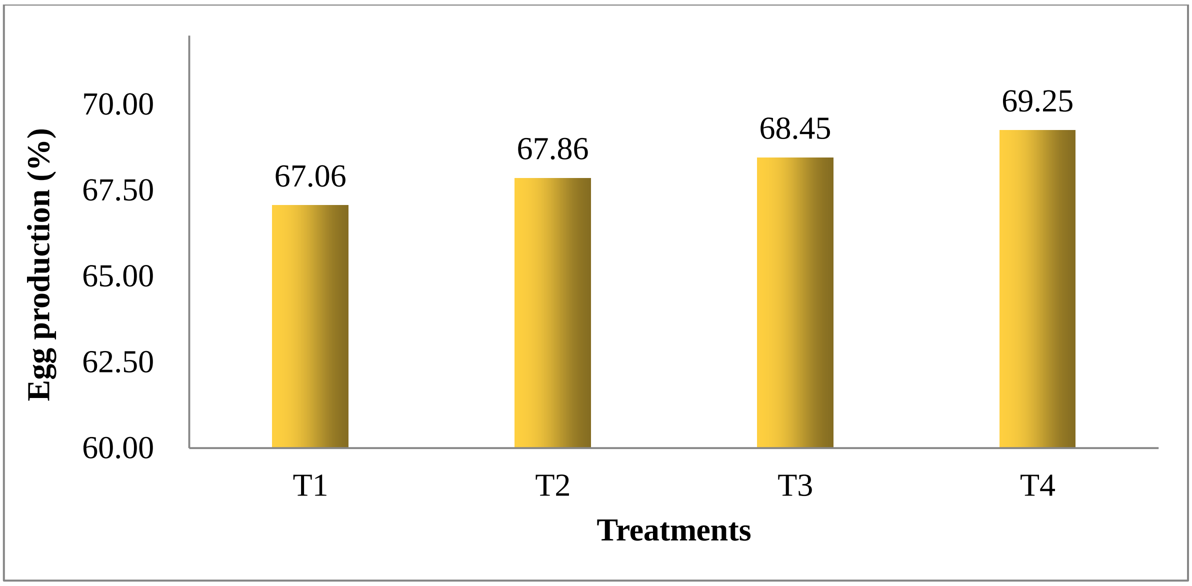

Fig. 3a: Average Egg production% Rhode Island Red laying hens during period 3 (36-40 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

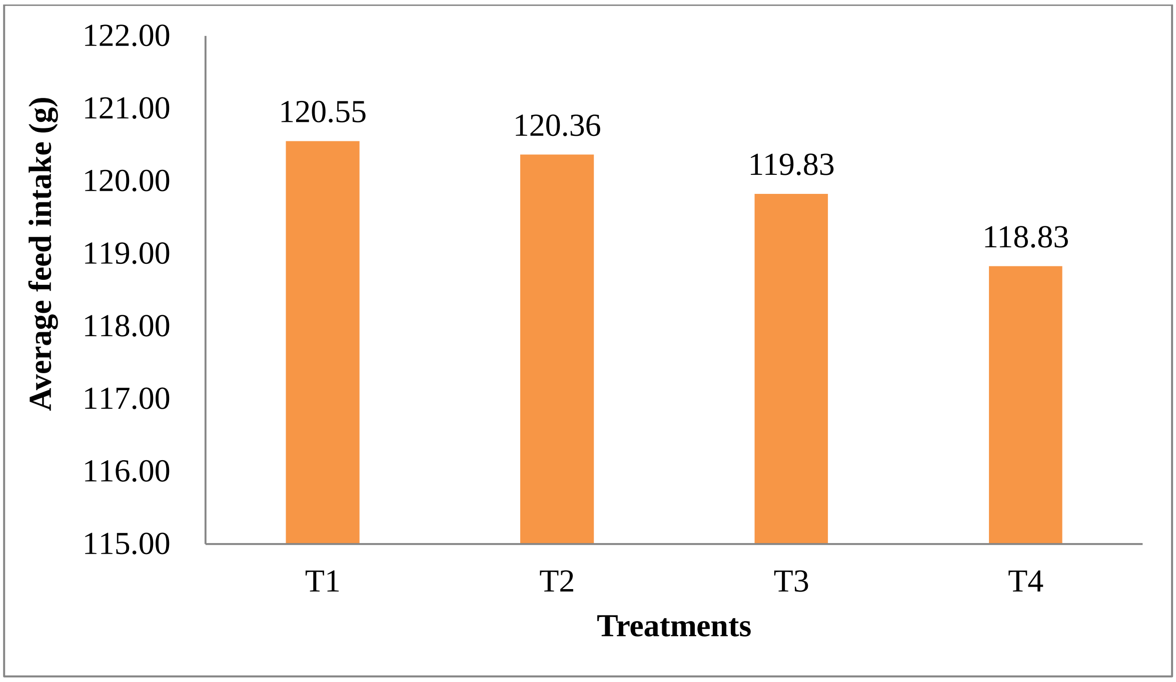

Fig. 3b: Average feed intake of Rhode Island Red laying hens during period 3 (36-40 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

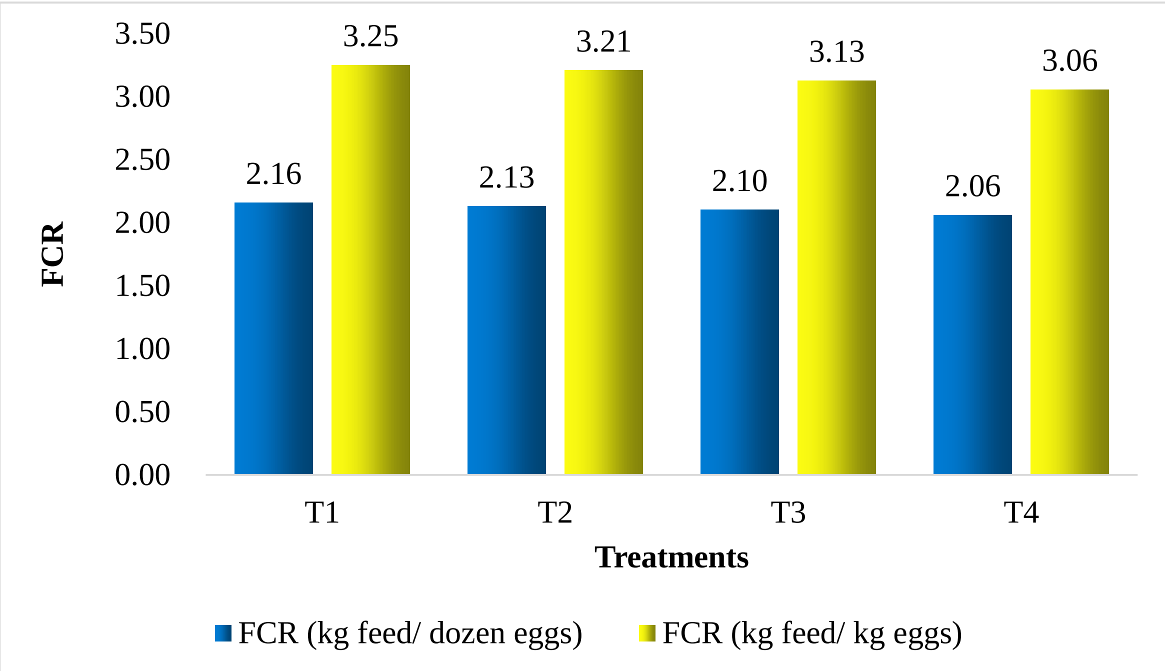

Fig. 3c: Average FCR (kg feed/ dozen eggs and kg feed/ kg eggs) of Rhode Island Red laying hens during period 3 (36-40 weeks) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.3.3.4 Overall period (28-40)

Table 4.9 shows the overall average production performance of Rhode Island Red laying hens during 28-40 weeks feeding trial in terms of egg production, feed intake, feed conversion ratio (kg/dozen eggs and kg/kg eggs), egg weight and body weight gain of different groups, presented in Figure 4a, 4b and 4c.

Table 4.9: Average production performance of Rhode Island Red laying hens from 28-40 weeks period fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Period (28-40 weeks)	Treatments/Groups				P-value
	T_1 Basal diet (control)	T_2 Basal diet+0.5% chili leaf powder	T_3 Basal diet+1.5% chili leaf powder	T_4 Basal diet+2.5% chili leaf powder	
Feed intake (g)*	120.40^{b}±0.31	120.15^{ab}±0.11	119.97^{ab}±0.11	119.45^{a}±0.33	0.03
Egg production (%)	62.50±2.34	63.16±2.38	63.62±2.49	64.62±2.45	0.95
FCR (kg/dozen egg)	2.32±0.08	2.29±0.08	2.26±0.08	2.23±0.09	0.95
FCR (per kg egg mass)	3.48±0.12	3.45±0.12	3.36±0.12	3.31±0.13	0.86

$^{a,\,b}$ Mean values bearing different superscripts in a row differ significantly, *P≤0.05

The overall average egg production in the feeding study did not differ significantly between treatment groups. The average egg production for overall period was 62.50±2.34, 63.16±2.38, 63.62±2.49 and 64.62±2.45 percent in laying hens of treatment groups T_1, T_2, T_3 and T_4, respectively. However, overall egg production during the experimental feeding period of 12 weeks, was slightly increased in T_4 group where 2.5% chili leaf powder was added into basal diet.

The average feed intake due to diet supplemented with chili (*Capsicum annuum*) leaf powder in treatment groups T_1, T_2, T_3 and T_4 groups was 120.40±0.31, 120.15±0.11, 119.97±0.11 and 119.45±0.33 g/day, respectively. As per above findings, it was found that average feed intake of laying hen of group T_4 was significantly ($P<0.05$) decreased than treatment group T_1. However, feed intake was statistically similar among the treatment groups T_1, T_2 and T_3.

The average feed conversion ratio (kg feed/dozen eggs) for overall period in treatment groups T_1, T_2, T_3 and T_4 was 2.32±0.08, 2.29±0.08, 2.26±0.08 and 2.23±0.09, respectively and did not differ significantly among treatment groups. The average feed conversion ratio (kg feed/kg eggs) for the entire study period revealed no significant differences between treatment groups. The average feed conversion ratio (kg feed/kg eggs) for the overall trial period was 3.48±0.12, 3.45±0.12, 3.36±0.12 and 3.31±0.13 in groups T_1, T_2, T_3 and T_4, respectively.

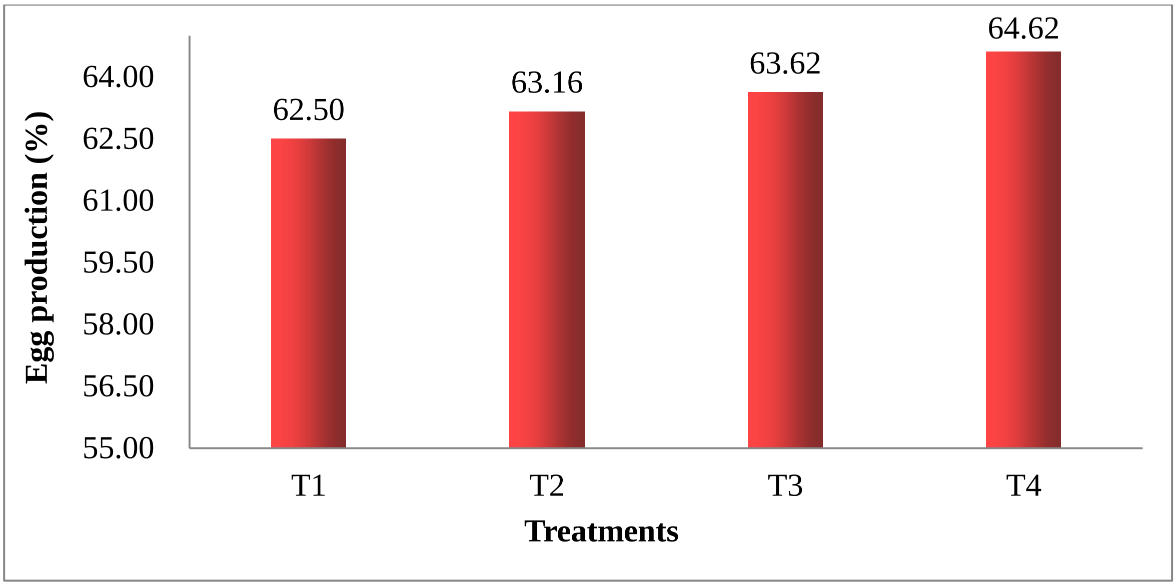

Fig. 4a: Average egg production % of Rhode Island Red laying hens from 28-40 weeks (overall) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

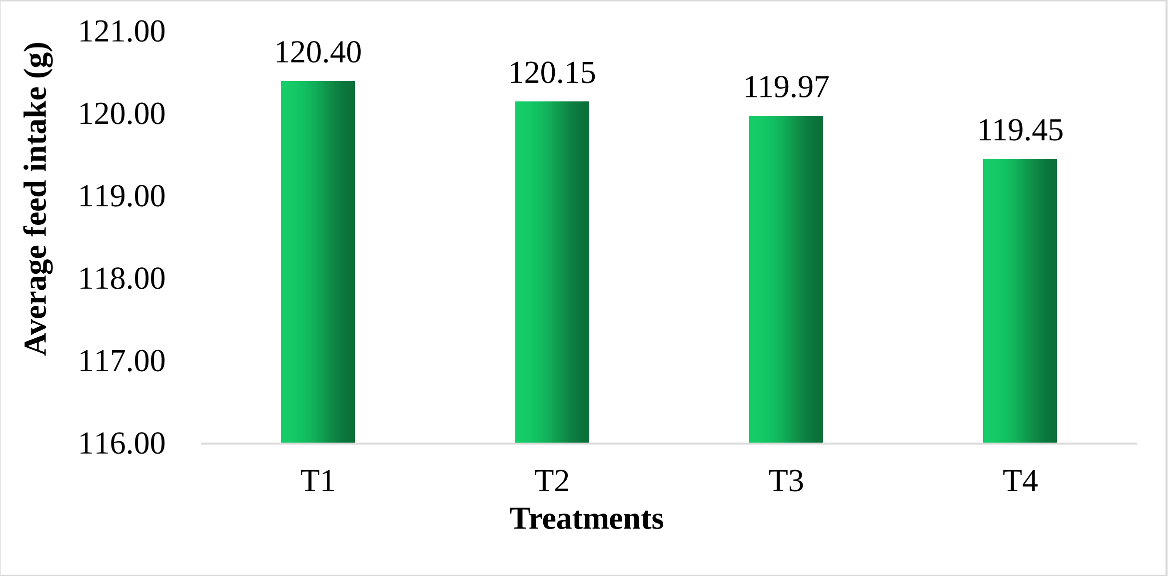

Fig. 4b: Average feed intake of Rhode Island Red laying hens from 28-40 weeks period fed diet supplemented with chili (*Capsicum annuum*) leaf powder

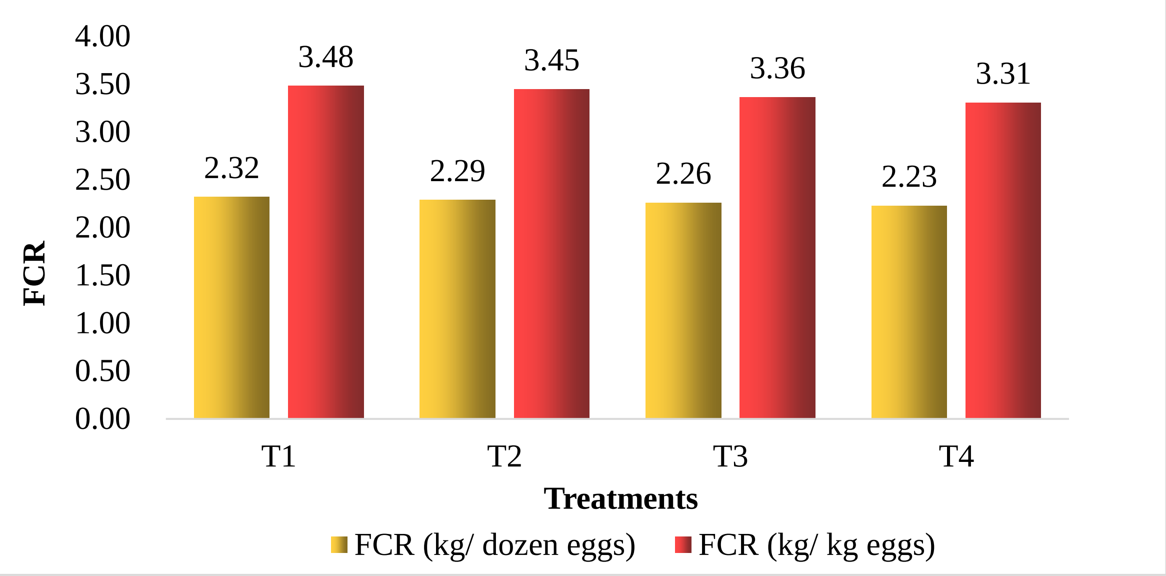

Fig. 4c: Average FCR (kg feed/ dozen eggs and kg feed/ kg eggs) of Rhode Island Red laying hens from 28-40 weeks (overall) fed diet supplemented with chili (*Capsicum annuum*) leaf powder

The present findings are in accordance with **Lokaewmanee (2019),** who reported that supplementing laying hens with dietary chili leaf powder at a concentration of 3% reduced feed intake (P<0.05) and feed conversion ratios when compared to a control and dietary chili leaf powder at a concentration of 1% which are corroborated with the present study. The improvement in the feed conversion ration may be attributed to the antioxidant capabilities of chili leaf **(Lokaewmanee (2019)**. Capsaicin has been proved to have antioxidant properties **(Liu *et al*., 2012).** Capsaicin had a significant free radical scavenging activity in vitro **Liu *et al*. (2015)**. Free radical-induced oxidative degradation, carbonylation modification, nitration modification, lipid peroxidation, and DNA oxidative damage have all been shown to be inhibited by capsaicin **(Zhang *et al*., 2017)**. Capsaicin's phenolic hydroxyl group can transfer hydrogen atoms from free radicals, thus lowering their activity **(Tsai *et al*., 2006)**. Furthermore, phenolic hydroxyl could react with metal ions to prevent the formation of free radicals. Metal ions are essential for the formation of free radicals **(Hua *et al*., 2010)**. As a result, they hypothesised that capsaicin's phenolic structure was responsible for its antioxidant properties.

In contrary, **Liu *et al*. (2021)** found no significance effect on daily feed intake and feed conversion ratio in broiler chickens supplemented with capsaicin. **Filik *et al*. (2020)** found that similar non-significant effect on feed conversion ratio and egg yield in Japanese quail. While, **Shahverdi *et al.* (2013)** found significant increase in feed intake in broiler chickens supplemented with red pepper.

Lokaewmanee (2019) found a non-significant effect on egg production in laying hens supplemented with chili leaf powder. **Liu *et al*. (2021)** also found non-significant effect on egg production in laying hens supplemented with capsaicin which is corroborated with present study.

In contrast, **Saleh *et al.* (2021)** who reported that egg production of laying hens supplemented with paprika powder was significantly improved. **Liu *et al*. (2021)** they observed that that dietary capsaicin supplementation at the rate of 150 mg/kg improved egg production performance, most likely via activating the calcium signalling pathway and increasing redox state. **Filik *et al*. (2020)** found significant increase in egg production of Japanese quail among all the hot pepper (*Capsicum annuum*) waste supplemented groups.

4.4 Nutrient Utilization

The data on average values of nutrient utilization of Rhode Island Red laying hens during the metabolic trial period, in terms of dry matter, crude protein, ether extract and organic matter fed diet supplemented with chili (*Capsicum annuum*) leaf powder presented in table 4.10 and Figure 5a, 5b, 5c and 5d.

Table 4.10: Average values of nutrient utilization (%) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*)

Nutrients	Treatments/Groups				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
DM*	$68.05^{b}\pm1.05$	$71.60^{ab}\pm1.46$	$72.18^{ab}\pm0.51$	$73.74^{a}\pm0.66$	0.02
CP*	$82.01^{b}\pm0.59$	$83.91^{ab}\pm0.83$	$84.22^{a}\pm0.29$	$82.57^{ab}\pm0.44$	0.05
EE	80.66±0.64	81.96±1.03	81.75±0.34	81.64±0.46	0.55
OM*	$72.20^{b}\pm0.93$	$73.24^{ab}\pm1.38$	$74.18^{ab}\pm0.48$	$75.18^{a}\pm1.48$	0.16

$^{a, b}$ Mean values bearing different superscripts in a row differ significantly,*P≤0.05.

4.4.1 Dry matter utilization

The dry matter utilization in laying hens of different treatment groups T_1, T_2, T_3 and T_4 during the metabolic trail was 68.05±1.05, 71.60±1.46, 72.18±0.51 and 73.74±0.66 percent, respectively. Dry matter utilization was significantly (P<0.05) increased in T_4 group (73.74±0.66 percent) compared to T_1 (68.05±1.05 percent) group. However, there was no statistically significant difference in dry matter utilization between the T_1, T_2, and T_3 groups.

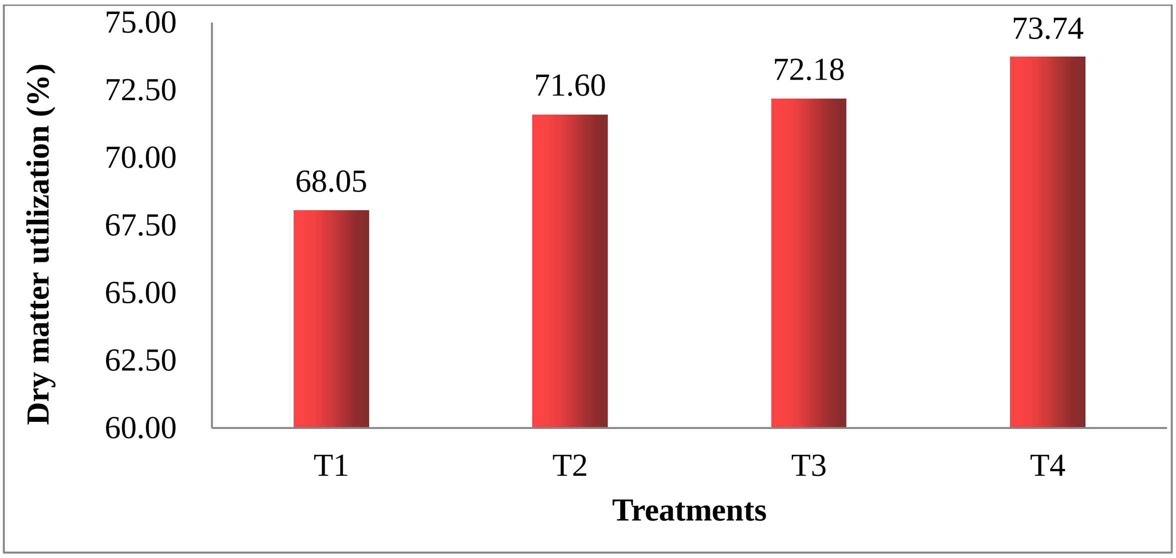

Fig. 5a: Average values of dry matter utilization (%) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.4.2 Crude protein utilization

The utilization of crude protein in Rhode Island Red laying hens due to diet supplemented with chili (*Capsicum annuum*) leaf powder was 82.01±0.59, 83.91±0.83, 84.22±0.29 and 82.57±0.44 percent, respectively, in treatment groups T_1, T_2, T_3 and T_4. Crude protein utilization was significantly ($P<0.05$) increased in T_3 group (84.22±0.29 percent) in which hens were fed basal diet along with 1.5% chili leaf powder compared to T_1 (82.01±0.59 percent) group. However, there was no significant difference in crude protein utilization between the T_1, T_2, and T_4 groups.

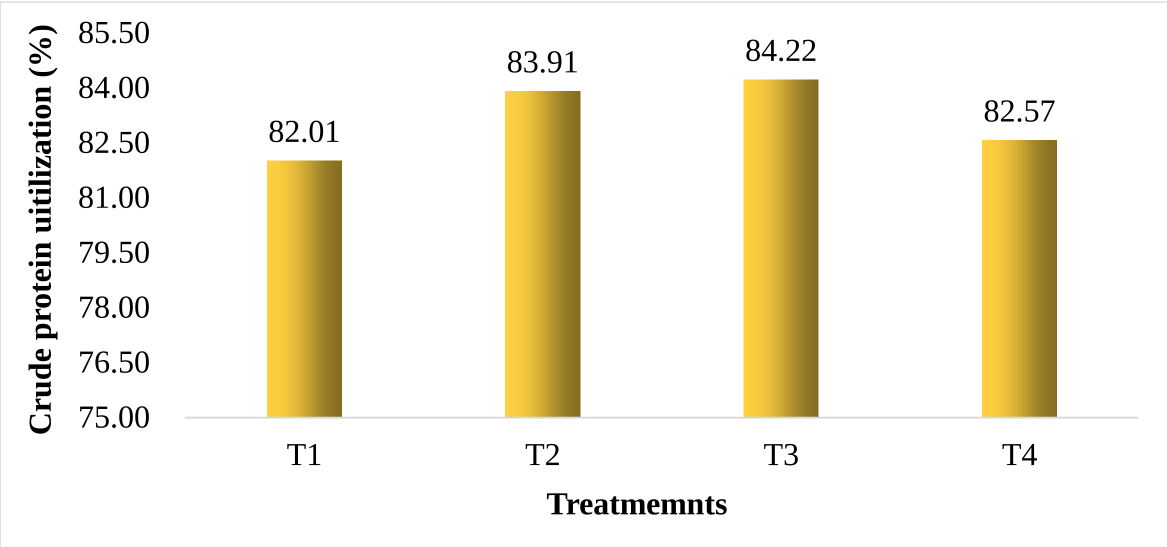

Fig. 5b: Average values of crude protein utilization (%) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder4.4.3 Ether extract utilization

4.4.3 Ether extract utilization

The ether extract utilization for treatment groups T_1, T_2, T_3 and T_4 were 80.66±0.64, 81.96±1.03, 81.75±0.34 and 81.64±0.46 percent, respectively. No significant difference was found in the utilization of ether extract among the various treatment groups.

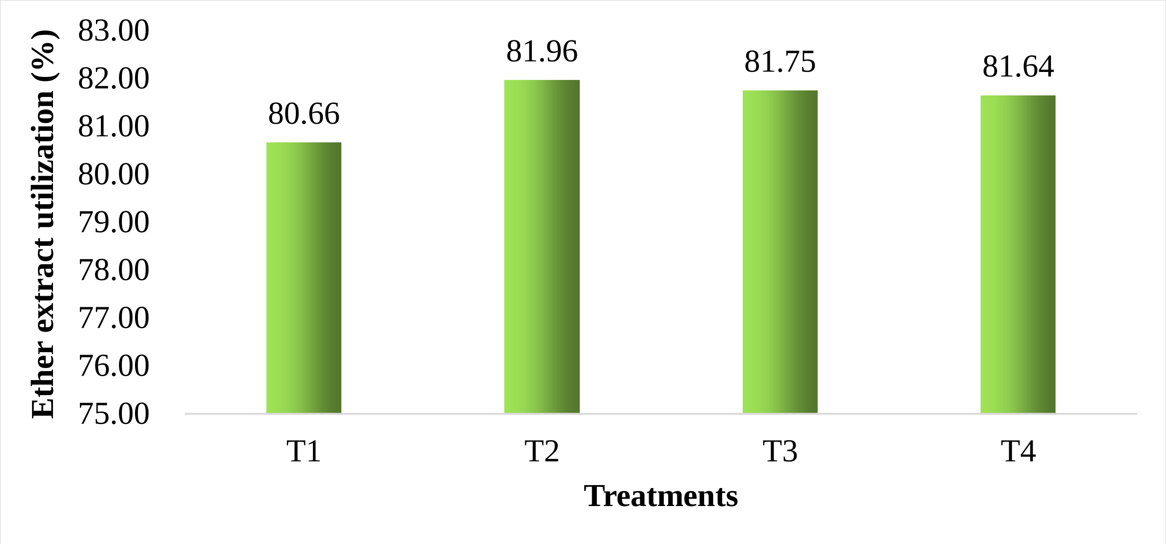

Fig. 5c: Average values of ether extract utilization (%) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.4.4 Organic matter utilization

The Organic utilization in laying hens of different treatment groups T_1, T_2, T_3 and T_4, during the metabolic trail was 72.20±0.93, 73.24±1.38, 74.18±0.48 and 75.18±1.48 percent, respectively. Organic matter utilization was significantly ($P<0.05$) increased in T_4 group (75.18±1.48 percent) compared to T_1 (72.20±0.93 percent) group. However, there was no statistically significant difference in Organic matter utilization between the T_1, T_2, and T_3 groups

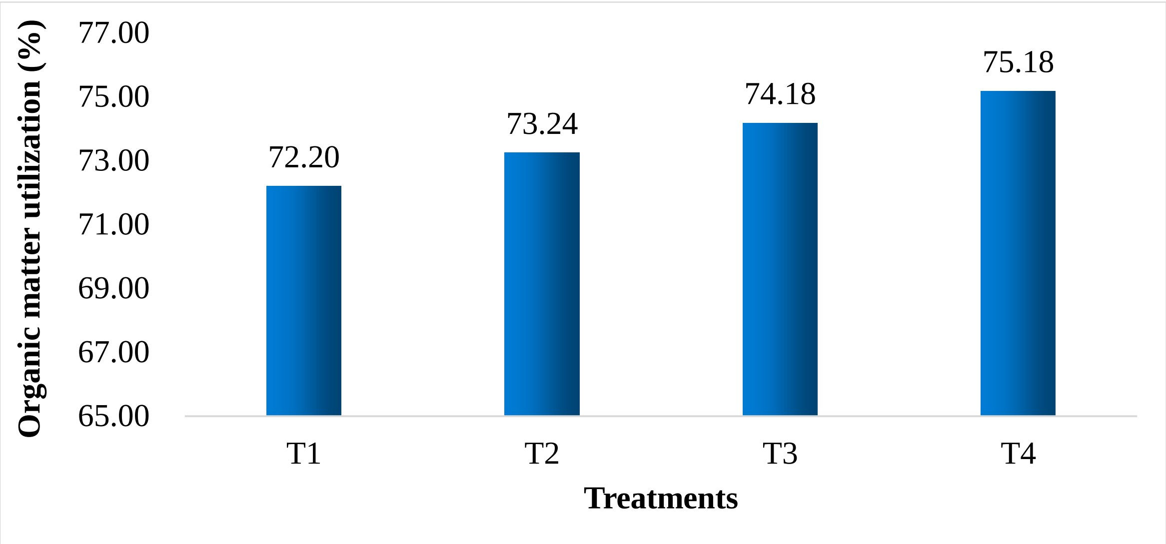

Fig. 5d: Average values of organic matter utilization (%) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

The results of present study are in corroboration with the findings of **Liu *et al*. (2021)** who noted a significance increase in utilization of organic matter and crude protein in Arbor Acre male broiler chickens supplemented with natural capsaicin extract at the rate of 80 mg/kg which is in agreement with the findings of the present study. **Li *et al*. (2022)** who observed that nutrient utilization was improved when broiler chicken was fed diet supplemented with natural capsaicin extract at the level of 2 and 4 mg/kg. **Ali *et al*. (2016)** reported that a significance increase in nutrient utilization was found in ducks supplemented with capsaicin at the level of 100 ppm to 150 ppm. **Atapattu and Belpagodagamage (2011)** hypothesised that chili powder improved the digestion and thereby the growth performance of broilers when broiler chickens were fed diet supplemented with 5% chili powder is increased.

Traditionally, herbs and spices have been used to help digestion by stimulating the synthesis of endogenous secretions in the small intestine mucosa, pancreas and liver. Bioactive compound such as capsaicin present in chili leaves can improve gut function by enhancing digestive enzyme activities such as pancreatic lipase and trypsin enzyme. **(Liu *et al*., 2021)**. They also theorised that higher digestibility of crude protein was also linked with the decrease of serum urea-nitrogen concentration in broilers. As a stressor, a high level of serum urea-nitrogen was thought to have negative impacts on broiler growth performance **(Glantzounis *et al*., 2005)**. Plant extract containing capsaicin improved crude protein digestibility in sows during the first week of lactation **(Ilsley *et al*., 2003)**, and the addition of plant extract resulted in increased dry matter and organic matter digestibility during this period. **Jamroz *et al.* (2005)** also found that broiler chickens fed a plant extract containing 3% capsaicin had higher crude fibre and protein digestibility than broiler chickens in the control group. The increase in digestive enzyme activity could be linked to the improvement in nutritional digestibility. **Platel and Srinivasan (2000)** also found that adding 1.5 mg/kg capsaicin to Wistar rats' diets increased pancreatic lipase and trypsin activity considerably.

The increase in endogenous cholecystokinin levels could be the underlying mechanism for higher digestive enzyme activity. Chronic cholecystokinin activation has been shown to improve pancreatic functioning, and capsaicin has been proved to increase cholecystokinin concentration by stimulating the capsaicin-sensitive afferent vagal pathway **(Otsuki *et al*., 1983)**. Supplementing dietary capsaicin may boost digestive enzymes, bile acids and endogenous cholecystokinin in the gut, all of which are necessary for efficient nutritional digestion. According to the findings, dietary capsaicin supplementation of 2 or 4 mg/kg could boost the lipase activity of jejunal and ileal contents, as well as trypsin and amylase activity of jejunal and ileal contents. Endogenous digestive enzymes serve a critical part in the digestion process. Amylase, lipase and trypsin are enzymes that aid in the breakdown, digestion, and absorption of carbohydrates, protein and lipids **(Li *et al.*, 2022).** Hence capsaicin could lead to increased nutrient utilization, resulting in improved growth and feed efficiency.

Literature is available on feeding of chili fruit which states that it contains capsaicin as its bioactive component which may increase digestive enzyme activity and ultimately nutrient utilization in laying hens. However, not much study has been done on feeding of chili leaves in poultry despite capsaicin’s ability to improve nutritional digestibility. Thus, there may be probability that leaves of chili contain capsaicin as well which may improve nutrient utilization in laying hens resulting in improved growth performance and feed efficiency.

4.5 Egg quality parameters

Average values of external egg parameters of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.11, 4.12 and 4.13.

Table 4.11: Average values of external egg parameters of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Parameters	Treatments/Groups				P- value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Egg wt. (g)	55.33±0.15	55.33±0.29	56.02±0.63	56.19±0.85	0.59
Shape index (%)	77.52±0.21	77.55±0.33	77.59±0.09	77.81±0.48	0.92
Shell wt. (g)	5.25±0.06	5.26±0.06	5.34±0.16	5.35±0.09	0.85
Shell wt. (%)	9.49±0.12	9.61±0.13	9.54±0.31	9.53±0.21	0.98
Shell thickness (mm)	0.40±0.04	0.39±0.06	0.38±0.06	0.37±0.00	0.85

4.5.1 Egg weight

Average values of egg weight among various treatment groups in presented in table 4.11. Egg weight obtained from different treatment groups T_1, T_2, T_3 and T_4 was 55.33±0.15, 55.33±0.29, 56.02±0.63 and 56.02±0.63g, respectively. No significant difference was found in egg weight among the various treatment groups.

These findings are in corroboration with **Lokaewmanee *et al.* (2019**), **Saleh *et al.* (2021)**, who reported non- significant effect on egg weight by supplementation of chili leaves in diets of laying hens at different levels. **Liu *et al*. (2021)** observed non-significant effect on egg weight in broiler chickens supplemented with natural capsaicin extract. **Filik *et al*. (2020)** found no significant effect on egg weight of Japanese quail supplemented with hot pepper *(Capsicum annuum)* waste powder at 1 and 4 g/kg diet. However, egg weight was increased when hot pepper *(Capsicum annuum)* waste powder was added into quail's diet at 2 g/kg diet.

4.5.2 Egg shell quality

Table 4.11 shows the average values of shape index, shell weight (gm), shell weight % and shell thickness (mm) in this investigation. The average shape index for the treatment groups T_1, T_2, T_3 and T_4 was 77.52±0.21, 77.55±0.33, 77.59±0.09 and 77.81±0.48percent, respectively and did not differ significantly among the treatment groups (Table 4.11).

No significant difference was found between the average values of shell weight, shell weight percentage and shell thickness among the various treatment groups (Table 4.11). Average values of shell weight were 5.25±0.06, 5.26±0.06, 5.34±0.16 and 5.35±0.09g and shell weight percentage were 9.49±0.12, 9.61±0.13, 9.54±0.31and 9.53±0.21 percent, in treatment groups T_1, T_2, T_3 and T_4, respectively. Shell thickness as measured among various treatment groups T_1, T_2, T_3 and T_4 came out to be 0.40±0.04, 0.39±0.06, 0.38±0.06 and 0.37±0.00mm, respectively. The average values of shell weight, shell weight percentage and shell thickness were found to be similar in all the treatment groups.

Results of present study for external egg quality are in similar line as observed by **Lokaewmanee *et al.* (2019**), found a non-significant effect on egg shell quality when laying hens fed diet supplemented with capsaicin. **Saleh *et al.* (2021)**

noted that there was no significance effect on shell weight which is in agreement with the present study.

However, **Filik *et al*. (2020)** found significant decline in shell thickness of Japanese quail egg among all the hot pepper (*Capsicum annuum)* waste supplemented groups. Fat burning in the body was the probable cause of the reduction in shell resistance in the hot pepper waste supplementation group compared to the control group. Vitamin D, calcium and phosphorus are essential for the production of the eggshell and they are transported throughout the body through fat. Because supplementing with hot pepper waste speeds up fat burning, it hinders the body from absorbing the necessary amounts of Vitamin D, calcium, and phosphorus for the production of the eggshell.

Hence, from the above results it can be concluded that supplementation of chili leaf powder had no adverse effect on the egg shell quality of the eggs.

4.5.3 Egg albumin quality

The average values of albumen quality and haugh unit of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.12.

Table 4.12: Average values of albumen quality and haugh unit of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Parameters	Treatments/Groups				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Albumen height (mm)	6.60±0.10	6.57±0.06	6.58±0.15	6.65±0.10	0.95
Albumen weight (gm)	32.85±0.19	32.78±0.37	33.25±0.65	33.38±0.88	0.85
Albumen weight (%)	59.37±0.33	59.25±0.41	59.34±0.51	59.39±0.73	0.99
Haugh unit	83.13±0.07	82.81±0.26	83.01±0.35	83.07±0.31	0.84

Albumen height of various treatment groups T_1, T_2, T_3 and T_4 was found to be 6.60±0.10, 6.57±0.06, 6.58±0.15 and 6.65±0.10 mm, respectively. According to the data, there was no statistically significant difference in the albumen height of eggs collected in different treatment groups, although it was numerically higher in all treatment groups when compared to the control group. The weight of albumen did not vary significantly across the treatment groups. The values of albumen weight (g) was 32.85±0.19, 32.78±0.37, 33.25±0.65 and 33.38±0.88 g, while for albumen weight (%) was 59.37±0.33, 59.25±0.41, 59.34±0.51 and 59.39±0.73 percent, among the various treatment groups T_1, T_2, T_3 and T_4, respectively.

The values for haugh unit were 83.13±0.07, 82.81±0.26, 83.01±0.35 and 83.07±0.31 for treatment groups T_1, T_2, T_3 and T_4, respectively. Although the results were statistically similar, there was no significant difference between the treated groups. Similar non- significant effect on albumen weight was reported by **Lokaewmanee *et al.* (2019**) who noted that albumen percentage, haugh unit were not significantly affected by the supplementation of chili leaves in diet fed to laying hens, **Rossi *et al.* (2015**) who reported that no significance difference was found in albumen height, haugh unit, albumen weight, albumen percentage by the addition of chili in diet of laying hens and **Filik *et al.* (2020)** who observed that albumen height, haugh unit, albumen weight, albumen percentage were not significantly affected when hot pepper (*Capsicum annuum*) power was supplemented in basal diet of laying hens. The results of albumin quality and haugh unit are in line with these findings. In contrary, **Saleh *et al*. (2021)** reported significant increase in albumin width while decrease in Albumin weight and albumin height with increased dietary supplementation of paprika powder.

Hence, from the above results it can be concluded that supplementation of chili leaf powder had no adverse effect on the albumen quality of the eggs.

4.5.4 Egg yolk quality

The average values of yolk weight, yolk weight percentage and yolk index of Rhode Island Red laying hens fed dietsupplemented with chili (*Capsicum annuum*) leaf powder presented in Table 4.13.

Table 4.13: Average values of egg yolk quality of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Parameters	Treatments/Groups				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Yolk weight (gm)	17.23±0.17	17.28±0.13	17.43±0.10	17.46±0.32	0.81
Yolk weight (%)	31.13±0.26	31.23±0.28	30.83±0.95	30.55±0.35	0.81
Yolk index (%)	43.82±0.30	43.89±0.83	43.91±0.10	43.92±0.75	0.99
Yolk colour*	6.35^{b}±0.66	6.20^{b}±0.15	6.60^{b}± 0.14	8.43^{a}±0.13	0.01
Yolk cholesterol (mg/g)	14.49±0.22	14.41±0.23	13.94±0.35	13.23±0.81	0.43
Yolk triglyceride (mg/g)	203.61±0.05	202.12±0.29	201.95±0.30	201.16±0.28	0.33

$^{a, b, c}$Mean values bearing different superscripts in a row differ significantly, *P≤0.05

The average values of yolk weight, yolk weight percentage and yolk index of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder leaf powder have been presented in Table 4.13.

The results suggested non- significant effect due to diet incorporated with chili (*Capsicum annuum*) leaf powder on yolk weight and yolk weight percent among various treatment groups T_1, T_2, T_3 and T_4. The average value of yolk weight (grams) was 17.23±0.17, 17.28±0.13, 17.43±0.10 and 17.46±0.32 g, while yolk weight percent was 31.13±0.26, 31.23±0.28, 30.83±0.95 and 30.55±0.35 in treatment groups T_1, T_2, T_3 and T_4, respectively. Yolk index of different treatment groups T_1, T_2, T_3 and T_4 was 43.82±0.30, 43.89±0.83, 43.91±0.10and 43.92±0.75 %, respectively, also did not differ significantly.

The present findings are in accordance with **Lokaewmanee *et al.* (2019**) who reported that yolk weight was not significantly affected by addition of chili leaves in diet of laying hens, **Saleh *et al.* (2021)** found no significant difference in yolk weight and yolk height with dietary supplementation of paprika powder, **Filik *et al.* (2020)** who observed that yolk weight and yolk index were not significantly changed by supplementation of hot pepper (*Capsicum annuum)*waste powder in the diet of Japanese quail layers which are in line with present study. In contrary**, Rossi *et al.*, (2015**) who reported that yolk weight percentage were significantly increased when sweet green pepper was incorporated in laying hens′ diet, which are not in agreement with present study.

Hence, from the above results it can be concluded that supplementation of chili leaf powder had no adverse effect on the yolk quality of the eggs.

Average values of egg yolk cholesterol and triglycerides due to diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.13 and Figure 6 and 7.

The average values of yolk cholesterol among various treatment groups T_1, T_2, T_3 and T_4 was 14.49±0.22, 14.41±0.23, 13.94±0.35 and 13.23±0.81 mg/g respectively. As per the findings, egg yolk cholesterol was not significantly affected. However, egg yolk cholesterol was slightly reduced in treatment group T_4 (13.23±0.81 mg/g) as compared to T_1 (14.49±0.22 mg/g) control group. No significant difference was observed between T_1 and T_2 group. Average values of egg yolk triglycerides were 203.61±0.05, 202.12±0.29, 201.95±0.30 and 201.16±0.28 mg/g in treatment groups T_1, T_2, T_3 and T_4, respectively. The values egg yolk triglycerides were not significantly affected by incorporation of chili leaf powder in the diet of laying hens.

The present findings are in accordance with **Lokaewmanee *et al.* (2019**) who reported that egg yolk cholesterol was not significantly affected by addition of chili leaves in the diet of laying hens. However, the layer diet supplemented with 2 and 3% chili leaves powder decreased the egg cholesterol level by approximately 10% as compared to the control eggs, while **Saleh *et al.* (2021)** stated that dietary supplementation of paprika considerably lowered egg yolk cholesterol in laying hens, **Abou-Elkhair *et al.* (2018)** found that hot red pepper intake decreased serum total cholesterol and egg yolk cholesterol of laying hens. In addition, **Puvaca *et al.* (2015)** stated that hot red pepper supplementation reduced blood total cholesterol content in broiler chicken.

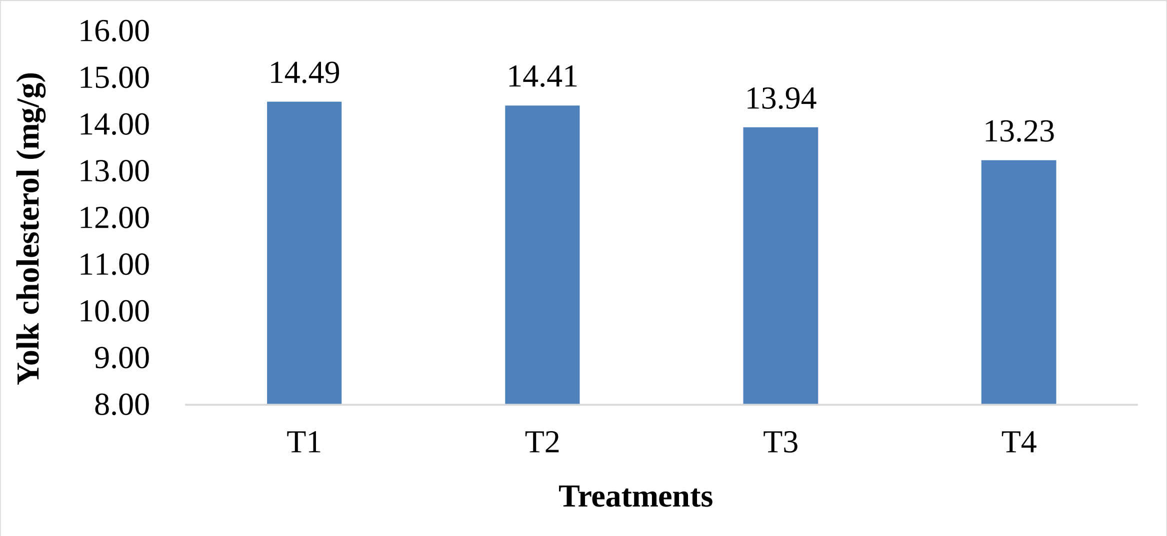

Fig. 6: Average values of egg yolk cholesterol (mg/g) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

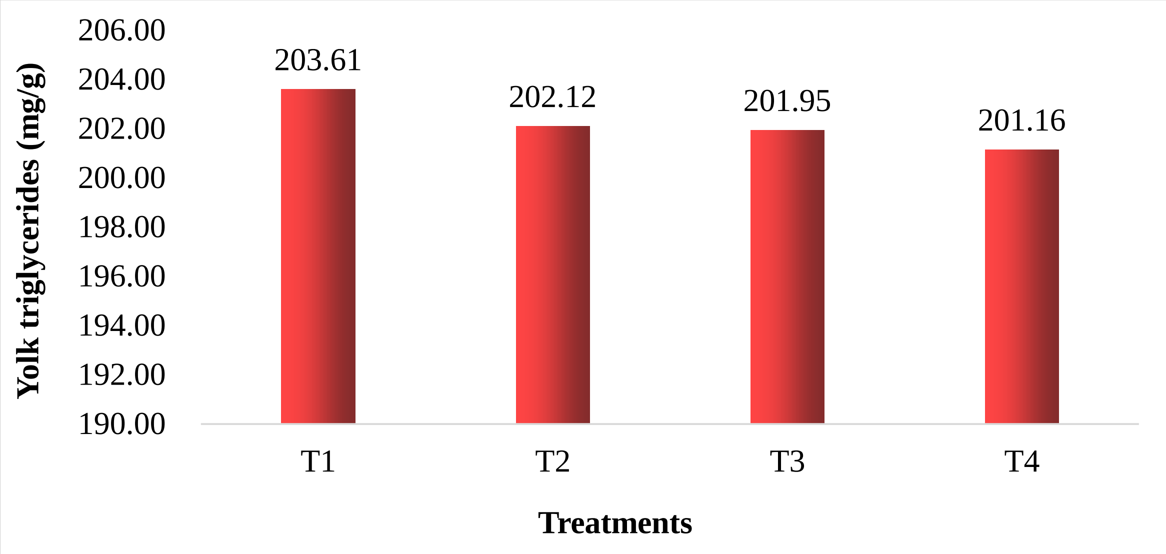

Fig. 7: Average values of egg yolk triglycerides (mg/g) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.5.4.1 Egg yolk colour

Average values of egg yolk colour due to diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.13 and Figure 8. Yolk colour of different treatment groups T_1, T_2, T_3 and T_4 was 6.35±0.66, 6.20±0.15, 6.60± 0.14 and 8.43±0.13, respectively. Yolk colour was found to be significantly ($P<0.05$) improved in T_4 (8.43±0.13) group, when compared to T_1 (6.35±0.66) control group, T_2 (6.20±0.15), T_3 (6.60± 0.14) groups. No significant difference was found between T_2 and T_3 groups. The egg yolk colour among treatment groups T_1, T_2 and T_3 also showed no significant difference and were statistically similar.

The above findings are in agreement with **Saleh *et al*. (2021)** who reported that egg-yolk colour was improved when the natural (paprika) colorant was added into layers diet. **Sözcü (2019)** who found that the yolk colour in the group supplemented with red pepper powder (*Capsicum annuum*) was greater than that in other groups. **Abou-Elkhair *et al*. (2018)** observed significant improvement in pigmentation of yolk colour on supplementation of red pepper in diets of laying hens. **Rossi *et al*. (2015)** also found improvement in yolk colour when laying hens were fed diet supplemented with sweet green peeper. **Li *et al*. (2012)** suggested that red pepper powder was found to be effective feed additives for improving egg yolk color for laying hens.

It is generally known that the carotenoid content of a yolk is directly and considerably linked with its colour. The type and chemical composition of carotenoids found in natural feed additives have a significant impact on carotenoid transport to the egg yolk and consequently their effect on yolk color. A large number of carotenoids found in paprika, such as capsanthin, capsorubin-cryptoxanthin, zeaxanthin, antheraxanthin, and beta-carotene, may be responsible for the improved egg yolk color of laying hens treated with paprika (**Saleh *et al*., 2021).**

The ingestion of zeaxanthin, lutein, alpha-carotene, beta-carotene and carotenoids has been shown to affect the colour of egg yolks. Capsanthin improved egg yolk colour and was responsible for the deep red colour of the egg yolk **(Lokaewmanee *et al*., 2019)**. The presence of carotenoids in red pepper could explain the higher egg yolk colour score in red pepper supplemented groups.

Supplementation of dietary capsaicin from paprika considerably boosted the deposition of red colour in the egg yolk, (**Vicente *et al*., 2007)**. They claimed that paprika peppers which contain natural capsaicin, are a good source of red pigments like trans-capsorrubin and trasn-capsantic, as well as yellow pigments like trans-lutein and trans-zeaxanthin, which could affect egg yolk pigmentation.

Numerous studies have been done to investigate the effect of feeding of chili on egg yolk colour. Zeaxanthin, lutein, alpha-carotene, beta-carotene, and carotenoids present in chilies has proven to enhance egg yolk colour of laying hens. Chili leaves are good source of beta- carotene which may reflect the increased egg yolk colour of laying hens.

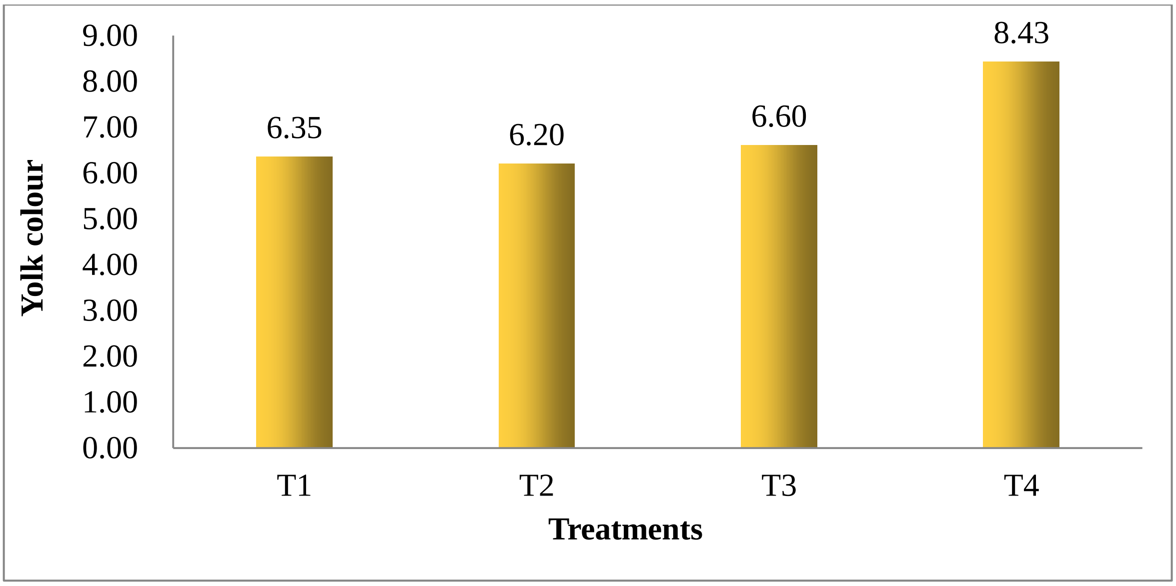

Fig. 8: Average values of egg yolk colour of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.6 Haematological Parameters

The data representing the haematological parameters in the laying hen fed diet supplemented with chili (*Capsicum annuum*) leaf powder on 90th day of the experimental feeding trial are summarized in table 4.14.

Table 4.14: Average values of haematological parameters of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Parameters	Treatments/Groups				P-value
	T_1	T_2	T_3	T_4	
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Haemoglobin (g/dl)	9.40±0.32	10.03±0.62	9.67±0.43	9.67±0.64	0.86
Packed cell volume (%)	30.00±1.76	30.17±1.48	30.33±0.67	30.50±2.00	0.99
Total erythrocyte count ($\times 10^6$ / µl)	3.08±0.04	3.10±0.06	3.13±0.11	3.23±0.14	0.77
Total leukocyte count ($\times 10^3$/ µl)	30.52±0.97	30.82±1.06	30.90±0.45	31.17±0.78	0.96
MCV (fl)	97.04±6.19	98.16±4.57	98.41±5.19	94.86±8.39	0.98
MCH (pg)	30.40±1.36	31.03±1.31	31.46±2.26	30.01±2.55	0.95
MCHC (g/dl)	31.47±0.86	31.66±0.92	31.84±0.79	31.67±0.12	0.99

4.6.1 Haemoglobin (g/dl)

Average values of blood haemoglobin content due to diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.13 and Figure 9.The average haemoglobin content in Rhode Island Red laying hens for treatment groups T_1, T_2, T_3 and T_4 was 9.40±0.32, 10.03±0.62, 9.67±0.43 and 9.67±0.64 g/dl, respectively. The dietary inclusion of 0.5%, 1.5% and 2.5% chili leaf powder revealed no significant difference in haemoglobin concentration among the various treatment groups.

These results are in agreement with **Shahverdi *et al*. (2013)** who found non-significant effect on blood haemoglobin concentration of broiler supplemented with red pepper. **Dougnon *et al*. (2014)** who reported the blood haemoglobin concentration of the Hubbard Broiler were not significantly affected by adding *Capsicum frutescens* powder in their diet. **Egbewande (2018)** also observed non- significant differences in blood haemoglobin concentration of broiler chicken fed a diet supplemented with *Capsicum annuum*. **Adedoyin *et al*. (2019)** also found no significant effect on blood haemoglobin content in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. Similar non- significant effect on haemoglobin level was reported by **Joseph *et al*. (2020)** on supplementation of diet of Wistar rats with capsaicinoids-rich red chili pepper.

On the contrary, **Firouzbakhsh *et al*. (2019)** reported a significant increase in blood haemoglobin level of rainbow trout (*Oncorhynchus mykiss*) where commercial feed was incorporated with chili pepper extract. **Elwan *et al*. (2020)** observed significant improvement in blood haemoglobin concentration on supplementation of red-hot powder in the diet of rabbits.

4.6.2 Packed cell volume (%)

The average values of packed cell volume for treatment groups T_1, T_2, T_3 and T_4 was 30.00±1.76, 30.17±1.48, 30.33±0.67 and 30.50±2.00 percent, respectively. There was no significant effect of dietary supplementation with chili (*Capsicum annuum*) leaf powder on the haemoglobin concentration in laying hens of different treatment groups.

The present findings are in agreement with **Dougnon *et al*. (2014)** who reported that blood packed cell volume of the Hubbard Broiler was not significantly affected by adding *Capsicum frutescens* powder in their diet, **Egbewande (2018)** also observed non-significant differences in blood packed cell volume of broiler chicken fed a diet supplemented *with Capsicum annuum*. **Adedoyin *et al*. (2019)** also found no significant effect on packed cell volume of broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. Similar non- significant effect on blood packed cell volume was reported by **Joseph *et al*. (2020)** on supplementation of diet of Wistar rats with capsaicinoids-rich red chili pepper.

In contrary, **Shahverdi *et al*. (2013)** found a significant decrease in packed cell volume of broiler supplemented with red pepper. However, **Firouzbakhsh *et al*. (2019)** found a significant increase in blood packed cell volume of rainbow trout (*Oncorhynchus mykiss*) where commercial feed was incorporated with chili pepper extract. **Elwan *et al*. (2020)** observed significant improvement in blood packed cell volume on supplementation of red-hot powder in diet of rabbits.

4.6.3 Total erythrocytes count ($\times 10^6/\mu l$)

Total erythrocytes count ($\times 10^6/\mu l$) for various treatment groups T_1, T_2, T_3 and T_4 was3.08±0.04, 3.10±0.06, 3.13±0.11 and 3.23±0.14, respectively. The overall erythrocyte count did not differ significantly between the different treatment groups.

The present findings are in line with **Dougnon *et al*. (2014)** who reported the red blood cell count of the Hubbard Broiler were not significantly affected by adding *Capsicum frutescens* powder in their diet, **Egbewande (2018)** also observed non-significant differences in red blood cells broiler chicken fed a diet supplemented *with Capsicum annuum*. **Adedoyin *et al*. (2019)** also found no significant effect on red blood cell count in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. Similar non- significant effect on red blood cell count was reported by **Joseph *et al*. (2020)** on supplementation of diet of Wistar rats with capsaicinoids-rich red chili pepper.

In contrast, **Firouzbakhsh *et al*. (2019)** reported a significant increase in red blood cell counts of rainbow trout (*Oncorhynchus mykiss*) where commercial feed was incorporated with chili pepper extract, **Elwan *et al*. (2020)** observed significant improvement in red blood cell counts on supplementation of red-hot powder in diet of rabbits.

4.6.4 Total leukocytes count ($\times 10^3/\mu l$)

The average values of total leukocytes count ($\times 10^3/\mu l$), for treatment groups T_1, T_2, T_3 and T_4 was30.52±0.97, 30.82±1.06, 30.90±0.45 and 31.17±0.78, respectively. There was no statistically significant change in total leukocyte count between treatment groups, and they were all statistically identical.

The above findings are in agreement with **Dougnon *et al*. (2014)** who reported that white blood cells count of the Hubbard Broiler was not significantly affected by adding *Capsicum frutescens* powder in their diet, **Egbewande (2018)** also observed non-significant differences in white blood cells of broiler chicken fed a diet supplemented *with Capsicum annuum*, **Adedoyin *et al*. (2019)** also found no significant effect on white blood cell count in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. Similar non- significant effect on white blood cells count was reported by **Joseph *et al*. (2020)** on supplementation of diet of Wistar rats with capsaicinoids-rich red chili pepper.

In contrast, **Firouzbakhsh *et al.* (2019)** reported a significant increase in white blood cell counts of rainbow trout (Oncorhynchus mykiss) where commercial feed was incorporated with chili pepper extract. **Elwan *et al.* (2020)** observed significant improvement in white blood cell counts on supplementation of red-hot powder in diet of rabbits.

4.6.5 Mean corpuscular volume (MCV)

The average values of mean corpuscular volume MCV (fl) of different treatment groups of laying hens viz., T_1, T_2, T_3 and T_4 was 97.04±6.19, 98.16±4.57, 98.41±5.19 and 94.86±8.39 fl, respectively. MCV (fl) values did not differ significantly amongst the different treatment groups in which chili leaf powder was supplemented.

The present findings are in accordance with **Dougnon *et al.* (2014)** who reported that blood mean corpuscular volume of the Hubbard Broiler were not significantly affected by adding *Capsicum frutescens* powder in their diet, **Egbewande (2018)** also observed non-significant effect on blood mean corpuscular volume of broiler chicken fed a diet supplemented *with Capsicum annuum*. **Adedoyin *et al.* (2019)** also found no significant effect on blood mean corpuscular volume of broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. **Elwan *et al.* (2020)** observed non- significant effect on blood mean corpuscular volume of rabbits fed diet supplemented with red-hot powder.

4.6.6 Mean corpuscular haemoglobin (MCH)

All of the treatments group had non-significant differences in mean corpuscular haemoglobin .The MCH (pg) values obtained among different treatment groups viz., T_1, T_2, T_3 and T_4 was30.40±1.36, 31.03±1.31, 31.46±2.26 and 30.01±2.55 pg, respectively.

The present results are in accordance with **Dougnon *et al.* (2014)** who reported that blood mean corpuscular hemoglobin of the Hubbard Broiler were not significantly affected by adding *Capsicum frutescens* powder in their diet. **Egbewande (2018)** also observed non- significant differences in mean corpuscular haemoglobin content of broiler chicken fed a diet supplemented *with Capsicum annuum*. **Adedoyin *et al.* (2019)** also found no significant effect on blood mean corpuscular haemoglobin level broiler chickens supplemented with hot red pepper (*Capsicum annuum.*) powder.

4.6.7 Mean corpuscular haemoglobin concentration (MCHC)

The average values of mean corpuscular haemoglobin concentration MCHC (g/dl) in different treatment groups T_1, T_2, T_3 and T_4 was31.47±0.86, 31.66±0.92, 31.84±0.79 and 31.67±0.12 g/dl respectively. The results indicated non- significant differences among the various treatment groups when compared with control group.

The above findings are in similar line with **Dougnon *et al*. (2014)** who reported that mean corpuscular hemoglobin concentration of the Hubbard Broiler were not significantly affected by adding *Capsicum frutescens* powder in their diet. **Egbewande (2018)** also observed non- significant differences in mean corpuscular haemoglobin concentration of broiler chicken fed a diet supplemented *with Capsicum annuum*. **Adedoyin *et al*. (2019)** also found no significant effect on blood mean corpuscular haemoglobin concentration of broiler chickens supplemented with hot red pepper (*Capsicum annuum.*) powder. **Elwan *et al*. (2020)** observed non- significant effect on blood mean corpuscular haemoglobin concentration of rabbits fed diet supplemented with red-hot powder.

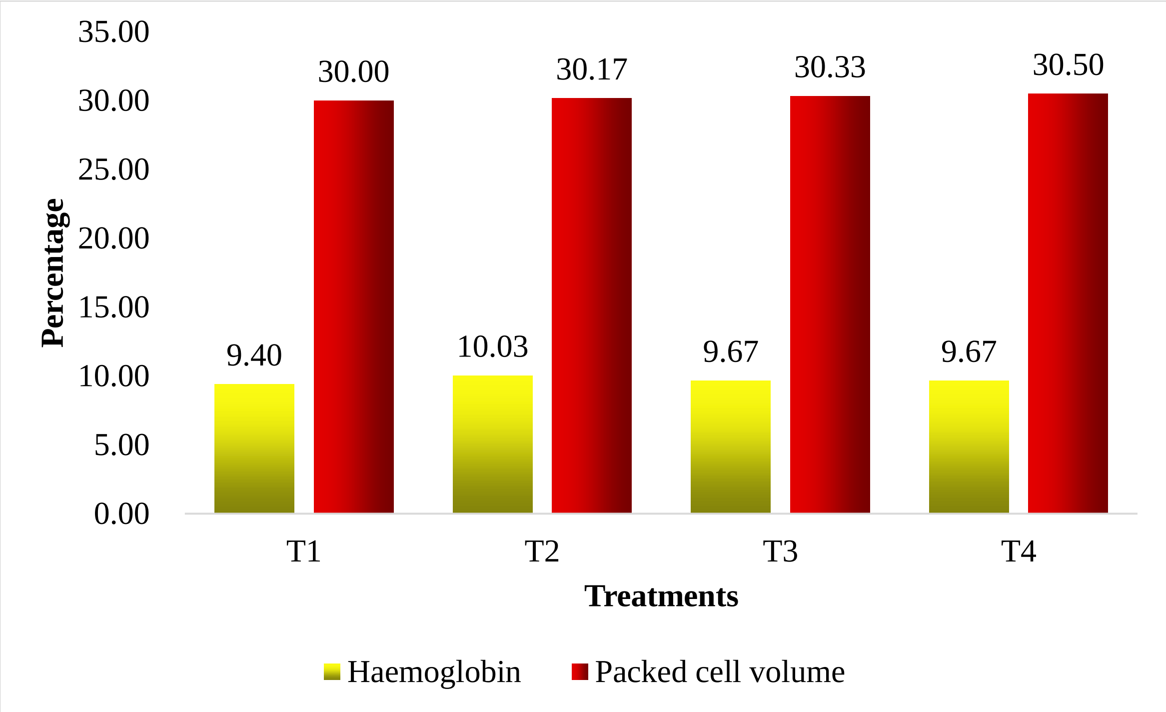

Fig. 9: Average values of haemoglobin and packed cell volume (%) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.7 Serum biochemical parameters

Average values of serum biochemical parameters due to diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.15

Table 4.15: Average values of serum biochemical parameters of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Parameters	Treatments/Groups				P-value
	T_1 Basal diet (control)	T_2 Basal diet+ 0.5% chili leaf powder	T_3 Basal diet+1.5% chili leaf powder	T_4 Basal diet+2.5% chili leaf powder	
Blood glucose (mg/dl)	210.71±8.25	205.53±3.30	206.04±5.09	209.39±5.08	0.91
Cholesterol (mg/dl)	151.69±1.20	150.24±2.26	148.48±2.59	143.33±1.20	0.07
Triglyceride (mg/dl)	211.39±0.20	211.03±1.76	211.11±1.92	207.75±0.63	0.26
Total protein (g/dl)	4.25±0.15	4.48±0.29	4.79±0.80	4.97±0.47	0.31
Globulin(g/dl)	2.94±0.19	3.07±0.20	3.37±0.85	3.60±0.46	0.74
Albumin(g/dl)	1.33±0.04	1.41±0.10	1.41±0.11	1.37±0.08	0.99
Calcium (mg/dl)	22.03±0.42	22.56±0.38	21.83±1.57	21.50±1.52	0.92
Phosphorus (mg/dl)	5.56±0.03	5.59±0.03	5.57±0.07	5.61±0.18	0.98
SGPT(U/I)	24.58±0.26	24.13±0.87	24.80±0.82	24.45±0.73	0.92
SGOT(U/I)	60.22±0.09	59.43±1.43	59.62±0.48	59.91±0.15	0.89

4.7.1 Serum glucose

The values of mean serum glucose concentration in laying hens fed diet supplemented with chili (*Capsicum annuum*)leaf powder in treatment groups T_1, T_2, T_3 and T_4 was 210.71±8.25, 205.53±3.30, 206.04±5.09 and 209.39±5.08 mg/dl, respectively. No significant difference was found in serum glucose content among the various treatment groups fed diet supplemented with chili leaves powder.

The present findings are in line with the findings of **Saleh *et al*. (2021)** found that serum glucose content was not affected when laying hens were fed supplemented with paprika. **Egbewande (2018)** also reported no significant effect on serum glucose concentrationin broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. However, **Shahverdi *et al*. (2013)** noted a significant decrease in serum glucose concentration in broiler supplemented with red pepper.

4.7.2 Serum cholesterol

Average values of Serum cholesterol and triglyceride content due to diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.13 and

Figure 10. The mean serum cholesterol concentration in the laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder of the treatment groups T_1, T_2, T_3 and T_4 was 151.69±1.42, 150.24±2.26, 148.48±2.59 and 143.33±1.20 mg/dl, respectively. Serum cholesterol content was found to be non- significantly decreased in treatment group T_4 (143.33±1.20 mg/dl) followed by treatment group T_3 (148.48±2.59 mg/dl) when compared to T_1 (151.69±1.42 mg/dl). Further, serum cholesterol concentration was lower in T_4 (143.33±1.20 mg/dl) group when compared to T_2 (150.24±2.26 mg/dl) group but the effect was statistically non- significant.

The results of present study are in accordance with **Dougnon *et al*. (2014)** as they reported that the blood total cholesterol concentration of the Hubbard Broiler was not significantly affected by adding *Capsicum frutescens* powder in their diet. **Adedoyin *et al*. (2019)** also found that serum cholesterol levels were non-significantly decreased in the birds fed diet supplemented with hot red pepper. **Egbewande (2018)** also observed non-significant differences in serum total cholesterol concentration of broiler chicken fed a diet supplemented with *Capsicum annuum*.

However, **Abou-Elkhair *et al*. (2018)** noted a significant reduction in serum cholesterol of laying hens supplemented with red pepper. **Pi *et al*. (2017)** found that supplementation of 15 mg/kg capsaicin significantly decreased serum total cholesterol low-density lipoprotein cholesterol contents compared with the control group. **Shahverdi *et al*. (2013)** also noted a significant reduction in serum cholesterol concentration and in broiler supplemented with red pepper. In comparison to the control group, feeding capsaicinoids in ovariectomized rats significantly reduced plasma total cholesteroland low-density lipoprotein cholesterol concentrations **(Zhang *et al*. 2013)**. **Atapattu and Belpagodagamage (2011)** found that broilers chicken supplemented with 1% chilli powder had lower serum cholesterol levels than the control group. **Marić *et al*. (2021)** who found a significant reduction in concentration of total cholesterol in broilers supplemented with chili pepper**. Liu *et al*. (2021)** also observed a significant reduction in broilers low-density lipoprotein cholesterol and total cholesterol concentration in broiler chickens supplemented with natural capsaicin extract.

4.7.3 Serum triglycerides

The mean serum triglyceride concentration (mg/dl) in the laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder was 211.39±0.20, 211.03±1.76, 211.11±1.92 and 207.75±0.63 mg/dl in treatment groups T_1, T_2, T_3 and T_4 respectively. The mean value of serum triglycerides in the different treatment groups were not significantly affected when chili leaf powder was added into laying hens' diet. However, Serum triglyceride content was lower in T_4 (207.75±0.63 mg/dl) group when compared with T_1 (211.39±0.20 mg/dl) group but the effect was statistically non- significant.

The results of present study are in accordance with **Dougnon *et al*. (2014)** as they reported that the serum triglycerides concentration of the Hubbard Broiler was not significantly affected by adding *Capsicum frutescens* powder in their diet. **Adedoyin *et al*. (2019)** also found that serum triglycerides levels were non-significantly decreased in the birds fed diet supplemented with hot red pepper. **Egbewande (2018)** also observed non-significant differences in serum triglycerides concentration of broiler chicken fed a diet supplemented with *Capsicum annuum*.

However, **Pi *et al*. (2017)** found that supplementation of 15 mg/kg capsaicin significantly decreased serum triglycerides contents compared with the control group. **Shahverdi *et al*. (2013)** also noted a significant reduction in serum triglyceride concentration and in broiler supplemented with red pepper. In comparison to the control group, feeding capsaicinoids in ovariectomized rats significantly reduced plasma triglycerides concentrations **(Zhang *et al*. 2013)**. **Marić *et al*. (2021)** who found a significant reduction in concentration of triglycerides in broilers supplemented with chili pepper**.**

4.7.4 Serum total protein

The mean serum total protein concentration (g/dl) in the laying hens was 4.25±0.15, 4.48±0.29, 4.79±0.80 and 4.97±0.47g/dl in treatment groups T_1, T_2, T_3 and $T_{4,}$ respectively. There was no significant effect observed in the serum total protein content of the treatment groups fed diet supplemented with chili (*Capsicum annuum*) leaf powder. However, the serum total protein content of all the treatment groups was higher than the control group, but the effect was statistically insignificant.

The results of present experiment are in accordance with the findings of **Egbewande (2018)** as they reported that no significant effect on total protein content in broiler chickens supplemented with hot red pepper(*Capsicum annuum*) powder. **Adedoyin *et al*. (2019)** as they found no significant effect on serum total protein concentration in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. **Saleh *et al*. (2021)** found that total protein content was not affected when laying hens were fed supplemented with paprika. **Marić *et al*. (2021)** who found non- significant difference in concentration of total protein content in broilers supplemented with chili pepper**.**

4.7.5 Serum globulin

The mean concentration of serum globulin in laying hens of treatment groups T_1, T_2, T_3 and T_4 groups were 2.94±0.19, 3.07±0.20, 3.37±0.85 and 3.60±0.46 g/dl, respectively and statistically were similar. However non- significant but numerically, the serum globulin content of all the treatment groups was higher than the control group.

The results of present experiment are in accordance with the findings of **Egbewande (2018)** as they reported that no significant effect on globulin content in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. **Adedoyin *et al*. (2019)** as they found no significant effect on serum globulin concentration in broiler chickens supplemented with hot red pepper (*Capsicum annuum.*) powder. **Saleh *et al*. (2021)** found that globulin content was not affected when laying hens were fed supplemented with paprika. **Marić *et al*. (2021)** who found non- significant difference in concentration of globulin content in broilers supplemented with chili pepper**.**

4.7.6 Serum albumin

No significant difference was found in the serum albumin concentration in the different treatment groups fed diet supplemented with chili (*Capsicum annuum*) leaf powder. The mean values of serum albumin in different treatment groups T_1, T_2, T_3 and T_4 group as shown in Table 4.15 were 1.33±0.04, 1.41±0.10, 1.41±0.11 and 1.37±0.08 g/dl, respectively.

The results of present experiment are in accordance with the findings of **Egbewande (2018)** as they reported that no significant effect on albumin content in broiler chickens supplemented with hot red pepper(*Capsicum annuum*) powder. **Adedoyin *et al*. (2019)** as they found no significant effect on serum albumin concentration in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. **Saleh *et al*. (2021)** found that albumin content was not affected when laying hens were fed supplemented with paprika. **Marić *et al*. (2021)** who found non- significant difference in concentration of albumin content in broilers supplemented with chili pepper**.**

4.7.7 Serum calcium and phosphorus

Table 4.15 shows the average serum calcium and phosphorus levels, which were found to be non-significant among treatment groups. Serum calcium concentration for treatment groups T_1, T_2, T_3 and T_4 was 22.03±0.42, 22.56±0.38, 21.83±1.57 and 21.50±1.52 mg/dl, respectively while serum phosphorus concentration was 5.56±0.03, 5.59±0.03, 5.57±0.07 and 5.61±0.18 mg/dl, respectively.

The results of present study are in line with **Aderemi *et al*. (2013)** as they found non- significant effect serum calcium and phosphorus levels when laying hens were supplemented with pepper (*Capsicum annuum*). **Shahverdi *et al*. (2013)** observed non-significant effect on blood calcium of broiler supplemented with red pepper. **Adedoyin *et al*. (2019)** found no significant effect on serum calcium and phosphorus concentration in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder.

4.7.8 Serum enzymes

The data representing the activity of serum enzymes viz., serum glutamic pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) in laying hens fed supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.15. The SGPT and SGOT level in laying hens of different groups T_1, T_2, T_3 and T_4 were 24.58±0.26, 24.13±0.87, 24.80±0.82 and 24.45±0.73 U/L; and 60.22±0.09, 59.43±1.43, 59.62±0.48 and 59.91±0.15 U/L, respectively. The results clearly showed that supplementation with chili leaf powder in the diet of laying hens did not induce any significant effect on the activity of SGPT and SGOT enzymes.

The results of present experiment are in support with **Adedoyin *et al*. (2019)** as they found no significant effect on serum SGOT and SGPT enzymes concentration in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. **Abou-Elkhair *et al*. (2018)** noted serum SGOT and SGPT enzymes concentration were not affected by adding hot red pepper in laying hen's diet. **Egbewande (2018)** also reported no significant effect on serum SGOT and SGPT enzyme concentration in broiler chickens supplemented with hot red pepper(*Capsicum annuum.*) powder.

In contrary, **Egbewande (2018)** reported significant increase in serum SGPT concentration in broiler chickens supplemented with hot red pepper (*Capsicum annuum*) powder. **Aderemi *et al*. (2013)** found significant increase in activity of SGPT and SGOT enzymes when laying hens were supplemented with pepper (*Capsicum annuum*).

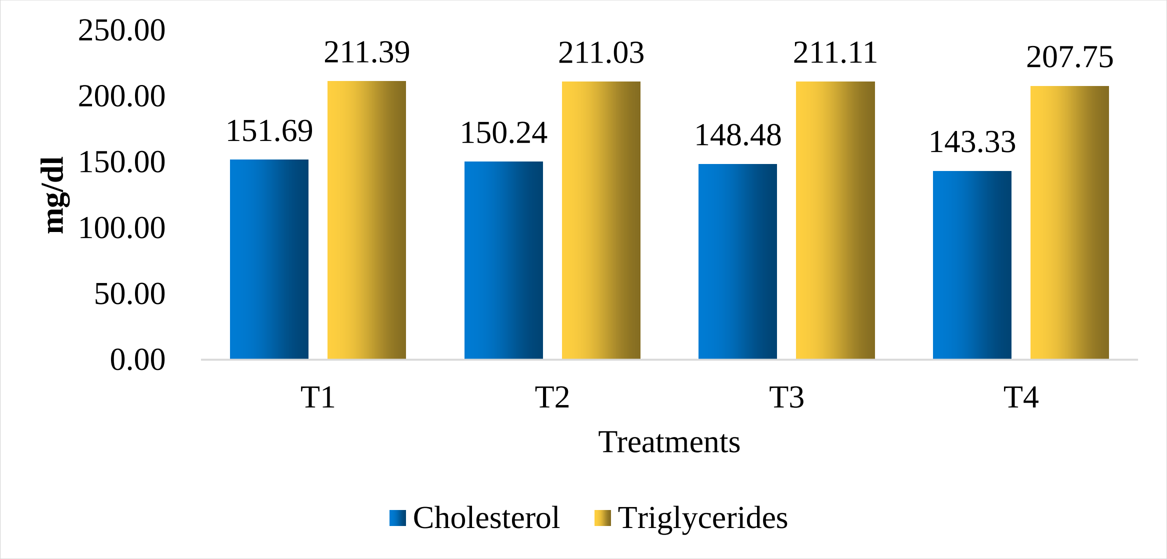

Fig.10: Average values of serum cholesterol and serum triglycerides (mg/dl) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

4.8 Economics of egg production

The average values of economics of egg production from 28-40 weeks of experimental feeding trial fed diet supplemented with chili (*Capsicum annuum*) leaf powder are presented in Table 4.16.

Table 4.16: Economics of egg production of Rhode Island Red laying hens during 28-40 (90 days) weeks period fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Parameters	Treatments/groups				P-value
	Basal diet (control)	Basal diet+0.5% chili leaf powder	Basal diet+1.5% chili leaf powder	Basal diet+2.5% chili leaf powder	
Cost/kg feed (Rs.)	25	25	25	25	
Avg. Dozen eggs per bird	4.38±0.05	4.42±0.08	4.47±0.09	4.52±0.06	0.55
Avg. Feed intake (kg) per bird*	10.84^{b}±0.02	10.81^{ab}±0.02	10.80^{ab}±0.01	10.75^{a}±0.01	0.05
Total feed cost (Rs.) *	270.89^{b}±0.41	270.33^{ab}±0.61	269.94^{ab}±0.28	268.76^{a}±0.19	0.03
Feed cost/dozen eggs (Rs.)	61.94±0.78	61.19±1.20	60.41±1.25	59.50±0.78	0.43

$^{a, b}$ Mean values bearing different superscripts within a row differ significantly from each other,*P≤0.05.

The calculated cost of per kg feed (in Rs.) of different treatment groups T_1, T_2, T_3 and T_4 fed diet supplemented with chili (*Capsicum annuum*) leaf powder was 25 Rs. The average dozen egg production for treatment groups T_1, T_2, T_3 and T_4 was 4.38±0.05, 4.42±0.08, 4.47±0.09 and 4.52±0.06, respectively. The average dozen egg production did not differ significantly among these groups. Average feed intake for treatment groups T_1, T_2, T_3 and T_4 are presented in Figure 11. The values of average feed intake for treatment groups T_1, T_2, T_3 and T_4 was 10.84±0.02, 10.81±0.02, 10.80±0.01 and 10.75±0.01 kg, respectively. Average feed intake was significantly ($P<0.05$) decreased in treatment groups T_4 (10.75±0.01 kg) when compare to control group T_1 (10.84±0.02 kg).

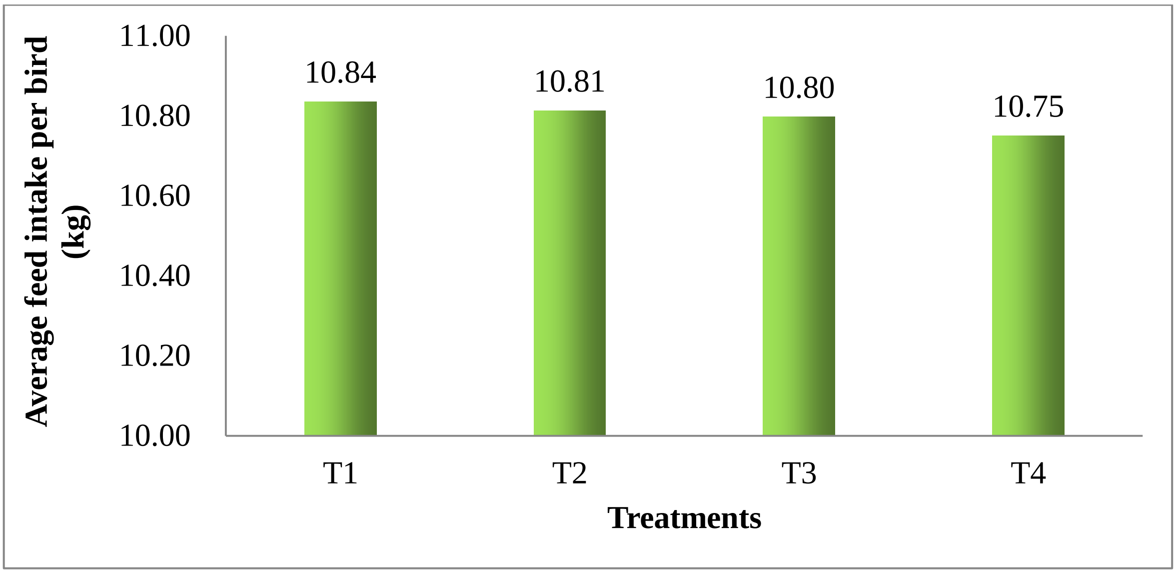

Fig. 11: Average values of Average feed intake (kg) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Total feed cost was found to differ significantly ($P<0.05$) among the various treatment groups and presented in Figure 12. The average total feed cost among various treatment groups T_1, T_2, T_3 and T_4 was 270.89±0.41, 270.33±0.61, 269.94±0.28 and 268.76±0.19 (Rs.), respectively. Total feed cost was significantly ($P<0.05$) decreased in treatment groups T_4 (268.76±0.19 Rs.) when compared to the control group T_1 (270.89±0.41 Rs.) however no significant difference was found in treatment T_2 and T_3. Average feed cost/dozen egg (Rs.) were 61.94±0.78, 61.19±1.20, 60.41±1.25 and 59.50±0.78 Rs., for treatment groups T_1, T_2, T_3 and T_4, respectively and was non-significant among various treatment groups. However, numerically, average feed cost/dozen egg (Rs.) was reduced in all the treatment group when compared to the control group, with maximum reduction in T_4 group followed by T_3 and T_2 group when compared with the control group (T_1).

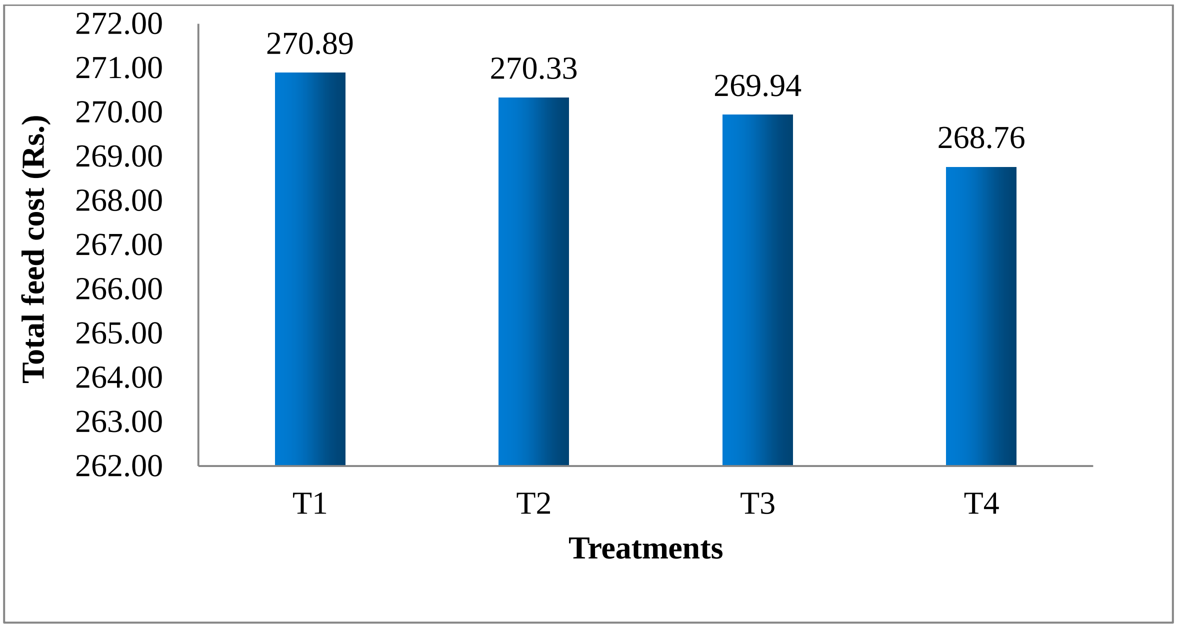

Fig. 12: Average values of total feed cost (Rs.) of Rhode Island Red laying hens fed diet supplemented with chili (*Capsicum annuum*) leaf powder

Egbewande (2018) observed that birds fed hot peppers had higher gross profits in terms of final live weights than those fed the control. Control group has the lowest gross profit, which could be due to the vaccination costs compared to the pepper costs in other treatments (without vaccines). As a result, using hot peppers instead of vaccines lowered broiler production costs.

Summary and Conclusion

Chapter 5 SUMMARY AND CONCLUSION

The present study was conducted in order to investigate the effect of dietary incorporation of chili (*Capsicum annuum)* leaf powder on productive performance, egg quality, haemato-biochemical parameters and economics of production in Rhode Island Red laying hens of 28 weeks of age for a period of 12 weeks (28-40 weeks). Chili (*Capsicum annuum*) leaves were obtained from plants in the local area. After collection, leaves were first shade dried for 3-4 days before being sun-drying on a clean concrete floor. Leaves were placed in a hot air oven at 70°C until its constant weight was achieved. The required amount of chili leaves was finely processed to powder and stored in sealed polythene bags in a cold, dry place.

Feeding trial of 12 weeks duration was conducted at Instructional Poultry Farm, Nagla, G. B. Pant University of Agriculture and Technology, Pantnagar, on 72 Rhode Island Red laying hens. Hens were randomly distributed into four treatments groups with 18 birds per treatment with three replicates in each. There were four dietary treatment groups viz., T_1: (control, containing basal diet); T_2: Incorporation of 0.5% chili leaf powder in basal diet; T_3: Incorporation of 1.5% chili leaf powder in basal diet; T_4: Incorporation of 2.5% chili leaf powder in basal diet. Proximate analysis of different feeds used in the experimental feeding trial was conducted for determining the nutrient composition such as, Dry Matter, Crude Protein, Crude Fiber, Ether Extract, Total Ash and Nitrogen free extract.

Effects of dietary interventions on production performance, egg quality parameters, haemato-biochemical parameters and economics of Rhode Island Red laying hens were evaluated. Weekly feed intake, daily egg production, FCR (feed conversion ratio), egg quality traits and feed cost of eggs produced by laying hens was calculated. A 7-day metabolic trial was performed at the end of the feeding experiment to determine nutrient utilization by the laying hens. Six hens were chosen at random from each treatment group to assess nutrient utilization. During this period of 7 days, 4 days were considered as adaptation period succeeded by excreta collection period of 3 days. Blood samples for hematological parameters and serum biochemical profile were taken at the end of the experimental trial, i.e., at the 12th week of the experimental feeding trial. Egg quality parameters were examined after 12 weeks of feeding trial.

The summary of results and conclusions drawn from the experiment are presented below:

1. Proximate analysis showed that chili leaf powder used in the experiment contained 6.78% crude protein, 7.89% ether extract, 14.52% crude fibre, 5.15% total ash and 65.66 % nitrogen free extract on dry matter basis.

2. Weekly average feed intake of Rhode Island Red hens fed diet containing chili leaf in different treatment groups T_1, T_2, T_3 and T_4 was found to be non- significant , except at 2^{nd}, 11^{th} and 12^{th} weeks during which average feed intake among the various treatment groups differed significantly (P<0.05). During 2^{nd} week, feed intake significantly (P<0.05) reduced in treatment group T_2 (120.16±0.27g) and T_4 (120.15±0.04g) as compare to T_1 (121.84±0.47g). No significant difference was observed between T_1 and T_3 group. The feed intake was also similar among T_2, T_3 and T_4 groups and no significant difference was seen among them. The average feed intake during 11^{th} and 12^{th} week differed significantly (P<0.05) among various treatment groups. Feed intake was significantly (P<0.05) lower in T_4 (119.44±0.14g/day) group, as compared to the T_1, T_2 and T_3 groups, whereas, feed intake in groups T_2 and T_3 was non-significantly different among each other. No significant difference was observed among T_1, T_2 and T_3 groups. Overall average feed intake per day in all treatments fed diet supplemented with chili (*Capsicum annuum*) leaf powder was found to be significantly (P<0.05) different. Overall average feed intake per day was significantly (P<0.05) reduced in treatment group T_4 (119.45±0.08g/day) as compared to T_1, T_2 and T_3 groups. However, no significant difference was observed among T_1, T_2 and T_3 groups. Fortnightly body weight was also similar among various treatment groups throughout the study. During 3^{rd} week, the average weekly egg production of group T_4 (61.90±0.79) was significantly (P<0.05) higher than the treatment groups $T_{1,}$ T_2 and $T_{3.}$

Overall cumulative performance of the Rhode Island Red hens of different treatment groups during the 12 weeks of experimental feeding period in terms of egg production and FCR was not significantly influenced due to dietary supplementation of chili leaf (*Capsicum annuum*) powder. However, overall egg production during the experimental feeding period of 12 weeks was slightly increased in T_4 group. Overall cumulative performance of the laying hens of different treatment groups during the 12 weeks of experimental feeding period in terms of feed intake revealed that average feed intake of laying hens of group T_4 (119.45±0.33g) was significantly ($P<0.05$) decreased than treatment group T_1.

3. Incorporation of diet containing chili (*Capsicum annuum*) leaf powder showed no significant effect in terms of ether extract utilization. However, dry matter and organic matter utilization was significantly ($P<0.05$) increased in T_4 group i.e., (73.74±0.66% for DM and75.18±1.48% for OM) compared to T_1 (68.05±1.05% for DM and 72.20±0.93% for OM) group. However, there was no significant difference in dry matter as well as organic matter utilization between the T_1, T_2, and T_3 groups. Crude protein utilization was significantly ($P<0.05$) increased in T_3 group (84.22±0.29 percent) as compared to T_1 (82.01±0.59 percent) group. However, there was no significant difference in crude protein utilization between the T_1, T_2 and T_4 groups.

4. The average values of egg weight (g) were non-significant among various treatment groups fed diet supplemented with different level of chili leaf powder.

5. The egg shell quality parameters in terms of shape index, shell weight, shell weight percentage and shell thickness of eggs did not differ significantly among treatment groups due to dietary supplementation of chili leaf powder in the diet of laying hens.

6. The egg albumen quality in terms of albumen height, albumen weight and haugh unit also did not differed significantly among T_1, T_2, T_3 and T_4 groups due to dietary supplementation of chili leaf powder in the diet of laying hens.

7. The egg yolk quality parameters including yolk weight, yolk percentage, yolk index, yolk cholesterol and yolk triglyceride did not differ significantly among different treatment group due to dietary supplementation of chili (*Capsicum annuum*) leaf powder in diet, however, significant ($P<0.05$) difference in yolk colour was observed. Yolk colour was found to be significantly ($P<0.05$) improved in T_4 (8.43±0.13) group, when compared to T_1 (6.35±0.66) control group. However, the treatment groups T_1, T_2, T_3 showed no significant difference and were statistically similar.

8. The hematological values *i.e.,* haemoglobin, packed cell volume, total erythrocyte count, total leucocytes count, MCV, MCH and MCHC in the blood were in normal range and showed no significant difference among the treatment groups of Rhode Island Red hens fed diet incorporated with chili leaf powder.

9. All serum biochemical constituents (Serum glucose, cholesterol, triglycerides, total protein, albumin, globulin, calcium, phosphorus, SGPT and SGOT) of laying hens did not differ significantly among dietary treatments. However, serum cholesterol content was found to be non-significantly decreased in treatment group T_4 (143.33±1.20 mg/dl) and T_3 (148.48±2.59 mg/dl) when compared to T_1 (151.69±1.42 mg/dl) and T_2 (150.24±2.26 mg/dl) group. Serum triglyceride content was low in T_4 (207.75±0.63 mg/dl) group when compared with T_1 (211.39±0.20 mg/dl) group but the effect was statistically non-significant. Serum total protein content of all the treatment groups was higher than the control group, but the effect was non-significant. Numerically, serum globulin content of all the treatment groups was higher than the control group.

10. The economics of egg production worked out due to dietary incorporation of chili leaf powder, it revealed that average dozen egg production and feed cost per dozen egg production was statistically similar in all the treatment groups. Average feed intake per bird was significantly ($P<0.05$) decreased in treatment group T_4 (10.75±0.01 kg) when compared to control group T_1 (10.84±0.02 kg). No significant difference was found among T_1, T_2 and T_3 groups. Similar findings were observed with total feed cost, which was significantly ($P<0.05$) low in treatment groupT_4 (268.76±0.19 Rs.) when compared to the control group T_1 (270.89±0.41 Rs). No significant difference was found between treatment group T_2 and T_3 for both parameters i.e., total feed cost and Average feed intake per bird.

Salient findings

1. Incorporation of chili leaf powder in basal diets showed similar performance with no adverse effects on the body weight gain. However, 2.5% chili leaf powder incorporation non- statistically improved egg production in laying hens.
2. Utilization of nutrients i.e., dry matter and organic matter was improved after supplementation of 2.5% chili leaf powder, however, crude protein utilization was improved after supplementation of 1.5% chili leaf powder in diets of laying hens. Ether extract utilization remained unchanged after supplementing with chili leaf powder.

3. Egg quality traits (egg shell, albumen and egg yolk quality parameters) were not affected by dietary supplementation of chili leaf powder in diets of laying hens. Also, egg yolk quality parameters including yolk weight, yolk percentage, yolk index, yolk cholesterol and yolk triglyceride were not affected by dietary supplementation of chili leaf powder in diets of laying hens. Furthermore, yolk colour pigmentation revealed appreciable improvement after supplementation of chili leaf powder at the rate of 2.5%.
4. Hematological constituents were not affected by supplementation of chili leaf powder in diets of laying hens.
5. Serum biochemical parameters (serum cholesterol, serum triglyceride content, serum total protein and globulin, serum glucose, albumin, calcium, phosphorus, SGPT and SGOT) were not significantly affected by addition of chili leaf powder in diets of laying hens.
6. Average feed intake per hen and total feeding cost reduced after adding 2.5% chili leaf powder in basal diet, as chili leaves was made available from the local area, at no cost.

Conclusion

From the above research findings, it can be concluded that chili (*Capsicum annuum*) leaf powder can be supplemented @ 2.5 % in laying hens' diet to improve egg yolk pigmentation and nutrient utilization as well as to reduce feed cost in Rhode Island Red laying hens

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ABSTRACT

The present study was carried out to discern the effect of dietary supplementation of chili (*Capsicum annuum*) leaf powder on growth, nutrient utilization, haemato- biochemical parameters, egg quality parameters and economics of egg production of Rhode Island Red laying hens. A feeding trial of 12 weeks duration was conducted on seventy- two, 28 weeks old Rhode Island Red laying hens. Hens were randomly distributed into four treatment groups with 18 birds per treatment having three replicates of 6 birds each. Hens of T_1 (control) group were fed basal diet, whereas in treatment groups T_2, T_3 and T_4, the basal diet was supplemented with chili leaf powder at the rate of 0.5, 1.5 and 2.5 percent, respectively. Results showed that overall cumulative performance of the laying hens of different treatment groups during the 12 weeks (28-40 weeks) of experimental feeding period in terms of weight gain, egg production and FCR was not significantly affected due to feeding of diet supplemented with chili (*Capsicum annuum*) leaf powder. However, overall average feed intake significantly ($P<0.05$) reduced in hens supplemented with 2.5% chili leaf powder. Dry matter and organic matter utilization was significantly ($P<0.05$) increased in T_4 group, while no significant difference was found in dry matter and organic matter utilization among T_1, T_2 and T_3 groups. Crude protein utilization was significantly improved in T_3 group as compared to T_1 group (control), whereas utilization of crude protein in group T_2 and T_3 was similar when compared with control group. However, ether extract utilization was similar in all the groups. The egg quality parameters in terms of egg weight, shape index, shell weight, shell weight percentage, shell thickness, albumen height, albumen weight and haugh unit also did not differed significantly among different dietary treatments. However, the egg yolk pigmentation was significantly ($P<0.05$) improved in T_4 group when compared with T_1, T_2 and T_3 groups while the colour of egg yolk was similar among T_1, T_2 and T_3 groups. Other yolk quality parameters like yolk weight, yolk weight percentage, yolk cholesterol, yolk triglycerides and yolk index were statistically similar in all the groups. The haematological parameters were also not affected by supplementation of chili leaf powder in basal diet. Serum biochemical constituents (serum glucose, cholesterol, triglycerides, total protein, albumin, globulin, calcium, phosphorus, SGPT and SGOT) of laying hens did not differ significantly among dietary treatments. Total feed cost and average feed intake in the group supplemented with 2.5 % chili leaf powder (T_4 group) was lower than the control group; however feed cost and average feed intake in T_2 and T_3 groups was not affected by addition of chili leaf powder. Further, feed cost per dozen egg production was statistically similar in all the treatments. From the present study, it is concluded that chili (*Capsicum annuum*) leaf powder can be supplemented @ 2.5 % in diet to improve egg yolk pigmentation and nutrient utilization as well as to reduce feed cost in Rhode Island Red laying hens.

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शोध का शीर्षक	:	**"रोड आइलैंड रेड अंडा उत्पादक मुर्गियों के प्रदर्शन पर मिर्च (*कैप्सिकम एनम*) के पत्ते के पाउडर युक्त आहार खिलाने का प्रभाव"**			
पृष्ठ संख्या	:	1-91	**सलाहकार**	:	डॉ. बी. सी. मण्डल

सारांश

वर्तमान अध्ययन को रोड आइलैंड रेड अंडा उत्पादक मुर्गियों के विकास, पोषक तत्वों के उपयोग, रक्त-जैव रासायनिक मापदंडों, अंडे की गुणवत्ता के मापदंडों और अंडा उत्पादन के अर्थशास्त्र पर मिर्च (कैप्सिकम एनम) के पत्तों के पाउडर से युक्त आहार के प्रभाव को समझने के लिए किया गया था। 12 सप्ताह की अवधि का एक आहार परीक्षण बहत्तर, 28 सप्ताह के रोड आइलैंड रेड अंडा उत्पादक मुर्गियों पर आयोजित किया गया था। मुर्गियों को चार उपचार समूहों में असंगत ढंग से वितरित किया गया, जिसमें प्रति उपचार 18 मुर्गियां थी जिनमें से प्रत्येक में 6 मुर्गियों की तीन प्रतिकृति थी। T_1 (नियंत्रण) समूह की मुर्गियों को को बेसल आहार दिया गया। जबकि उपचार समूहों T_2, T_3 और T_4 में बेसल आहार को क्रमशः 0.5,1.5 और 2.5 प्रतिशत की दर से मिर्च के पत्ते के पाउडर के साथ पूरक किया गया। परिणामों से पता चला कि वजन बढ़ाने, अंडा उत्पादन और एफसीआर के संदर्भ में प्रायोगिक आहार अवधि के 12 सप्ताह (28-40 सप्ताह) के दौरान विभिन्न उपचार समूहों की मुर्गियों का समग्र संचयी प्रदर्शन मिर्च पूरक आहार के कारण महत्वपूर्ण रूप से प्रभावित नहीं हुआ था। हालांकि 2.5 प्रतिशत मिर्च के पत्ते के पाउडर के साथ पूरक मुर्गियों में समग्र औसत आहार सेवन काफी ($P<0.05$) कम हो गया। T_4 समूह में शुष्क पदार्थ और कार्बनिक पदार्थ का उपयोग उल्लेखनीय रूप से ($P<0.05$) बढ़ा, जबकि T_1, T_2 और T_3 समूहों के बीच शुष्क पदार्थ और कार्बनिक पदार्थों के उपयोग में कोई महत्वपूर्ण अंतर नहीं पाया गया। T_1 समूह (नियंत्रण) की तुलना में T_3 समूह में कच्चे प्रोटीन के उपयोग में काफी सुधार हुआ था। जबकि समूह T_2 और T_3 में कच्चे प्रोटीन का उपयोग नियंत्रण समूह की तुलना में समान था। हालाँकि, ईथर के अर्क का उपयोग सभी समूहों में समान था। अंडे के वजन, आकार सूचकांक, खोल वजन, खोल वजन प्रतिशत, खोल मोटाई, एल्ब्यूमिन ऊंचाई, एल्ब्यूमिन वजन और हॉग इकाई के संदर्भ में अंडे की गुणवत्ता पैरामीटर भी विभिन्न आहार उपचारों के बीच महत्वपूर्ण रूप से भिन्न नहीं थे। हालांकि T_1, T_2 और T_3 समूहों की तुलना में T_4 समूह में अंडे की जर्दी रंजकता में काफी सुधार हुआ था ($P<0.05$) जबकि अंडे की जर्दी का रंग T_1, T_2 और T_3 समूहों के बीच समान था। जर्दी वजन, जर्दी वजन प्रतिशत, जर्दी कोलेस्ट्रॉल, जर्दी ट्राइग्लिसराइड्स और जर्दी सूचकांक जैसे अन्य जर्दी गुणवत्ता पैरामीटर सभी समूहों में सांख्यिकीय रूप से समान थे। मूल आहार में मिर्च के पत्ते के पाउडर पूरक से रुधिर संबंधी पैरामीटर भी प्रभावित नहीं हुए। मुर्गियों के सीरम जैव रासायनिक घटक (सीरम ग्लूकोज, कोलेस्ट्रॉल, ट्राइग्लिसराइड्स, कुल प्रोटीन, एल्ब्यूमिन, ग्लोब्युलिन, कैल्शियम, फास्फोरस, एसजीपीटी और एसजीओटी आहार उपचारों में महत्वपूर्ण रूप से भिन्न नहीं थे। 2.5 प्रतिशत मिर्च पत्ती पाउडर (T_4 समूह) के साथ पूरक समूह में आहार की लागत और औसत आहार सेवन नियंत्रण समूह की तुलना में कम था; हालांकि T_2 और T_3 समूहों में आहार की लागत और औसत आहार सेवन मिर्च पत्ती के पाउडर को जोड़ने से प्रभावित नहीं हुआ था। इसके अलावा, प्रति दर्जन अंडा उत्पादन आहार की लागत सभी उपचारों में सांख्यिकीय रूप से समान थी। वर्तमान अध्ययन से, यह निष्कर्ष निकाला गया है कि अंडे की जर्दी रंजकता और पोषक तत्वों के उपयोग में सुधार के साथ-साथ रोड आइलैंड रेड अंडा उत्पादक मुर्गियों में आहार की लागत को कम करने के लिए मिर्च (कैप्सिकम एनम) के पत्तों के पाउडर को 2.5 प्रतिशत की दर से पूरक किया जा सकता है।

(बी.सी. मंडल)
सलाहकार

(अभिलाषा)
लेखिका