EFFECT OF DIETARY SUPPLEMENTATION OF HERBAL CHOLINE ON PERFORMANCE OF BROILER CHICKEN

T H E S I S

Submitted

In partial fulfillment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE

IN

ANIMAL NUTRITION

BY

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DECLARATION OF STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"EFFECT OF DIETARY SUPPLEMENTATION OF HERBAL CHOLINE ON PERFORMANCE OF BROILER CHICKEN"** or part thereof has not been submitted for any other degree or diploma of any University, not the data have been derived from any thesis/publication of any University or scientific organization. The sources of materials used and all assistance received during the course of investigation have been duly acknowledged.

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DECLARATION OF ADVISORY COMMITTEE

Mr. DHAIGUDE MANGSH VILAS has satisfactorily prosecuted his course of research for a period of not less than one semester and that the thesis entitled **"EFFECT OF DIETARY SUPPLEMENTATION OF HERBAL CHOLINE ON PERFORMANCE OF BROILER CHICKEN"** submitted by his is the result of research work is sufficient to warrant its presentation to the examination in the subject of **ANIMAL NUTRITION** for the award of **MASTER OF VETERINARY SCIENCE** degree by the Maharashtra Animal and Fishery Sciences University, Nagpur.

We also certify that the thesis or part thereof has not been previously submitted by him for a degree of any other University.

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PGR- ANNEXURE – XV

CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF DIETARY SUPPLEMENTATION OF HERBAL CHOLINE ON PERFORMANCE OF BROILER CHICKEN"** submitted by **Mr. DHAIGUDE MANGSH VILAS** to the Maharashtra Animal and Fishery Sciences University in partial fulfillment of the requirement for the degree of **MASTER OF VETERINARY SCIENCE (ANIMAL NUTRITION)** has been approved by the Student's Advisory Committee after oral examination in collaboration with the External Examiner.

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Place:
Date:

(Dhaigude Mangesh Vilas)

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LIST OF ABBREVIATIONS

Abbreviations		**Long Form**
%	:	Per cent
@	:	At the rate
°C	:	Degree Celsius
°F	:	Degree Farenite
AOAC	:	Association of Analytical Chemist
BIS	:	Bureau of Indian Standards
Ca	:	Calcium
CF	:	Crude Fibre
cm	:	Centimeter
CP	:	Crude Protein
CPCSEA	:	Committee for Purpose of Control and Supervision of Experiments on Animals
DM	:	Dry matter
DMB	:	Dry matter basis
EE	:	Ether extract
et al.	:	et alia (and others)
FCR	:	Feed Conversion Ratio
Ft.	:	Feet
g/dl	:	Gram per deciliter
gm	:	Gram
HDL	:	High-Density Lipoprotein
Kg	:	Kilogram
LDL	:	Low-Density Lipoprotein
VLDL	:	Very Low-Density Lipoprotein
mg/dL	:	Milligram per deciliter
ml	:	Milliliter
ND	:	New Castle disease

NFE	:	Nitrogen free extract
OIE	:	Office international des epizootics
RD	:	Ranikhet disease
Rs	:	Rupees
SGOT	:	Serum glutamic-oxaloacetic transaminase
SGPT	:	Serum glutamic pyruvic transaminase
Sq.m.	:	Square meter
TA	:	Total Ash
Wk	:	Week
µg	:	Microgram
APEDA	:	Agricultural and Processed Food Products Export Development Authority
etc	:	Et cetera
Hb	:	Hemoglobin
AST	:	Amino transaminase
IBD	:	Infectious Bursal Disease
viz.	:	Namely
N	:	Nitrogen
DF	:	Degrees of freedom
SS	:	Sum of squares
MSS	;	Mean sum of squares
NS	:	Non-significant
wt.	:	weight

CHAPTER -1

INTRODUCTION

India is one of the biggest producers of eggs and broiler meat in the world. India is the third-largest producer of eggs and the fifth-largest producer of meat. In 2019, 39% of the world's meat production was chicken meat (FAO, 2019). One of the fastest-growing segments of India's agricultural economy is poultry production, which is growing at an annual rate of 8 to 10% (APEDA, 2020). The total output of commercial poultry production in 2019 is 534.74 million, rise 4.5 percent from previous year. Commercial poultry grew by 17.95% in urban regions compared to 3.95% in rural regions (20^{th} livestock census, 2019). A genetic potential, management practices and balanced diet play an important role in improving the performance of broiler bird.

A balanced diet will provide the bird with the precise quantities of biologically available nutrients that it requires. Formulations include supplements to supply minerals, vitamins and particular amino acids in addition to energy and protein. All diets need to include these supplements since they offer the vital nutrients required for health and performance (Thirumalaisamy *et al*., 2016). As synthesis of all vitamin B-complex occurs in rumen of ruminant animals with the help of rumen micro-organisms, dietary supply of vitamin B-complex is not required by them. However, chicken requires dietary supply of all B-complex vitamins, failing to which performance of birds get affected. Furthermore, deficiency of vitamin B-complex may produce various types of deficiency symptoms.

Choline is among the B-group vitamins which play significant role in fat metabolism and also chickens require dietary choline as an essential amino acid. Strecker isolated choline from pig bile for the first time in 1962. Choline is an ammonium hydroxide with the formula beta-hydroxy ethyl trimethyl ammonium (Xu *et al*., 2010). Choline supplementation in chicken feed has been shown to improve broiler chicken growth performance and carcass quality (Attia *et al*., 2005). Choline has an important role in maintaining the structural and functional integrity of cells, organs and bodies (Sidransky, 1985). Free choline, phosphocholine, glycerophosphocholine, phosphatidylcholine and sphingomyelin are all forms of choline that can be transformed to choline or reverted to their original form in food (Zeisel and DaCosta, 2009). In the body, phosphatidylcholine is responsible for removing lipids from the liver as it is essential for the synthesis of very low-density lipids (VLDL), which transport fat to the peripheral tissues (Yao and Vance, 1988). Phosphatidylcholine also makes up 35% of the phospholipids in cell membranes (Battaglia *et al*., 1997). Choline, a component of phospholipids, is required for cell construction and maintenance, as well as appropriate development of the cartilage matrix of bone, including the prevention of perosis in broiler chickens (Workel *et al*., 2002).

Choline is required for the synthesis of acetylcholine, a neurotransmitter that allows nerve impulses to be transmitted. At the end of the parasympathetic nerves resulting in release acetylcholine (Xu *et al*., 2010). Choline, commonly known as lipotropic agent, stimulates the flow of fat and bile to and from the liver, reducing undesired fat build-up in the liver (fatty liver) and also facilitates its transport as a lecithin or enhances its utilization in the liver itself (Wen *et al*., 2014). In the synthesis of betaine from homocysteine and numerous methylation processes, choline is an active donor of labile methyl groups. It has three chemically reactive methyl groups attached to nitrogen atom of glycine molecule. Choline, betaine and methionine are all nutrients that can help improve liver function and detoxification (Ghasura *et al*., 2021). Choline conserves energy by lowering maintenance requirements, resulting in improved overall growth and productivity (Schrama *et al*., 2001).

Commercial choline chloride (CCC), the most typical form of choline, is commonly utilized in poultry diet formulations (Zeisel *et al*., 1989). Choline in standard feedstuffs isn't totally absorbable, but choline chloride is a good source of choline in poultry diets because it is 100 % bio-available. Choline chloride is converted into choline in the body (Wu and Davis, 2005). However, this product has several drawbacks, such as being highly hygroscopic (Selvam *et al*., 2018) because it raises the free water content in the mixture, resulting in a higher reactive potential, it may result in the loss of water-soluble vitamins added to premixes.

Furthermore, the product may form lumps, this presents operational issues in the feed mill (Albers *et al.*, 2002). Trimethylamine (TMA) is formed in the gastrointestinal tract of the birds as a result of this TMA is a short-chain aliphatic amine made from dietary choline and catalysed by enzymes found in the gut bacteria (Selvam *et al*., 2018). Choline requirements for chicken are in the range of 300–2000 mg/kg feed (McDowell, 2000). The young chick has a high dietary choline need (which cannot be supplied by high levels of dietary methionine or other methyl donors), which lowers as chick gets older (NRC, 1994).

Choline deficiency causes fatty liver syndrome and perosis in birds, as well as problems with liver fat mobilisation due to a lack of carrier lipoproteins (Pompeu *et al*., 2011). Fatty liver syndrome is common in fast-growing commercial broiler chicks fed high-energy diets with limited exercise. This is caused by a lack of choline or biotin in the feed. As young chicks are unable to produce choline in the liver, they are more susceptible to this disease than adult birds. Supplementing choline to high-energy broiler diets has been shown to boost feed consumption, body weight gain and feed conversion efficiency (Emmert and Baker, 1997). Choline deficiency causes growth retardation and bone deformation in fast-growing broiler strains (Igwe *et al*., 2015). Tibial-tarsal rotation, tendon dislocation (gastrocnemius) and more typically leg joint hypertrophy are common in affected birds, in adults, ascites and cirrhosis can also occur (Selvam *et al*., 2018).

Some herbal compounds now include large levels of choline in their composition, making them viable alternatives to choline chloride for choline supplementation in broiler diet. Such product contains large levels of phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine that are readily available (Calderano *et al*., 2015; Farina *et al*., 2017), due to which choline bioavailability is higher than choline chloride due to its high gut-receptor affinity. In herbal choline glycerol's, phosphatidyl inositol and phosphatidylserine play important roles in the metabolism, enzymic control and production of phosphatidylcholine, as well as in producing a significant growth response and improving the bio-activity of herbal choline (Khosravinia *et al*., 2015).

To improve energy metabolism and nutrient utilization, herbal choline has a stronger physiological effect. This helps prevent energy from being transformed into lipids and fatty liver syndromes (Koujalagi *et al*., 2018). These vegetable substances are less hygroscopic, which means they don't harm the integrity of other molecules in nutrients like water-soluble vitamins. Various experiments have revealed that adding herbal choline products like Bio Choline to the feed can help broiler chickens avoid the adverse metabolic effects of a high energy diet. Finally, the molecule is not converted to trimethylamine in the gut and does not produce any harmful chemicals.

Thus, many researchers have reported improvement in performance of broiler chicken after dietary inclusion of herbal choline in poultry feed due to its high bioavailability and positive effect on lipid metabolism and energy utilization. The inclusion of herbal choline in poultry diet also regulated in overcoming various problem related with feed manufacturing which commonly occurred in feed mill because of hygroscopic nature of synthetic choline like choline chloride. Considering high bioavailability of herbal choline, it was thought worth to assess the performance of broiler chicken by dietary supplementation of lower, higher and standard level of choline through herbal choline. Hence, the present research work was planned to evaluate the effect varying levels of herbal choline on the performance of broiler chicken with following objectives.

OBJECTIVES:

1. To study effect of herbal choline supplementation on performance of broiler chicken
2. To study effect of herbal choline supplementation on biochemical aspects of broiler chicken
3. To study economics of broiler chicken production

CHAPTER - 2

REVIEW OF LITERATURE

The term "organic" describes the methods used to raise and process agricultural and livestock products, which excludes the use of agrichemicals such as artificial fertilizers and pesticides. Organic poultry farming has more of an emphasis on the health and wellbeing of the animals, responsible environmental practices and product quality than on economic measures like reducing costs and production augmentation (Fanatico *et al*., 2009). To a greater extent, synthetic vitamins and amino acids have been used to improve the health of poultry. However, as humans become more aware, there is an increasing interest in producing animals without synthetic nutrients worldwide. Unconventional management, feeding practices, nutritional strategies and additives including use of neutraceuticals like enzyme, probiotics, synbiotics and organic acids that act as alternative to antibiotics have been tested by several researcher. Similarly herbal amino acids and vitamins are the alternatives to the synthetic ones. Therefore, newer approach of supplementing poultry feed with herbal amino acids and vitamins such as herbal methionine, choline, lysine and biotin is being implemented on large scale in poultry sector.

Choline is quaternary saturated amine with the chemical formula $(CH_3)_3N+CH_2$ CH_2OHX-, where X- is coumterion such as chloride, hydroxide or tartrate. Choline is water soluble essential nutrient. It is grouped within the B-complex vitamins. Choline must be consumed through the diet in order to remain healthy.

Choline and its metabolites are needed for three main physiological purposes: structural integrity and signing roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis) and major source for methyl groups via its metabolites, trimethylglycine (betaine) which participate in the S-adenosylmethionine (SAMe) synthesis pathways.

Commanly use herbal choline products contains selected herbs rich in non-toxic and highly bioavailable choline in conjugated or esterified form (Phosphatidylcholine) and phospholipids. Esterification has the benefit to provide receptor recognition that improves bioavailability and reduces transformation of choline to TMA. Herbal choline also contains glycerol's, phosphatidyl inositol and phosphatidyl serine which play significant role in metabolism, enzymatic modulation and biosynthesis of phosphatidylcholine. Along with PUFA (s) and phospholipids, they optimize fat metabolism and efficient dispersion of liver lipids and produce significant growth response (Koujalagi *et al*., 2018). Herbal choline products are non hygroscopic and does not cause deterioration of vitamins and other feed ingredients. Herbal choline molecule is not converted to trimethylamine in gut and in fact, generates no toxic compounds in digestive tract. The most promissor plants with these proprieties are *Trachyspermum amni*, *Citrullus colocynthis*, *Achyranthus áspera* and *Azadirachta indica*. Many researchers had shown that herbal choline can replace choline chloride in diets for poultry.

The previous research work related to the effect of supplementation of herbal choline on the performance of broiler have shown encouraging results. Therefore, literature pertaining to influence of dietary supplementation of varying levels of herbal choline on the performance of broilers in terms of growth, feed intake, feed conversion ratios, carcass quality, blood biochemical parameters and cost effectiveness of use of herbal choline was reviewed and presented. Similarly, literature pertaining to other herbal feed supplements related with choline is also reviewed and presented herewith.

2.1 Growth Performance

Hassan *et al*. (2005) evaluated the response of slow growing chicks to different dietary levels of choline and betaine during the starter-grower period from 1 to 56 days of age. A basal all-mash corn-soybean meal diet was formulated to contain 872 mg of choline based on native one. This diet was supplemented with 0, 300 and 600 mg of choline, from feed grade choline chloride (50%), which is equal to 872, 1172 and 1472 mg total choline/kg feed. Each choline level was supplemented with, 0.0, 0.072 and 0.144% betaine. Thus, there were nine experimental diets; each diet was fed to 45 chicks divided equally among 5 replicates of nine unsexed chicks each. Results indicated that betaine addition at 0.072% to 872, 1172 or 1472 mg choline- containing diet increased BWG by 3.9, 4.1 and 5.1% and improved FCR by 4.1, 4.3 and 4.8% compared to their respective controls, respectively. Also, betaine addition at 0.144% to 872, 1172 and 1472 mg/kg diet increased BWG by 5.0, 4.9 and 4.4% and improved FCR by 4.8, 4.3 and 4.1% compared to their respective controls, respectively. Betaine addition at 0.072 or 0.144% to the basal diet containing 872 mg choline resulted in similar BWG and FCR.

Waldroup and Fritts (2005) conducted study to evaluate the effects of supplementation of corn-soybean meal-based diets with betaine and choline on performance of male broiler chicks. Diets formulated to meet nutrient levels of top broiler companies were supplemented with either 0 or 1000 mg/kg betaine in combination with either 0 or 1000 mg/kg choline. Each dietary treatment was assigned to eight replicate pens of 60 male chicks of a commercial strain. The results of this study indicated little or no positive benefit in terms of body weight gain and feed conversion.

Chattopadhyay *et al.* (2006) conducted study to determine the comparative efficacy of DL-methionine and herbal methionine on performance of broiler chickens. Two thousand and four hundred day-old commercial broiler (VenCobb) chicks were randomly divided into four dietary treatment groups of 600 birds each. Each treatment group was further subdivided into three replicates of 200 broilers per replicate. The treatments groups were control; control plus 10 g DL-methionine/kg diet; control plus 10 g herbal methionine (Herbomethion, supplied by Indian Research and Supply Co. Ltd.) /kg diet and control plus 15 g herbal methionine/kg diet. There were significant effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio at 0 to 41 days. The body weight and body weight gain of the broilers fed the 15 g herbal methionine/kg diet were heavier than other treatments. Feed conversion ratio of broiler fed 15 g herbal methionine/kg diet was significantly better than that of broilers fed on 10 g herbal methionine. Study demonstrates that herbal methionine can replace DL-methionine very effectively when used at the rate 15 g/kg diet of commercial broiler chicken.

Jadhav *et al.* (2009) conducted experiment in one hundred and eighty VenCobb broiler chickens to evaluate comparative efficacy of herbal and synthetic choline on body weight, weight gain, FCR, nutrient balance and serum activities for 35 days on deep litter. The study indicated that increase in body weight gain was observed in synthetic and herbal fed groups, improvement in FCR in choline supplemented broilers when compared with low choline diet fed (control) birds and elevation in serum glucose levels and notable reduction in serum cholesterol levels in herbal choline incorporated broilers.

Ravinder (2009) conducted study to assess the effect of Herbal biocholine: a better alternative than synthetic choline chloride for profitable production of commercial broilers. 660 unsexed broilers were randomly divided into three groups (N=220 birds/group). The birds were supplemented with synthetic choline chloride or Biocholine as follows: T1-standard diet, with synthetic choline chloride (60%) @ 1 kg/ton of feed, T2-standard diet, without synthetic choline chloride, with Biocholine @ 250 g/ton of feed, T3-standard diet, without synthetic choline chloride, with Biocholine @ 350 g/ton of feed. After the feeding period, results showed that the growth rate and feed conversion efficiency were better in birds supplemented with Biocholine (T2 and T3).

Rodelas *et al*. (2011) conducted feeding trial for 42 days to determine the efficacy of replacing choline chloride (60%) with biocholine alone or in combination with herbal vitamin C and herbal vitamin E on the performance of broilers. A total of 280 straight-run, Cobb day-old chicks were randomly distributed to 28 cages with 10 chicks in each cage. Four treatments, basal diet with synthetic choline chloride (CC), basal diet with biocholine (BC), BC with herbal vitamin C and BC with Herbal vitamin E, were randomly assigned to different cages following a Completely Randomized Design (CRD). Each treatment was replicated 7 times. Results showed that, except for the overall feed consumption, feed efficiency and dressing percentage, the other performance parameters did not differ significantly among treatments. The results obtained in this study indicated that supplementation of diet with biocholine in combination with herbal vitamins C or E significantly reduced feed consumption and improved feed efficiency of the broilers.

Nimkar *et al*. (2012) conducted study to evaluate the effect of supplementation of herbal methionine on growth performance of broiler chickens. Two hundred forty, day old 'Vencob' chicks were distributed into control and three dietary treatments. In Control (C) Maize-Soya based ration was supplemented with synthetic L-Lysine and DL-Methionine, whereas in T1, T2 and T3 the DL-Methionine was replaced by Herbal Methionine at 10, 15 and 20 g/kg feed. The feed intake was significantly higher in T3 treatment group followed by T2, T1 and Control groups. The average weekly live weight of birds in T3 treatment group was found to be significantly higher. The feed conversion ratio was found to be narrow in T3 treatment group. In T1, T2 and control groups the feed conversion ratio was wider than T3 treatment group. This indicates the positive effect of herbal methionine feeding at 20 g/kg levels as compared to lower levels.

Kathirvelan *et al*. (2013) conducted trial to assess the effect of replacement of synthetic choline with herbal choline on growth performance of broilers. A total of 144 commercial broiler (Vencobb 400) day old chicks were divided into three treatment groups namely T1, T2 and T3 each group with six replicates of 8 birds per replicate. T1 group birds are fed with feed devoid of choline (Control), T2 group birds fed with synthetic choline (1kg/ton of feed) and T3 group birds fed with natural choline (1 kg/ton of feed). Results showed that body weight of T2 and T3 birds did not differ significantly during pre-starter, starter and finisher phases, the body weight of control group birds was significantly ($P<0.05$) less as compared to T2 group and the feed conversion ratio (FCR) did not differ in all the treatment groups during all phases.

Hossain *et al.* (2014) studied the effect of varying level of choline chloride supplementation on performance parameters of broiler. Two hundred and forty Hubbard Classic broiler chicks were used. Birds were fed four types of diet, without choline chloride (T0), diet containing 0.1 g / 100 g choline chloride (T1), 0.2 g / 100 g choline chloride (T2) and 0.3 g / 100 g choline chloride (T3). Results indicated that weight gain differed ($P<0.05$) at 5th week irrespective of the level of supplemental choline and feed intake also differed ($P<0.05$) at 5th week. From the results of the study, it was concluded that increasing levels of supplemental choline may progressively improve weight gain and feed efficiency in commercial broiler.

Ibrahim (2014) carried out study to assess the effect of herbal methionine (Phytonin) supplementation on performance and carcass characteristics of broiler chicks. A number of 112 one day old broiler chicks Arber Ackers hybrid were used in this study and divided into 4 equal groups subdivided into 4 replicates with 7 birds each. Birds were fed a basal diet supplemented with 100:0, 50:50, 25:75 and 0:100% synthetic methionine: herbal methionine for a period of 6 weeks. Experimental rations were formulated to furnish the nutrient requirements in the starter and finisher period as recommended by the National Research Council (NRC, 1994). No significant difference was observed among the dietary treatments in terms of performance indices, feed intake, body weight gain and feed conversion ratio.

Reddy (2014) conducted experiments to compare the performance of commercial broilers supplementated with synthetic choline chloride vis-a-vis BioCholine. In most of the experiments, BioCholine was used at half the dose (0.35 to 0.50 kg per ton feed) of choline chloride 60%. The study revealed that BioCholine was more efficacious in improving the performance of broilers in terms of growth and FCR.

Ahmed and Abbas (2015) studied the effects of supplementation of Phytonin herbal natural methionine versus DL methionine on broiler performance. One day old Ross 308 broiler chicks (N=150) were assigned to 5 dietary treatments, each was replicated 3 times. Diet A was the control without supplementing methionine, Diet B was supplemented with 0.15% DL-methionine, Diet C was supplemented with 0.15% herbal methionine, Diet D was supplemented with 0.20% DL methionine and Diet E was supplemented with 0.20% herbal methionine. Result showed that feed intake by birds fed different sources and levels of methionine was not significantly ($P \geq 0.05$) different compared to the control. Birds fed 0.15% herbal methionine diet consumed significantly ($P \leq 0.05$) higher amount of feed versus 0.15% DL methionine. Overall body weight gain of broilers fed 0.15% herbal methionine diet increased by 9.5% compared to those on 0.15% DL methionine.

Calderano *et al.* (2015) assessed the use of a vegetal source of choline as a replacement of choline chloride in the diet for broilers from one to 21 and 22 to 42 days of age. In the first experiment used 1500 Cobb male broilers at one day of age, distributed in a completely randomized design with ten treatments, five repetitions and 30 birds per experimental unit. In the second experiment used 1000 Cobb male broiler at 22 days of age, distributed in a completely randomized design with ten treatments, five repetitions and 20 birds per experimental unit. The treatments consisted of five levels of choline chloride 60% (400, 500, 600, 700 and 800 mg kg-1) and five levels of vegetal source of choline (100, 150, 200,250 and 300 mg kg-1) and concluded that no difference between the performance parameters of broilers in the periods of 1 to 21 and 22 to 42 days of age.

Filho and Possamai (2015) evaluated natural and alternative sources of methionine and choline which can be allowed to use in organic livestock systems to feed broilers produced in Brazil. Seven hundred and twenty one-day-old male Cobb broilers were randomly allocated to four treatments with six replicates of 24 birds each. The treatments consisted in substituting the commonly used DL-methionine 99% by a vegetable source of methionine and choline chloride 60% by alternative source of choline in the form of phosphatidylcholine and daily weight gain, body weight, feed intake, feed conversion ratio, and mortality were evaluated for the periods of 1 to 21 and 1 to 42 days of age. When only choline chloride was substituted by the alternative choline source, broiler performance was not different compared with that of the control group and concluded that group fed the diet with substitution of both DL-methionine 99% and choline chloride 60% by natural sources presented lower daily weight gain, final body weight and feed intake.

Hayat *et al*. (2015) conducted trial to evaluate a natural methionine source on broiler growth performance. A total of 360 straight-run Cobb 500 day-old broiler chicks were distributed to 24 pens containing an of chicks (15) per pen. Six experimental pens were randomly assigned to each treatment. The final two diets were prepared by replacing 100% and 50% by weight of supplemental DLM with MM: MM (basal starter diet with 0.20% MM) and MM + DLM (basal finisher diet with 0.1%

DLM and 0.1% MM). Results revealed that the basal diet has a lower growth performance ($P < 0.05$) than all other diets. However, replacement of synthetic methionine with a naturally sourced methionine has no significant effect ($P > 0.05$) on weight gain, feed consumption and feed conversion ratio.

Igwe *et al*. (2015) conducted study to investigate the effect of graded levels of choline on the growth performance, dressed carcass yield, internal organs and hematological parameters of broiler chickens. A total of three hundred, day old mixed sex broiler chicks were shared into five treatment groups of sixty birds each and twenty birds for each of the three replicates. Birds in (T1) belonged to the control. Those in T2 had 500 mg/kg choline chloride supplementation of their feed, those in T3 had 1,000 mg/kg choline chloride supplementation, and birds in T4 had 1,500 mg/kg choline chloride supplementation, while those in T5 had 2,000 mg/kg choline chloride supplementation. There were significant differences ($P<0.05$) in the average final weight, average weight gain, daily weight gain, daily feed intake and feed conversion ratio with improvement as the choline supplementation increased from 500 to 2,000 mg/kg of feed.

Khosravinia *et al.* (2015) conducted 42-days experiment in that 576 one- day-old Vencobb 308 broiler chicks were used to investigate the effects of lecithin extract (0.5 g/kg), choline chloride 60% (1 g/kg) and Bio choline (1 g/kg) in diets of moderate and high energy in a 4×2 factorial arrangement on performance and certain physiological traits in broiler chickens. It was Concluded that inclusion of Bio choline and lecithin extract in the diet significantly increased average daily gain and improved feed conversion ratio in overall (1 to 42 days) period. The addition of Bio choline and lecithin extract to diet significantly decreased serum γ–glutamyltransferase activity and also revealed that dietary supplementation of commercial lipotropic compounds could remove potential detrimental effects from high energy diets through reducing liver fat and maintaining liver health.

Sharma and Ranjan (2015) conducted study to evaluate the effect of herbal and chemically synthetic choline on physio-biochemical characteristics of chicks. The vaccinated chicks were randomly divided into three groups with one control (T0) and two treatments (TN and TC) having 50 birds in each group with 3 replicates. Chicks in Group- T0 were offered basal broiler starter and grower feed (as per NRC requirements) without any additional source choline chloride, TN was supplemented with N- Choline (supplied by Natural Herbs and Formulations Pvt. Ltd. India) 500 gm/ton of feed and TC with synthetic choline chloride (60% pure) 1000 gm/ton of feed. The results showed that the maximum mean weight gain (749.20 gm. and 1623.80) was observed in TN after 21 and 42 days respectively whereas minimum mean weight gain (612.30 gm. and 1333.20) was observed in T0 among the treatments and control. At 42 days, the mean body weight gain of TN group was significantly higher than TC suggesting that the supplementation of herbal product led to comparatively higher body weight gain than the group fed combination of synthetic choline in basal ration.

Dhumal *et al.* (2016) carried out experiment to evaluate the effect of herbal amino acids in improving performance in broilers. Total 400 day old Vencobb400 broiler chicks were weighed and distributed randomly into five treatment groups A, B, C, D and E with four replicates of 20 chicks each. For treatment group A rations was prepared as per BIS Indian Standards (2007) and treatment group B (a negative control group) ration was formulated with reduced protein (10%). For treatment groups C & D rations were prepared by supplementing the Methiorep @ 0.10% and 0.15% respectively, in negative control group (Treatment group B). Similarly, for treatment group E, negative control group (Treatment group B) ration was supplemented with Superliv @0.05%. The weight gain, FCR for different treatments groups were non-significant. The feed consumption was significantly higher for various treatment groups compared to control A. It was concluded that Methiorep @ 0.1% of feed and Superliv @ 0.05 % of feed can be used efficiently for compensating the performance of broiler in term of body weight gain, feed consumption, feed conversion ratio.

Farina *et al.* (2017) conducted two experiments to evaluate the bioequivalence of a commercial phosphatidylcholine source (Biocholine™) as an alternative to choline chloride and the choline requirements of broilers of a fast- growth strain. In Experiment I, 672 broilers were fed four Biocholine™ levels (0, 100, 200, 300 mg/kg) and three choline levels (200, 400, 600 mg/kg) supplied as choline chloride between 4 and 28 days (d) of age and concluded that birds fed choline chloride presented higher feed intake than those fed Biocholine™. In Experiment II, 462 broilers received diets supplemented with 0, 200, 400, 600 or 800 mg/kg choline as choline chloride and concluded that the supplementation of choline had a quadratic effect on weight gain (WG) but did not affect FCR.

Onel *et al.* (2017) investigated the effects of supplementary choline on amount of choline passing to quail meat and reduction of fat deposition in liver. A hundred and thirty-six one-day-old Japanese quails were divided into eight equal groups of 17 quails each. The experiment was designed based on completely randomized design in two controls and six treatment groups: high stocking density, basal diet, control group (HSD; control 1); normal stocking density, basal diet, control group (NSD; control 2); HSD, basal diet + 900 mg kg−1 choline (A); NSD, basal diet + 900 mg kg−1 choline (B); HSD, basal diet + 1800 mg kg−1 choline (C); NSD, basal diet + 1800 mg kg−1 choline (D); HSD, basal diet + 3600 mg kg−1 choline (E); and NSD, basal diet + 3600 mg kg−1 choline (F). The result showed that total lipid content of liver in E and F groups was reduced by 42% compared with the control groups.

Khose *et al.* (2018) conducted trial to evaluate the efficacy of supplementation of herbal choline as a replacement of synthetic choline chloride in diets on performance of broiler chickens. Three hundred straight-run Cobb-400 day-old broiler chicks were randomly distributed into five treatment groups having three replicates having 20 chicks in each and reared up to 6 weeks of age. The treatment group T1 (control) offered basal diet (BIS, 2007) without synthetic choline chloride-60% (CC) or herbal choline (HC), group T2 basal diet with choline chloride-60% @ 1 kg/ton of feed, groups T3, T4 and T5 offered basal diets with herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed, respectively. It may be concluded that supplementation of herbal choline at 0.350 and 0.500 kg/ton of feed replaces synthetic choline chloride-60% routinely added at 1 kg/ton of broiler feed. The overall performance of the birds under treatment group T5 was found to be better and this supplementation of herbal choline at 0.500 kg/ton of feed was more beneficial in terms of improving the bird’s performance as revealed by European performance efficiency factor.

Selvam *et al.* (2018) designed experiment to establish choline deficiency model (CDM) in broilers for evaluating efficacy of polyherbal formulation (PHF) in comparison with synthetic choline chloride (SCC). A total of 2,550 one-day-old Cobb 430 broiler chicks were randomly assigned to different groups in three experiments. In experiment 1, G1 and G2 served as normal controls and were fed a basal diet with 100% soybean meal (SBM) as a major protein source supplemented with and without SCC, respectively. In G3, G4, G5 and G6 groups, SBM was replaced at 25%, 50%, 75%, and 100% by soy protein isolate to induce a graded level of choline deficiency. In experiment 2, PHF (500 and 1,000 g/ton) in comparison with SCC (1,000 g/ton) were evaluated. In experiment 3, dose-response of PHF (200, 400, and 500 g/ton) with SCC (400 g/ton) was determined. In experiment 2, PHF (500 and 1,000 g/ton) and SCC (1,000 g/ton) showed a similar performance in BWG, FCR and relative liver weight and in experiment 3, PHF produced an optimum efficacy at 400 g/ton and was comparable to SCC in the restoration of serum aspartate aminotransferase activity, abdominal fat, breast muscle lipid content and liver histopathological abnormalities.

Surwade and Rani (2018) assessed the effect of substitution of herbal methionine with DL methionine on the performance of broilers. 120 day-old broiler chicks were randomly assigned into 3 treatment groups (G1, G2 & G3) with 40 chicks in each group, having 4 replicates of 10 birds in each replicate. The diets included Corn soybean meal-based, control diet supplemented with DLM or HM. G1 as a control supplemented with DLM (3.4 and 2.3 kg/ton, respectively in starter and finisher ration) feed only, G2 was given feed supplemented with HM @ 6.5 and 5.2 kg/ton of feed, respectively in starter and finisher ration and G3 was provided commercial feed supplemented with combination of DLM and HM @ 1.7 + 3.2 and 1.15 + 2.60 kg/ton each, respectively in starter and finisher ration. The DL methionine fed group showed significantly increased growth performance in live body weight and weekly body weight gain, feed consumption and feed conversion ratio as well as antioxidant parameters like TABARS, SOD, CAT and GPx significantly higher in group 1 as compared to group 2 and 3. on these findings, it can be suggested that herbal methionine can be effectively substituted for synthetic methionine in the poultry broiler diets up to the extent of 50%.

Marimuthu and D’Souza (2019) conducted trial to evaluate field performance of Kolin Plus, a poly herbal formulation to replace synthetic choline chloride. Studies were conducted in eight different commercial broiler farms across India. Farms were randomly designated as synthetic choline chloride (SCC) and Kolin Plus (KP). Groups were raised on commercial broiler feed supplemented with SCC at 1000 g/ton and KP at 400 g/ton of feed respectively. The results showed that the performance parameters (FCR and body weight) in all the eight KP (400 g/ton) supplemented farms was at par with that of SCC (1000 g/ton) supplemented farms.

Abdullahi *et al*. (2020) carried out study to evaluate growth performance of broiler birds fed with varying level of choline inclusion in their diets; T1 (control), T2 (10g/10kg) and T3 (20g/10kg). A total of 225 marshall broiler chicks were randomly divided into three treatment group of 75 birds each. Each group was divided into five replicates of fifteen birds each laid in a completely randomized design and concluded that treatment two (10g/10kg) performed better in terms of total body weight (TBW) and feed conversion ratio (FCR).

Rana *et al*. (2020) studied the effect of herbal methionine supplementation on growth performance and economics of broiler chicken. Broiler chickens (200 in number) were randomly distributed into 5 treatment groups offered standard diet formulations. T1 birds were offered basal diet without herbal/synthetic methionine. T2, T3, T4 and T5 birds were offered basal diet supplemented with 100% synthetic, 50% synthetic and 50% herbal, 100% herbal methionine and herbal methionine supplementation 50% higher than T4, respectively. Feed intake (g/bird), body weight gain (g/bird) and feed conversion ratio revealed significantly ($P<0.05$) better values for birds fed 100% herbal methionine supplemented diet as compared to control group. Better economic returns were obtained when birds fed diets supplemented with 100% herbal methionine. Thus, it was concluded that supplementation of herbal methionine at 100% level was found to improve growth performance in terms of body weight gain and feed conversion ratio along with better profitable poultry production.

Petrolli *et al.* (2021) conducted trial to determine choline chloride replacement effects by a vegetable choline source, compost by *Trachyspermumamni, Citrullus colocynthis, Achyranthus aspera,* and *Azadirachta indica* in broiler feed. A total of 640 broilers birds were randomly allocated in a completely randomized design, with four treatments and eight repetitions (n = 20), and zootechnical performance (body weight, weight gain, feed conversion ratio, and productive efficiency index), carcass yield, cuts yield and organs (heart, liver, proventriculus, gizzard, and small intestine) and relative weights were evaluated in two choline chloride levels (600 and 800 mg kg-1) and two vegetable choline levels (100 and 200 mg kg-1), added in a corn-soybean meal basal diet, during 42 days of raising and revealed that better feed conversion ratio and production efficiency index in broilers fed vegetable choline, with no differences on body weight and weight gain among broilers and it concludes that vegetable choline can adequately replace choline chloride in broiler feed, with improvement on performance and no compromising carcass, cuts or organ development.

Dias *et al*. (2022) conducted study to assess the use of a plant source of choline to replace choline chloride in broiler diets. Male Cobb broilers (n = 1120) were reared from Day 1 to Day 42 in boxes, experimental design was completely randomized with four treatments, eight replicates and 35 birds per replicate. Treatments were control, choline source 100% choline chloride; and replacement of 25%, 50% and 100% of choline chloride with herbal choline. Herbal choline was derived from *Ocimum sanctum*, *Andrographis paniculata*, *Silybum marianum*, *Glycine max* and *Azadirachta indica*. The results revealed no differences among the treatments for weight gain, feed intake and feed conversion.

Gregg *et al*. (2022) carried out experiment to determine the effect of dietary choline chloride supplementation on growth performance and carcass characteristics of modern broilers reared to 32 days of age. Corn and soybean meal-based diets were formulated to contain an additional 0, 400, 800, 1200, 1600, or 2000 mg of choline chloride per kg of feed above the choline present in the basal diet ingredients. As-hatched Ross 708 × Yield Plus broiler chicks (n = 2160; 30 birds per pen; 44 ± 0.2 g initial BW) were randomly allotted to the experimental diets, reared for 31 days, and processed at day 32. The results indicated that feeding additional choline does not impact growth performance of broilers.

2.2 Blood Biochemistry

Muthukumarasamy *et al*. (2004) conducted experiment to determine effect of biocholine supplementation in Four hundred and fifty day-old straight-run Cobb broiler chicks with body weights ranging from 40.5 to 42.2 g were randomly distributed into six groups, each group having three replicates. Each replicate contains twenty-five chicks. The dietary treatments were: TI - control (CT) prepared as per IS 1347/92 specifications. T2 - CT + choline chloride (60% @ 1 kg/ton of ration, T3 CT+ biocholine @ 0.5 kg/ton of ration, T4 - CT + palm oil @ 25kg/ ton of ration (FT), T5 FT+ choline chloride (60%) @ 1.5 kg/ton of ration and T6, - FT + biocholine @ 0.75 kg/ton of ration. The serum concentration of SGOT, SGPT, ALP, cholesterol, HDL cholesterol and liver lipid contents were significantly lower in T6 compared to T4 supplemented with oil and similar to other treatments and there was no change in serum triglyceride concentration among the treatments. It was concluded that supplementation of biocholine to commercial broiler diet with high fat increased the performance of commercial broiler birds.

Jadhav *et al*. (2009) conducted an experiment in broiler chickens to evaluate comparative efficacy of herbal and synthetic choline on serum activities. One hundred & Eighty broiler chicks of day-old age were randomly divided into three groups with one control and three treatments having 60 birds in each group with 4 replicates of 15 broilers each. The control (T0) was offered low choline feed whereas, other three groups were supplemented with synthetic choline (T1) and AV/BCP/15 (polyherbal formulation) (T2) at the rate of 0.5 g per kg of feed from day old age till the end of trial. It was evident that there was numerical decrease in SGOT and SGPT in treated groups whereas the triglycerides decrease significantly ($P<0.05$) in those birds when compared with control. The protein level was not affected but the cholesterol levels in treated broilers were significantly reduced, the lowest being in herbal choline supplemented group.

Azadmanesh and Jahanian (2014) performed study to investigate the effects of supplemental choline and carnitine on performance and some serum metabolites related to liver health in broiler chicks fed on high-energy diets. A total of 540 day-old Ross 308 chicks were randomly distributed between 9 experimental diets according to a 3 × 3 factorial arrangement of treatments including three dietary metabolizable energy (ME) levels (control, 0.42 and 0.84 MJ/kg greater) and three statuses of dietary supplementation with lipotropic agents (control diet, diets supplemented with either 1000 mg/kg choline as choline chloride or 100 mg/kg carnitine as l-carnitine) with 5 replicates of 12 birds each. Increase in dietary ME level resulted in a linear increase in serum ALT activity. The results revealed that activities of both ALT and AST reduced as the result of introducing lipotropic agents into the diet. A significant ($P<0.05$) interaction occurred between dietary ME level and lipotropic agents; whereby dietary supplementation of lipotropic factors decreased ALT activity in birds fed on diets with the highest ME level.

Hadinia *et al*. (2014) conducted study to compare Bio-efficacy comparison of herbal-methionine and DL-methionine based on performance and blood parameters of broiler chickens on 160 “Ross 308”. DL-Met and H-Met were added to the basal diet in eight experimental treatments with three and four concentrations respectively in starter, grower and finisher period. Blood parameters which were measured at 24 and 42 days of age consisted of serum proteins (total protein, albumin and globulin), serum uric acid, serum fats (low density lipoprotein, high density lipoprotein, triglyceride and cholesterol) and serum enzymes (alanine amino transaminase and aspartate amino transaminase). The results showed that supplemented methionine (Met) sources had no significant effect on blood parameters at 24 day of age. At 42 day of age the amounts of globulin and serum high density lipoprotein (HDL) increased with supplemented methionine ($P < 0.05$).

Sharma and Ranjan (2015) designed an experiment to study the effect of herbal and chemically synthetic choline on physio-biochemical characteristics of chicks. The chicks were randomly divided into three groups with one control (T0) and two treatments (TN and TC) having 50 birds in each group with 3 replicates. Chicks in

Group- T0 were offered basal feed (as per NRC requirements) without any additional source choline chloride, TN was supplemented with N- Choline (supplied by Natural Herbs and Formulations Pvt. Ltd. India) 500 gm/ton of feed and TC with synthetic choline chloride (60% pure) 1000 gm/ton of feed. The results showed that the least mean cholesterol (88.50 mg/dl) was observed in TC after 21 days whereas 114.73 mg/dl was observed in TN after 42 days among the treatments and control. Mean cholesterol was recorded to be significantly lower in treatment groups (TN and TC) than control, however, the values were non-significantly different among two treatments from 1st to 21 days. At 42 days, the mean cholesterol of TN group was significantly lower than TC suggesting that the supplementation of N- Choline (herbal product) lead to reduce cholesterol more efficiently than the group fed combination of synthetic choline in basal ration.

Khose *et al.* (2017) conducted experiment to evaluate influence of herbal choline as a replacement of synthetic choline chloride in broiler diets on serum biochemical profile. Three hundred straight-run Cobb-400 day-old broiler chicks were randomly distributed into five treatment groups having three replicates containing 20 chicks in each and reared up to 6 weeks of age on deep letter housing system. The treatment group T1 (Negative control) offered basal diet (BIS, 2007) without synthetic choline chloride 60% (CC) or herbal choline (HC), group T2 basal diet with choline chloride 60% @ 1kg/ton of feed (Control), groups T3, T4 and T5 basal diets with herbal choline @ 250, 350 and 500 g/ton of feed. The cholesterol level in treatment group T2 and T5 was reduced as compared to treatment group T1. At the end of 6th week, there was increase in level of serum total protein and globulin in groups T2, T4 and T5 as compared to treatment groups T1 and T3. The total serum cholesterol and LDL-cholesterol level in treatment groups T2, T4 and T5 was decreased than groups T1 and T3 also the HDL- cholesterol and triglycerides levels were numerically lower in treatment groups supplemented with choline chloride or herbal choline as compared to non- supplemented groups. It was concluded that the broilers fed diets with herbal choline or synthetic choline chloride found to improve biochemical profile compared to non-supplemented groups.

Khose *et al*. (2019) conducted trial to evaluate the effect of replacement of synthetic choline chloride by herbal choline in diets on liver function enzymes, carcass traits and economics of broiler production. Three hundred straight-run Cobb 400 day-old broiler chicks were randomly distributed into five treatment groups having three replicates having 20 chicks in each and reared up to 6 weeks. The treatment group T1 (control) offered basal diet (BIS, 2007), T2 offered basal diet with choline chloride 60% @ 1 kg/ ton of feed, groups T3, T4 and T5 offered basal diets with herbal choline @ 0.25, 0.35 and 0.50 kg/ton of feed, respectively. The SGPT, SGOT and ALP were significantly ($P<0.05$) reduced in treatment group T5 as compared to control group. The results revealed that the birds fed diet with supplementation of herbal choline (@ 0.350 or 0.500 kg/ton of feed or synthetic choline chloride (@ 1 kg/ton of feed) in broilers showed decreased liver enzymes indicating hepatoprotective property of the choline. Thus, the use of herbal choline and synthetic choline protected the liver function as evident by normalization of SGPT, SGOT and ALP enzyme levels in broilers.

2.3 Liver Histopathology

Gangane *et al.* (2010) conducted an experiment for 0-42 days in day old 150 Vencobb broiler chickens to determine comparative effects of synthetic choline and herbal sources of choline on hepatic lipid metabolism in broilers. Birds were randomly distributed into three groups (T0 - T2), one untreated control and two treatments. Chicks in group T0 were given feed without any additional source choline chloride. Chicks of group T1 were fed with feed mixed with herbal product (Repchol supplied by Ayurvet Ltd., Baddi, India) @ 500 g/ton of feed and T2 was given combination of synthetic choline chloride @ 1 kg/ton (60%) and biotin @ 150 mg/ton of feed. Gross pathological changes in liver were recorded on representative birds per group at the end of the study. It was revealed that inclusion of either synthetic choline or herbal source of choline exerted a hypocholesterolemic effect and also decreased the level of triglycerides as compared to untreated control thus minimizing the incidence of fatty liver and study also revealed no significant changes in the architecture of liver as compared to control.

Sonkusale *et al*. (2011) conducted 6 week long experimental trial to study the supplementation of herbal products as Superliv liq. and Repchol (supplied by M/S Ayurvet Limited, Baddi, India) as herbal sources of choline are efficacious in treatment of liver and kidney due to CCL4. A total of 75 Cobb Day old broilers were divided into three equal groups (n = 25). Group A served as negative control, group B (positive control) and C (treatment group) were induced with FLKS with CCL4 @1 ml/kg body weight orally, administered every 3 days during 15-28 days of trial, treated group C were given Superliv Liq. @ 5 ml/100 chicks/day for first 2 weeks and @ 10ml/100 chicks/day for 3-6 weeks along with drinking water in combination with Repchol @ 500 gm/ton of feed from 0-6 weeks. Gross and histopathology of liver and kidney revealed that there was mild congestion and negligible degenerative changes in group C in contrast to severe changes of FLKS syndrome in group B, concluded that liver tonic Superliv liquid along with phytoadditive Repchol (herbal supplement for choline and biotin) efficaciously ameliorated the CCL4 induced toxicity, when administered prophylactically in broilers.

Selvam *et al*. (2018) designed study to establish choline deficiency model (CDM) in broilers for evaluating efficacy of polyherbal formulation (PHF) in comparison with synthetic choline chloride (SCC). A total of 2,550 one-day-old Cobb 430 broiler chicks were randomly assigned to different groups in three experiments. In experiment 1, G1 and G2 served as normal controls and were fed a basal diet with 100% soybean meal (SBM) with and without SCC. In G3, G4, G5, and G6 groups, SBM was replaced at 25%, 50%, 75% and 100% by soy protein isolate (SPI) to induce a graded level of choline deficiency. In experiment 2, PHF (500 and 1,000 g/ton) in comparison with SCC (1,000 g/ton) were evaluated. In experiment 3, dose response of PHF (200, 400, and 500 g/ton) with SCC (400 g/ton) was determined. The histopathological studies revealed a moderate cell swelling with decreased sinusoidal spaces in hepatocytes of birds reared on CDM. In contrast, reduced cell swelling and mild vacuolar changes were observed in hepatocytes of birds supplemented with SCC or PHF (400 g/ton).

2.4 Carcass Traits

Fouladi *et al*. (2008) carried out experiment to evaluate different levels of choline chloride supplement (CCS 60%) (0, 500 and 1000 mg kg-1) in the basal diet (corn and soybean meal) and their effects on the different parts of carcass weight (breast and thigh) in broiler chicks. A total of 90 Ross 308 strain male broiler were randomly divided into 3 experimental treatments with 3 replicates (10 chicks per pen) and arranged in a completely randomized design. Experimental diets consisted of: Basal diet 0 mg kg-1 choline chloride supplement, basal diet with 500 mg kg-1 choline chloride supplement and basal diet with 1000 mg kg-1 choline chloride supplement. Result showed choline chloride supplement in levels of 1000 and 500 mg kg-1 (T3 and T2, respectively) significantly decrease the liver, spleen and heart weight.

Hossain *et al*. (2014) evaluated the effect of choline chloride supplementation on performance parameters and carcass characteristics of broiler. Two hundred and forty Hubbard Classic broiler chicks were used. Birds were fed four types of diet i.e., diet without choline chloride (T0), diet containing 0.1 g / 100 g choline chloride (T1), 0.2 g / 100 g choline chloride (T2) and 0.3 g / 100 g choline chloride (T3). Results revealed that as either weight gain or feed intake increased progressively, the weight of shank, drumstick bone, thigh bone, dressed carcass, breast meat, thigh meat, drumstick meat, skin, wing meat, heart and lung also increased simultaneously in the same fashion.

Khosravinia *et al*. (2015) conducted experiment on 576 one-day-old Vencobb 308 broiler chicks to evaluate the effects of lecithin extract (0.5 g/kg), choline chloride 60% (1 g/kg) and Bio choline (1 g/kg) in diets of moderate and high energy in a 4×2 factorial arrangement on performance and certain physiological traits in broilers. Carcass yield percentage did not change by supplementation of Bio choline, choline chloride and Lecithin extract to the moderate and also high energy diets. No difference was observed in carcass yield of the birds fed on diets with and without lipotropic agents.

Khose *et al*. (2018) evaluated the effect of supplementation of herbal choline in diets on hematological indices and development of vital organ in broilers. Total three hundred Cobb-400 straight-run day-old broiler chicks were randomly distributed into five treatment groups with three replicates containing 20 chicks in each and reared up to 6 weeks. The group T1 (control) offered basal diet, while group T2 basal diet with choline chloride 60% @ 1 kg/ton of feed, groups T3, T4 and T5 offered basal diets with herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed. It was concluded that the broiler diet supplemented with synthetic choline or herbal choline did not alter the hematological parameters at the 21^{st} day and internal organ weight at 42^{nd} day.

Khose *et al.* (2019) conducted trial to evaluate the effect of replacement of synthetic choline chloride by herbal choline in diets on liver function enzymes, carcass traits and economics of broiler production. Three hundred straight-run Cobb-400-day-old broiler chicks were randomly distributed into five treatment groups having three replicates having 20 chicks in each and reared up to 6 weeks. The treatment group T1 (control) offered basal diet (BIS, 2007), T2 offered basal diet with choline chloride 60% @ 1 kg/ ton of feed, groups T3, T4 and T5 offered basal diets with herbal choline @ 0.25, 0.35 and 0.50 kg/ton of feed and revealed that the supplementation of herbal choline at 0.5 kg/ton of feed was more beneficial in terms of improved liver protection, carcass traits and economics of broiler production and concluded that the herbal choline supplemented at dose levels of 0.35 kg/ton and 0.5 kg/ton of feed was found to be useful to improve the overall performance due to better growth resulting into increase in profit margin of broiler production replacing synthetic choline chloride (1kg/ton) in broiler diets.

Gregg *et al.* (2022) carried out study to determine the effect of dietary choline chloride supplementation on growth performance and carcass characteristics of modern broilers reared to 32 days of age. Corn and soybean meal-based diets were formulated to contain an additional 0, 400, 800, 1200, 1600, or 2000 mg of choline chloride per kg of feed above the choline present in the basal diet ingredients. As-hatched Ross 708 × Yield Plus broiler chicks (n = 2160; 30 birds per pen; 44 ± 0.2 g initial BW) were randomly allotted to the experimental diets, reared for 31 days, and processed at day

32. Results revealed that a potential benefit of choline supplementation as carcass weight may be diverted to other more profitable carcass parts such as breast meat. However, no other impacts of choline supplementation of individual carcass part yields were observed.

2.5 Economics

Ghasura *et al.* (2021) conducted experiment to investigate the effect of dietary supplementation of choline from different sources on the performance and economics of commercial broiler chicken. Day old broiler chicks (Vencobb, n=216) were randomly distributed into nine experimental groups with four replicates of six birds each. The experimental groups (T1 to T9) were fed with diet containing choline chloride 60% @ 1000 g/ton, 750 g/ton and 500 g/ton feed in T1 (Control), T2 and T3 ration and herbal choline @ 1000 g/ton, 750 g/ton and 500 g/ton feed was given in T4, T5, T6 ration and phosphatidylcholine @ 1000 g/ton, 750 g/ton and 500 g/ton feed-in T7, T8, T9 ration and revealed no adverse effects of feeding choline from different sources in diet also return over feed cost was highest in broilers fed a diet with choline chloride 60 % @ 1000 g/ton feed.

CHAPTER - 3

MATERIALS AND METHODS

The proposed research programme entitled “Effect of dietary supplementation of herbal choline on performance of broiler chicken” was carried out at the Department of Animal Nutrition and poultry shed of the Department of Poultry Science, College of Veterinary and Animal Sciences, Udgir. The experimental work of present study was carried out for 42 days.

3.1 Procurement of Herbal Choline Powder

The herbal choline powder was purchased from local market at Udgir. It was present in free flowing powder form easy to mix homogenously in basic feed.

3.2 Experimental Design and Treatments

Total two hundred and forty (“VenCobb430y” strain), day old broiler chicks were purchased from commercial hatchery.

Table 3.1 Dietary treatments allotted for various experimental groups

Sr. No.	Dietary Treatments	No. of birds in each replica	Total No. of Birds
1	Group T_0 Standard broiler diet (BIS, 2007) with commercial choline chloride 60% supplement @ 0.09% of feed	15 15 15 15	60
2	Group T_1 Standard broiler diet (BIS, 2007) with herbal choline @ 0.075% of feed (Equivalent to synthetic choline)	15 15 15 15	60
3	Group T_2 Standard broiler diet (BIS, 2007) with herbal choline @ 0.055% of feed (25% less than synthetic choline equivalent)	15 15 15 15	60
4	Group T_3 Standard broiler diet (BIS, 2007) with herbal choline @ 0.095% of feed (25% more than synthetic choline equivalent)	15 15 15 15	60
	Total		240

The experimental chicks were allocated randomly into four groups viz. T_0, T_1, T_2 and T_3 of uniform size, each consisting of 60 chicks with four replicates and 15 chicks in each replica. The standard broiler diet formulated as per with BIS (2007) was provided to all groups. All the diets were iso-nitrogenous and iso-caloric. The experiment was conducted for the period of six weeks. The experimental birds were reared on deep litter system with standard management practices. The dietary treatments allocated to birds from various experiment groups was as per Table 3.1.

3.3 Housing Management

The experimental chicks were reared under deep litter system. Before scattering the litter, the shed's floor was properly cleaned, scrubbed, and disinfected. Total sixteen compartments were made by using chicken net. The compartments of birds were made by considering space requirement of birds. The compartment was measured 4x5 square feet in dimension per 15 birds. The 1.5 sq. ft. for every bird, space was allotted and the remaining area was used for feeders and drinkers. The sixteen compartments had all been properly labelled. Provisions for brooding were made (Plate 3.1 and 3.2). After making all the necessary arrangements and cleaning the shed, limestone powder was applied to the floor as a disinfectant. The biosecurity measures to protect the newly arrived chicks from infection were followed properly. Before the arrival of chicks, pre-starter, starter and finisher broiler feeds were prepared as per BIS (2007).

Chicks were properly unboxed and weighed after arriving (Table 3.2 and Plate 3.3) and randomly distributed to each compartment containing fifteen birds. Extra care was taken during the initial period to ensure efficient feeding and watering of chicks. Day before arrival of chicks, rice straw was uniformly spread on the floor. Newspapers were spread on rice straw. Brooder area was stabilized for 95°F temperature before arrival of chicks (Plate 3.1). The stress alleviator agent and electoral was supplied to the birds through water. The feed was kept in chick feeder plate as well as spread on newspaper. Up to the age of three weeks, appropriate brooding was carried out. The birds were provided with clean and fresh water throughout the experimental duration. *Ad-libitum* feed was provided to the birds. The recommended vaccination schedule

Plate 3.1 Preparation of shed before arrival of chicks

Plate 3.2 Shed after arrival of chicks (Day old)

(Table 3.3) was followed for birds from all the experimental groups to protect them from various diseases.

Table 3.2 Average body weights of day-old experimental chicks

Groups	Weight (g)
T_0	50.87
T_1	46.37
T_2	49.25
T_3	47.55

Table 3.3 Vaccination schedule followed for experimental birds

Sr. No	Vaccine	Age of Vaccination	Dose/ Route
1	Ranikhet	7^{th} day	One drop in eye (Intraocular)
2	Gumbaro (IBD)	14^{th} day	One drop in eye (Intraocular)
3	Ranikhet booster dose	21^{st} day	Through drinking water

3.4 Feeding Management

The raw feed ingredients were procured from local market. Feed formulation was done according to BIS, (2007). Procured feed ingredients were ground at feed mill of the Department of Animal Nutrition, COVAS, Udgir and diets were prepared as pre-starter (1^{st} week), starter (2-3 weeks) and finisher (4-6 weeks) and formulation of the same is presented in Table 3.4, 3.5 and 3.6, respectively. Major ingredients used for feed formulation were maize and soybean meal. Group T_0 (control) received standard broiler diet (BIS, 2007) with commercial choline chloride (60%) @ 0.09% of feed. Whereas, group T_1, T_2 and T_3 received standard broiler diet (BIS, 2007) with herbal choline @ 0.075% (supplying choline equivalent to synthetic choline), 0.055% (supplying 25% less choline than synthetic choline) and 0.095% (supplying 25% more choline than synthetic choline). The diets of all experimental groups were iso-caloric and iso-nitrogenous.

Thus, group T_0 (control) received choline as per BIS (2007) through feed containing choline chloride (60%) @ 0.09%. Group T_1 received choline as per BIS (2007) through feed containing herbal choline @ 0.075%. Group T_2 received 25% less choline than BIS (2007) through feed in which herbal choline was included @

0.055%. Whereas group T_3 received 25% more choline than BIS (2007) with inclusion of herbal choline @ 0.095% in feed.

Table 3.4 Ingredients composition (%) of pre-starter diets

Sr. No.	Ingredients	Experimental groups			
		T_0	T_1	T_2	T_3
1	Maize	52.2	52.2	52.2	52.2
2	Soybean meal	40.6	40.6	40.6	40.6
3	Vegetable oil	2.8	2.8	2.8	2.8
4	Di-calcium phosphate	1.9	1.9	1.9	1.9
5	Limestone powder	1.5	1.5	1.5	1.5
6	Salt (NaCl)	0.25	0.25	0.25	0.25
7	DL- methionine	0.17	0.17	0.17	0.17
8	L-Lysine	0.14	0.14	0.14	0.14
9	Trace mineral mixture	0.09	0.09	0.09	0.09
10	Coccidiostat	0.09	0.09	0.09	0.09
11	Choline chloride (60%)	0.09	0	0	0
12	Herbal choline	0	0.075	0.055	0.095
13	Toxin binder	0.09	0.09	0.09	0.09
14	Acidifier	0.11	0.11	0.11	0.11
15	Vitamin premix	0.03	0.03	0.03	0.03

Table 3.5 Ingredient composition (%) of starter diets

Sr. No.	Ingredients	Experimental groups			
		T_0	T_1	T_2	T_3
1	Maize	52.2	52.2	52.2	52.2
2	Soybean meal	38.4	38.4	38.4	38.4
3	Vegetable oil	5	5	5	5
4	Di-calcium phosphate	1.9	1.9	1.9	1.9
5	Limestone powder	1.5	1.5	1.5	1.5
6	Salt (NaCl)	0.25	0.25	0.25	0.25
7	DL- methionine	0.18	0.18	0.18	0.18
8	L- Lysine	0.1	0.1	0.1	0.1
9	Trace mineral mixture	0.09	0.09	0.09	0.09
10	Coccidiostat	0.09	0.09	0.09	0.09
11	Choline chloride (60%)	0.09	0	0	0
12	Herbal choline	0	0.075	0.055	0.095
13	Toxin binder	0.09	0.09	0.09	0.09
14	Acidifier	0.11	0.11	0.11	0.11
15	Vitamin premix	0.03	0.03	0.03	0.03

Table 3.6 Ingredient composition (%) of finisher diets

Sr. No.	Ingredients	Experimental groups			
		T_0	T_1	T_2	T_3
1	Maize	56.9	56.9	56.9	56.9
2	Soybean meal	33	33	33	33
3	Vegetable oil	5.9	5.9	5.9	5.9
4	Di-calcium phosphate	1.9	1.9	1.9	1.9
5	Limestone powder	1.5	1.5	1.5	1.5
6	Salt (NaCl)	0.25	0.25	0.25	0.25
7	DL- methionine	0.15	0.15	0.15	0.15
8	L- Lysine	0.08	0.08	0.08	0.08
9	Trace mineral mixture	0.09	0.09	0.09	0.09
10	Coccidiostat	0.09	0.09	0.09	0.09
11	Choline chloride (60%)	0.09	0	0	0
12	Herbal choline	0	0.075	0.055	0.095
13	Toxin binder	0.09	0.09	0.09	0.09
14	Acidifier	0.11	0.11	0.11	0.11
15	Vitamin premix	0.03	0.03	0.03	0.03

3.5 Proximate Composition of Experimental Broiler Diets

The nutrient composition of experimental diets during various phases viz. pre-starter, starter and finisher were as per BIS (2007). The nutrient composition of experimental broiler diets is given in Table 3.7

Table 3.7 Nutrient composition of experimental broiler diets (DMB%)

Nutrient %	Different phases of broilers		
	Pre-starter (0-7 days)	Starter (8-20 days)	Finisher (21-42 days)
DM	92.95	92.52	92.78
CP	23.07	22.05	20.04
EE	3.16	3.44	3.81
CF	5.38	5.58	5.21
TA	6.93	6.65	6.16
NFE	61.46	62.28	64.78
ME Kcal/kg (calculated)	3000	3100	3200

*****Nutrients: DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fibre, TA, total ash; NFE, nitrogen free extract; ME, metabolisable energy.

3.6 Ethical Permission

The present experiment was conducted in the Department of Animal Nutrition, College of Veterinary and Animal Sciences, Udgir, Dist. Latur. All the experimental procedures carried out during trial period were approved by the Institutional Animal Ethical Committee constituted by Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Department of Animal Welfare, Ministry of Forest, Environment and Climate Change, Government of India which is established under Chapter 4 Section 15 (1) of the Prevention of Cruelty to Animals Act,1960. (Approved No. I/22, Dated.06/05/2022).

3.7 Growth Performance

3.7.1 Weekly feed intake

Records of feed offered and refused were maintained daily throughout experimental period of 42 days. The feed consumption was calculated by subtracting refusal from the total feed offered on the previous day. Weekly feed consumption was calculated by adding the daily average feed consumption on particular weeks.

3.7.2 Weekly body weight gain

The chicks were weighed individually at the start of the experiment and thereafter at weekly intervals for six weeks. From this data average weekly body weight gain for different experimental groups was calculated.

3.7.3 Feed conversion ratio

Feed conversion ratio was calculated by dividing the weekly feed consumption by weekly weight gain.

3.7.4 Mortality

The death of birds during an experiment, if any, was recorded as and when the event happened and the post-mortem examination was conducted to know the exact cause of the death so that necessary precautionary measures can be taken to avoid further mortality.

3.8 Proximate Analysis

Proximate analysis of ground feed of various treatment groups including control group was done as per the method of the Association of Official Analytical Chemists (AOAC, 1996) (Plate 3.4).

3.8.1 Moisture content

For determination of moisture content, hot air oven method was utilized. The percentage of dry matter was measured using electronic balances (least count 0.1 mg) and moisture content was calculated as

$$\text{Moisture (\%)} = \frac{\text{Weight of sample before drying} - \text{Weight of sample after drying}}{\text{Weight of sample before drying}} \times 100$$

3.8.2 Crude Protein percentage (CP)

Protein content was determined by using Kelplus Automatic Protein Analyzer. Nitrogen content was calculated by following equation

$$\text{Nitrogen (\%)} = \frac{14 \times \text{Normality of acid} \times \text{Titration reading} \times 100}{\text{Sample weight} \times 1000}$$

The crude protein of sample was calculated by the following equation:

Crude Protein (%) = N X 6.25 (protein factor specific to sample)

3.8.3 Crude Fat percentage (Ether Extract)

Fat content was determined by using Kelplus Automatic Fat Analyzer. It was calculated by following equation

$$\text{Ether Extract (\%)} = \frac{W2 - W1}{SW} \times 100$$

Where, W_2 = Weight of receiver flask with fat

W_1 = Weight of empty receiver flask

SW = weight of sample

3.8.4 Total Ash content

2 gm sample was taken into the preweighed crucible and weighed. Then, the sample was charred and placed in muffle furnace at 600°C for 4-6 hrs., cooled in desiccator and reweighed. The total ash content was determined by using following equation

$$\text{Total Ash (\%)} = \frac{\text{Weight of ash} \times 100}{\text{Weight of sample}}$$

3.8.5 Crude Fibre percentage (CF)

The finely ground feed sample was boiled with 1.25% sulphuric acid solution (W/V) for 45 minute and wash with distilled water to make it acid free. This was followed by boiling the residue with 1.25% sodium hydroxide for 45 minutes and washed with hot distilled water to make it alkali free. Residue sample with crucible was dried, weight and then ignited in muffle furnace (600°C ± 30°C) to obtain its ash content. Crude fibre was calculated by deducting ash from the weight of dried residue.

$$\text{Crude Fibre (\%)} = \frac{\text{Loss in weight} \times 100}{\text{Weight of sample}}$$

3.8.6 Nitrogen free extracts (NFE)

NFE percentage was calculated by subtracting sum of the percentage of CP, EE, CF and total ash from 100.

$$\text{NFE (\%)} = 100 - (\text{CP\%} + \text{CF\%} + \text{EE \%} + \text{Ash\%})$$

3.9 Metabolic Trial

A metabolic trial of seven days duration was conducted during 6^{th} week of experimental period to determine digestibility of DM, EE, CP, CF and NFE. A preliminary period of 2 days was given for adaptation to the birds to new housing system and management followed by collection period. Two birds from each replicate were randomly selected and transferred to metabolic cages. Weighed feed was offered to the birds for seven days and droppings were collected separately daily at 9 AM for each bird. The total amount of excreta obtained in 24 hours period was weighed. The fecal sample for protein estimation was collected daily in separate container and preserved with 5% sulphuric acid (W/V) 10 ml/50 gm fecal sample. The fecal samples from each bird were oven dried to constant weight for estimation of moisture daily during seven days metabolic trial and the pooled oven dried samples for seven days were used for proximate analysis. Analysis of fecal sample was done as per AOAC (1996).

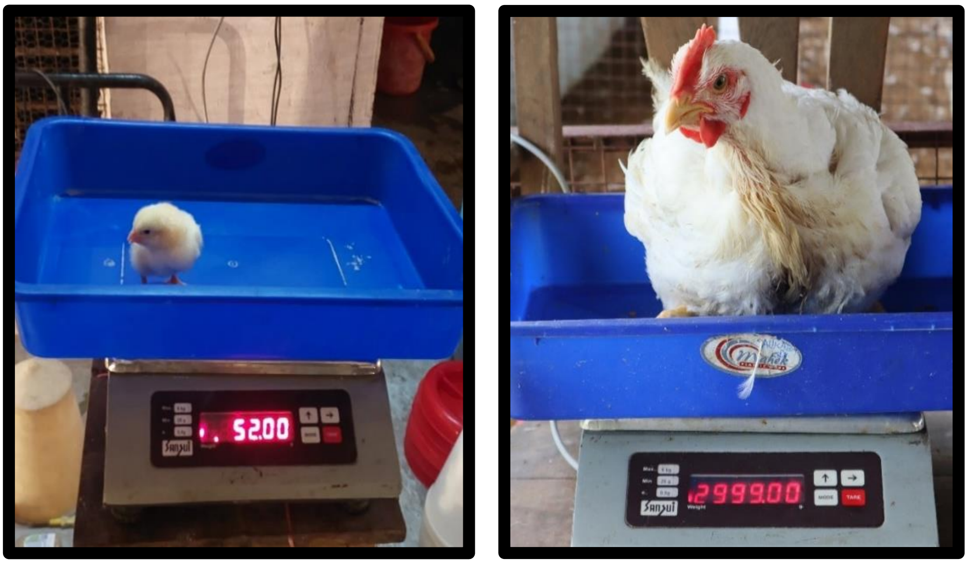


Plate 3.3 Weighing of broiler birds @ day old and 42 days

Plate 3.4 Proximate Analysis

3.10 Carcass Traits

For carcass evaluation studies, four birds from each group were sacrificed at the end of 6th week. The birds were sacrificed by severing the jugular vein and allowed to bleed completely following Halal method. The heads of slaughtered birds were removed at the atlanto-occipital joint and shank at hock joint. Defeathering of slaughtered birds was done manually. Manual evisceration was carried out to determine carcass traits (Plate 3.5).

3.10.1 Dressing percentage

The dressed weight thus obtained was recorded as follows

Dressed weight= Live weight – (blood+ feathers+ head shank+ skin losses+ viscera)

Dressing percentage was calculated as follows

$$\text{Dressing percentage} = \frac{\text{Carcass weight}}{\text{Pre} - \text{slaughter live weight}} \times 100$$

3.10.2 **Eviscerated yield** percentage

The eviscerated weight was recorded and its percentage was calculated.

Eviscerated weight = Dressed weight – Weight of giblet

$$\text{Eviscrated yield \%} = \frac{\text{Dressing weight without giblet}}{\text{Pre} - \text{slaughter live weight}} \times 100$$

3.10.3 Giblet weight

The weight of heart, liver and gizzard were recorded on digital weighing balance in grams.

3.10.4 Other weights

The weight of other organs such as breast, thigh, and drumstick were also recorded on digital weighing balance having least count of 0.1 mg.

3.11 Serum biochemistry

Blood samples were collected at the end of trial from one bird of each replicate thus four birds from each group. Blood samples were withdrawn in serum vials then centrifuged for 1500 rpm for 15 minutes. The upper layer of serum drawn and transferred to eppendorf tubes. Serum stored at -20°C in a deep freezer until the time of biochemical estimation. The biochemical estimation of blood serum was determined with the help autoanalyzer using appropriate kits.

The individual serum samples were analyzed for total protein, albumin and globulin, SGPT, SGOT and Lipid profile. The methodology and the set of reagents used in respect of each parameter were as per the recommendation of the manufacturer of the analyzer system.

3.12 Liver Histopathological Examination

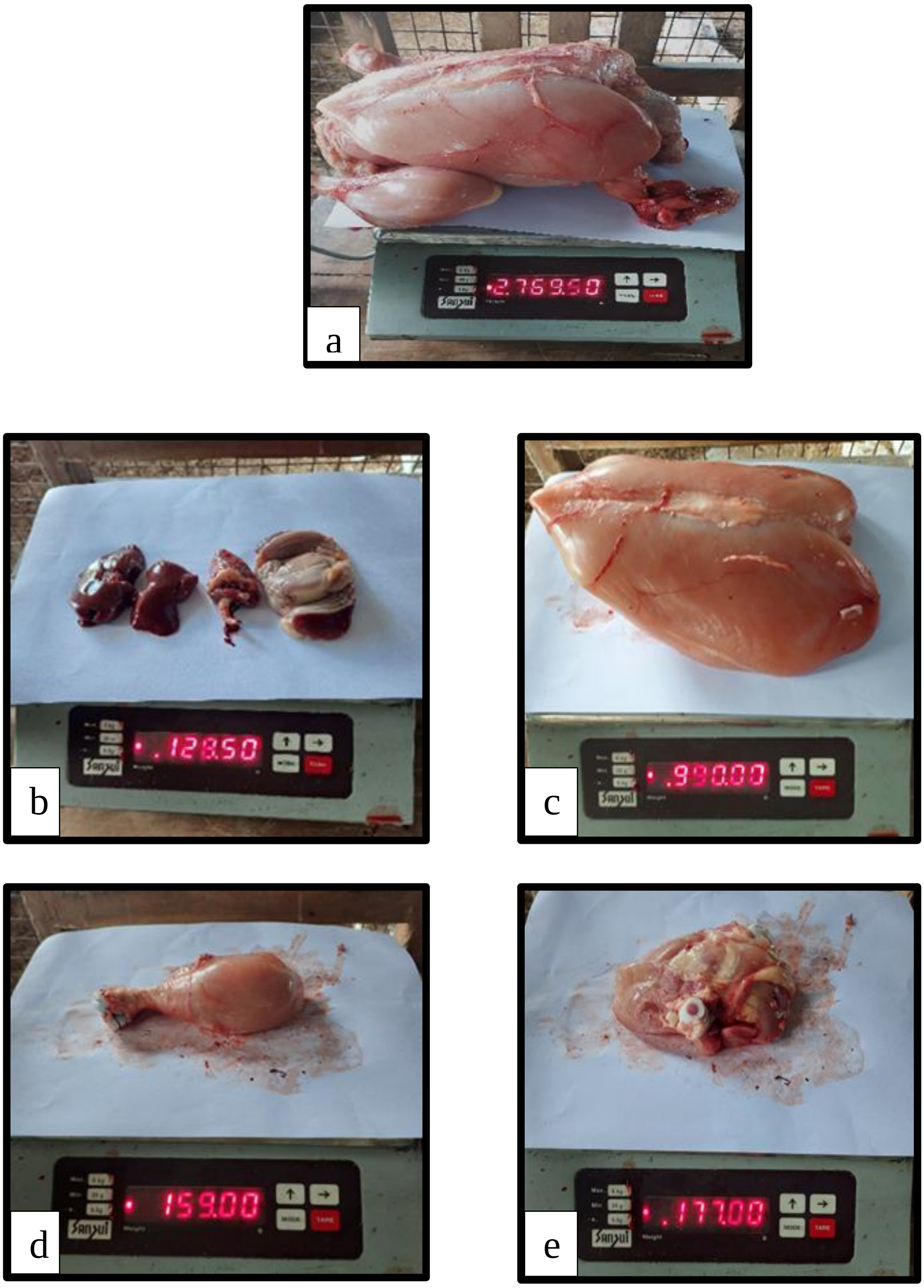
The 0.5 cm tissue section of liver was collected after detailed necropsy examination for Histopathology. The tissue section was taken from two birds from each group regardless of presence or absence of pathological changes. The tissue was fixed in 10% buffered formalin for 48 hours. Then, the tissue sections were embedded in paraffin following standard procedure. Cross-sections of 4-6 µm were stained with H&E and the detail microscopic examination was recorded for each group as per standard protocol described by Bancroft and Gamble (2007).

3.13 Economics of Broiler Production

Economics of supplementation of herbal choline on broiler production was calculated at the end of 6^{th} week. For calculation the cost of chick, cost of total feed consumed by birds, cost of litter material, vaccination, medication and other miscellaneous expense were considered. The cost of herbal choline was also considered for calculating economics of broiler production of birds from treatment groups.

3.14 Statistical Analysis

The data collected throughout experiment period were tabulated and subjected to statistical analysis as per Snedecore and Cochran (1989) by using Completely Randomized Design.



(a: Dressed weight, b: Giblet weight, c: Breast weight, d: Drumstick weight, e: Thigh weight)

Plate 3.5 Carcass Traits

CHAPTER – 4

RESULTS AND DISCUSSION

The objective of present study was to assess the effect of dietary supplementation of varying levels of herbal choline on performance of broiler chicken. The trial involved 240 broiler birds randomly distributed into four uniform groups viz, T_0, T_1, T_2 and T_3 with each group having 60 chicks divided in four replicates. Group T_0 (control) received standard broiler diet (BIS, 2007) with commercial choline chloride 60% @ 0.09%, while groups T_1 received standard broiler diet with herbal choline @ 0.075% (Equivalent to synthetic choline), T_2 received standard broiler diet with herbal choline @ 0.055% (25% less than synthetic choline equivalent) and T_3 received standard broiler diet with herbal choline @ 0.095% (25% more than synthetic choline equivalent). The diets of all the experimental groups were iso-caloric and iso-nitrogenous. Each experimental group was fed with standard broiler diet formulated as per BIS (2007). The assessment of inclusion of varying levels of herbal choline in broiler pre-starter, starter and finisher diet was carried out by determining growth performance (body weight and weight gain), feed intake, feed conversion ratio, nutrient metabolizability, blood biochemistry (total protein, albumin, globulin, SGOT, SGPT, Lipid profile), histopathology of hepatic cells and carcass traits. Economics of broiler production was also studied. The data obtained were statistically analyzed for interpretation of the results.

4.1 Growth Performance

The growth performance of experimental broiler birds was evaluated on the basis of average weekly body weight, average weekly body weight gain, feed consumption and FCR.

4.1.1 Weekly live body weight

The data related to average weekly live body weights (g) of the experimental birds from day old to six weeks of age from various experimental groups are presented in Table 4.1. The same is depicted graphically in Fig.4.1.

It is seen from Table 4.1 that average initial live body weights of day-old chicks for groups T_0, T_1, T_2 and T_3 were 50.88 ± 1.25, 46.38 ± 2.47, 48.98 ± 1.14 and 47.55 ± 1.01 g, respectively. At the end of six weeks trial period the average live body weights for groups T_0, T_1, T_2 and T_3 were 2745.97 ± 32.62, 2786.33 ± 12.46, 2705.80 ± 26.64 and 2718.66 ± 38.71 g, respectively. From the data it is observed that group T_1 showed the highest average live body weight at the end of six weeks followed by groups T_0 (control), T_3 and T_2, however, difference among various experimental groups was non-significant.

It is observed that during second week of experimental period, the average weekly live body weights (g) of birds from group T_1 was highest followed by groups T_3, T_2 and T_0. The statistical analysis revealed significantly ($P<0.01$) higher average live body weights of birds from group T_1 than groups T_0, T_2 and T_3. Further, it is observed that average live body weights of birds from group T_0 and T_3 were significantly ($P<0.01$) higher than group T_2, however, difference between group T_0 and T_3 was comparable.

Table 4.1 Average weekly live body weight (g) of birds from various experimental groups

Weeks	Experimental Groups				CD
	T_0	T_1	T_2	T_3	
0	50.88 ± 1.25	46.38 ± 2.47	48.98 ± 1.14	47.55 ± 1.01	NS
1	180.29 ± 5.69	182.63 ± 1.63	169.98 ± 1.60	180.63 ± 4.28	NS
2	495.88^b ± 7.18	521.63^a ± 6.24	463.50^c ± 2.84	501.50^b± 5.78	17.70
3	954.75 ± 15.86	986.13 ± 8.46	969.50 ± 5.10	1019.75 ± 28.56	NS
4	1636.13 ± 28.50	1645.56 ± 15.64	1685.10 ± 28.64	1705.88 ± 12.01	NS
5	2260 ± 12.42	2264.59 ± 16.88	2255 ± 27.50	2244.90 ± 7.63	NS
6	2745.97 ± 32.62	2786.33 ± 12.46	2705.80 ± 26.64	2718.66 ± 38.71	NS
Mean	1189.13 ± 3.83	1204.75 ± 6.67	1185.41 ± 11.07	1202.69 ± 9.88	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet+ herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton.
Values bearing different superscripts in a row differ significantly ($P<0.01$). NS: Non significant

The mean average weekly live body weights for group T_0, T_1, T_2 and T_3 were 1189.13 ± 3.83, 1204.75 ± 6.67, 1185.41 ± 11.07 and 1202.69 ± 9.88 g, respectively. From the data it is seen that the birds from group T_1 showed the highest mean average weekly live body weights followed by groups T_3, T_0 and T_2.

The average weekly live body weight data of various experimental groups obtained at the end of experimental period were statistically analyzed and the findings are shown in Table 4.2. Though there was a numerical difference among the mean average weekly live body weights of the four experimental groups, on statistical analysis it revealed no significant difference among various experiment groups.

Table 4.2 Analysis of variance mean for average weekly live body weights (g)

Source of variation	DF	SS	MSS	F cal
Treatments	3	1118.977	372.992	1.336^{NS}
Error	12	3350.056	279.171	-
Total	15	-	-	-

Coefficient of Variation = 1.398 Treatments found to be Non Significant

Finding of present study are matching with Calderano *et al*. (2015) who observed no significant differences in live body weights on dietary supplementation of vegetal source of choline @ 100, 150, 200, 250 and 300 mg kg-1 in male broiler birds. Filho and Possamai (2015) also revealed no significant difference in body weights of broiler birds on dietary supplementation of natural and alternative sources of methionine and choline. Also, Kathirvelan *et al*. (2013) reported no significant difference in body weights in broilers on dietary supplementation of natural choline @ 1 kg/ton of feed.

On contrary, Khose *et al*. (2018) observed significant improvement in live body weights on dietary supplementation of herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed. Muthukumarasamy *et al*. (2004) also reported significant ($P<0.05$) improvement in the body weights of broilers on supplementation of Biocholine @ 0.75 kg/ton.

4.1.2 Weekly weight gain

The data concerned with average weekly body weight gain of the experimental birds from different groups from first to sixth week of experimental period are presented in Table 4.3. The same is depicted graphically in Fig 4.2.

It is observed from Table 4.3 that the mean of average weekly body weight gains for groups T_0, T_1, T_2 and T_3 were 449.18 ± 5.55, 456.66 ± 1.67, 442.80 ± 4.58 and 445.18 ± 6.47 g, respectively. At the end of present study, the cumulative body weight gain for the groups T_0, T_1, T_2 and T_3 were 2695.11 ± 33.33, 2740.37 ± 10.03, 2656.83 ± 27.50 and 2671.11 ± 38.83 g, respectively.

Table 4.3 Average weekly body weight gain (g) of birds from various experimental groups

Weeks	Experimental Groups				CD
	T_0	T_1	T_2	T_3	
1	129.42 ± 6.82	136.25 ± 2.68	121.00 ± 2.11	133.08 ± 4.90	NS
2	315.58^b ± 6.30	339.00^a ± 5.88	293.53^c ± 3.30	320.87^b ± 4.90	16.09
3	458.88 ± 20.38	464.50 ± 14.09	506.00 ± 6.22	518.25 ± 27.18	NS
4	681.38 ± 14.72	659.44 ± 16.05	715.60 ± 28.86	686.13 ± 16.68	NS
5	623.88^a ± 27.85	619.03^a ± 5.55	569.90^b ± 10.50	539.03^b ± 6.59	47.74
6	485.97 ± 13.92	521.74 ± 2.77	450.80 ± 5.25	473.75 ± 3.30	NS
Mean	449.18 ± 5.55	456.66 ± 1.67	442.80 ± 4.58	445.18 ± 6.47	NS
CWG	2695.11 ± 33.33	2740.37 ± 10.03	2656.83 ± 27.50	2671.11 ± 38.83	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet + herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton.
Values bearing different superscripts in a row differ significantly (P<0.01). NS: Non significant.

The average live weight gain during the second and fifth week of experimental period revealed a significant (P<0.01) difference among various experimental groups. During second week of experimental period, the average weekly body weight gain of birds from group T_1 was significantly (P<0.01) higher than all other experimental groups, however, difference between group T_0 and T_3 was comparable. Further, it is seen that average body weight gain of birds at 2nd week from T_1 and T_3 was significantly higher than group T_2.

The data of average weekly body weight gain (g) obtained during fifth week revealed significantly (P<0.01) higher body weight gain for control and T_1 group than group T_2 and T_3, however, difference between T_0 and T_3 and that of T_2 and T_3 was non-significant.

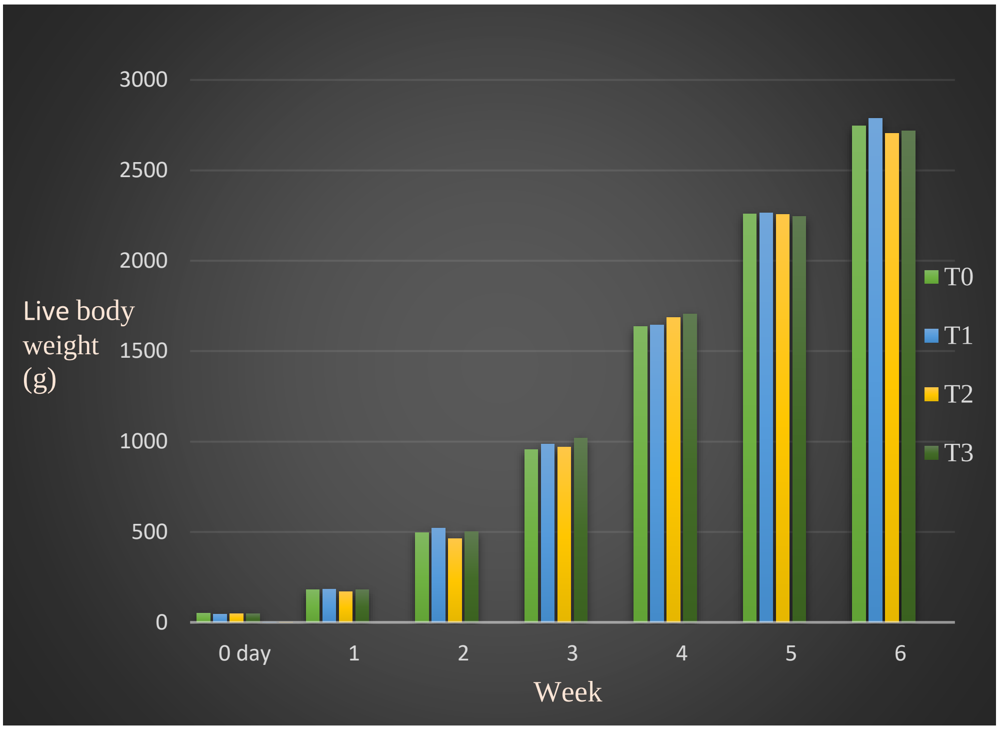


Fig. 4.1 Average weekly live body weights (g) of birds from various experimental groups

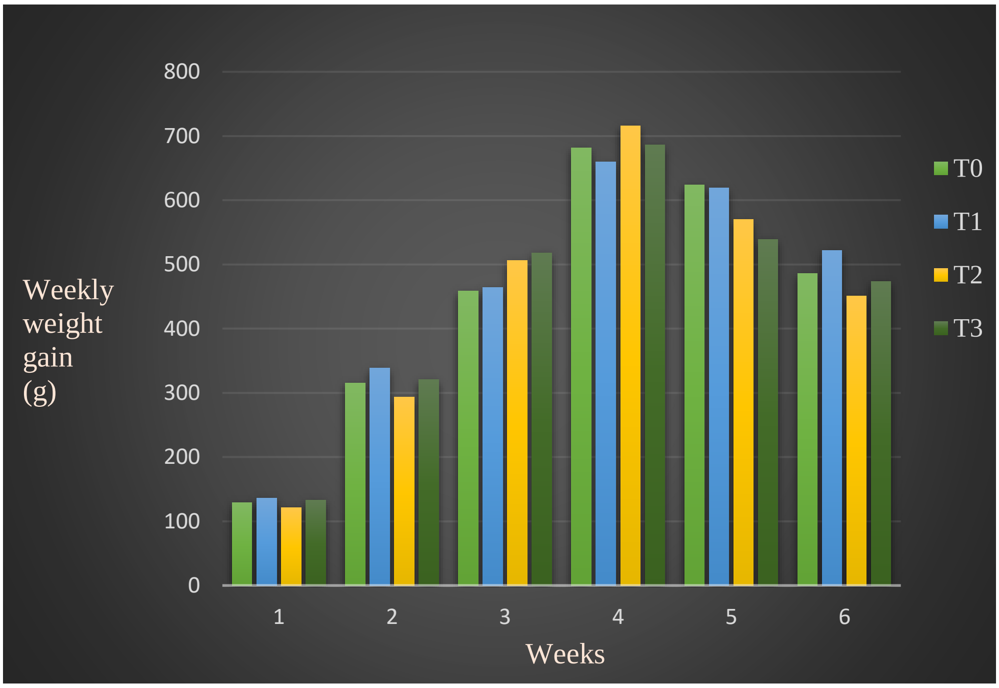


Fig.4.2 Average weekly body weight gain (g) of birds from various experimental groups

The data for average weekly body weight gain was subjected to statistical analysis and the results of the same are presented in Table 4.4. On statistical analysis of the data, it is revealed that mean and cumulative weight gain of birds from various experimental groups showed statistically non-significant effect of feed treatments.

Similar results were obtained by Kathirvelan *et al*. (2013) who found no significant difference in body weight gain of broilers on dietary supplementation of natural choline @ 1 kg/ton of feed. Dias *et al*. (2022) also observed no significant difference in body weight gain in broilers on supplementation of plant source of choline as replacement of 25%, 50% and 100% of choline chloride with herbal choline. Similarly, Petrolli *et al*. (2021) found no significant difference in body weight gain in broilers on dietary supplementation of vegetable choline @ 100 and 200 mg kg-1.

Table 4.4 Analysis of variance mean for average weekly body weight gain (g)

Source of variation	DF	SS	MSS	F cal
Treatments	3	441.845	147.282	1.526^{NS}
Error	12	1158.248	96.521	-
Total	15	-	-	-

Coefficient of Variation = 2.191 Treatments found to be Non Significant

The result of present study are not matching with, Khose *et al*. (2018) who reported significant ($P<0.05$) improvement in body weight gain in broiler birds on dietary supplementation of herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed. Khosravinia *et al*. (2015) also showed improvement in body weight gain in broiler birds on dietary supplementation of Bio choline @ 1 g/kg. Jadhav *et al*. (2009) found significant ($P<0.05$) increase in body weight gain on dieatary supplementation of herbal choline as Repchol (polyherbal formulation) @ 500 g/ton of broiler feed.

4.1.3 <u>Weekly feed consumption (g)</u>

Average weekly feed consumption (g) of the experimental birds from first to sixth week of experimental period is presented in Table 4.5. The same is depicted graphically in Fig 4.3.

Weekly feed consumption at first week was 155.86 ± 7.69, 157.75 ± 1.81, 154.91 ± 2.5 and 158.71 ± 2.12 g for the groups T0, T_1, T2 and T3, respectively. At the end of experiment the cumulative feed consumption for the groups T_0, T_1, T_2 and T_3 was 4471.43 ± 48.90, 4391.45 ± 3.69, 4441.91 ± 10.93 and 4383.27 ± 48.01 g, respectively. The mean of average weekly feed consumption was 745.24 ± 8.15, 731.91 ± 1.15, 740.32 ± 1.82 and 730.54 ± 19.03 g for the groups T_0, T_1, T_2 and T_3, respectively. From the data it is revealed that feed consumption was highest in group T_0 followed by groups T_2, T_1 and T_3.

Table 4.5 Average weekly feed consumption (g) of birds from various experimental groups

Weeks	Experimental Groups				CD
	T_0	T_1	T_2	T_3	
1	155.86 ± 7.69	157.75 ± 1.81	154.91 ± 2.50	158.71 ± 2.12	NS
2	412.96 ± 4.55	423.90 ± 11.83	400.89 ± 2.66	414.75 ± 7.02	NS
3	772.21^{a} ± 5.72	700.32^{b} ± 18.00	780.80^{a} ± 3.82	794.71^{a} ± 18.69	41.37
4	812.00^{b} ± 2.85	838.21^{b} ± 9.06	895.01^{a} ± 13.03	820.70^{b} ± 8.35	27.97
5	1084.20^{a} ± 19.68	1044.30^{b} ± 14.72	1018.02^{bc} ± 2.77	996.32^{c} ± 4.59	38.76
6	1234.20 ± 19.68	1226.98 ± 14.50	1192.27 ± 2.02	1198.07 ± 19.03	NS
Mean	745.24 ± 8.15	731.91 ± 1.15	740.32 ± 1.82	730.54 ± 19.03	NS
CFI	4471.43 ± 48.90	4391.45 ± 3.69	4441.91 ± 10.93	4383.27 ± 48.01	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet + herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton.
Values bearing different superscripts in a row differ significantly ($P<0.01$). NS: Non-significant. CFI=Cumulative Feed Intake.

Significant ($P<0.01$) differences in average feed consumption were observed among various experimental groups during 3rd, 4th and 5th week of experimental period. During 3rd week, the average feed consumption of birds from T_0, T_2 and T_3 groups was significantly ($P<0.01$) higher than group T_1, however, difference among group T_0, T_2 and T_3 was comparable. In the 4th week of experimental period, the average feed consumption was significantly ($P<0.01$) higher in T_2 group than other three experimental groups, however, difference among groups T_0, T_1 and T_3 was non-significant. The fifth week of experiment revealed significantly ($P<0.01$) higher feed consumption in birds from control group than group T_1, T_2 and T_3, however, difference between group T_1 and T_2 and that of group T_2 and T_3 was comparable.

The data for average weekly feed consumption of birds from various experimental groups was subjected to statistical analysis and results of the same are presented in Table 4.6. From statistical analysis of the data, it is observed that mean and cumulative feed consumption of various experimental groups was comparable.

Table 4.6 Analysis of variance mean for average weekly feed consumption (g)

Source of variation	DF	SS	MSS	F cal
Treatments	3	585.901	195.3	1.456^{NS}
Error	12	1609.928	134.161	-
Total	15	-	-	-

Coefficient of Variation = 1.572 Treatments found to be Non Significant

The results of present study corroborated with Petrolli *et al*. (2021) who observed no significant difference in feed intake in broiler birds on dietary supplementation of vegetable choline @ 100 and 200 mg kg-1. Also, Dias *et al*. (2022) found no significant difference in feed intake in broilers on supplementation of plant source of choline.

Finding of present study are not resembling with, Khose *et al*. (2018) who reported significant (P<0.05) improvement in feed intake in broilers on supplementation of herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed. Farina *et al*. (2017) also observed improvement in feed intake in broilers on supplementation of Biocholine @ 100, 200, 300 mg/kg feed.

4.1.4 Weekly Feed Conversion Ratio (FCR)

Average weekly feed conversion ratio (FCR) of the experimental birds during entire experimental period of six weeks is presented in Table 4.7. The same is depicted graphically in Fig 4.4.

The mean of average weekly feed conversion ratio for birds from groups T_0, T_1, T_2 and T_3 were 1.62±0.03, 1.57±0.01, 1.65±0.01 and 1.61±0.02, respectively. The cumulative FCR of the experimental birds were 1.66±0.02, 1.62±0.01, 1.67±0.01 and 1.64±0.01 in T_0, T_1, T_2 and T_3 groups, respectively. The average weekly feed conversion ratio of group T_1 was found numerically superior followed by group T_3, T_0 and T_2. During first week of experimental period, the average FCR of group T_0 and T_3 was significantly (P<0.01) better than group T_2, however, difference among groups T_0, T_1 and T_3 and that between group T_1 and T_2 was non significant.

The data for average weekly FCR was subjected to statistical analysis and the results of the same are presented in Table 4.8. The statistical analysis of means of average feed conversion ratio and cumulative feed conversion ratio showed no significant difference among the various experimental groups. Non-significant effect on feed conversion ratio was reported in this experiment due to similar feed intake among all the experimental groups.

Table 4.7 Average weekly feed conversion ratio (FCR) of birds from various experimental groups

Weeks	Experimental Groups				CD
	T_0	T_1	T_2	T_3	
1	$1.21^{b} \pm 0.01$	$1.24^{ab} \pm 0.02$	$1.28^{a} \pm 0.01$	$1.20^{b} \pm 0.03$	0.05
2	1.31 ± 0.02	1.25 ± 0.06	1.37 ± 0.01	1.29 ± 0.03	NS
3	1.69 ± 0.07	1.51 ± 0.02	1.54 ± 0.03	1.54 ± 0.08	NS
4	1.19 ± 0.02	1.27 ± 0.03	1.26 ± 0.06	1.20 ± 0.03	NS
5	1.75 ± 0.10	1.69 ± 0.04	1.79 ± 0.03	1.85 ± 0.03	NS
6	2.59 ± 0.19	2.44 ± 0.03	2.65 ± 0.09	2.58 ± 0.19	NS
Mean	1.62 ± 0.03	1.57 ± 0.01	1.65 ± 0.01	1.61 ± 0.02	NS
Cumulative	1.66 ± 0.02	1.62 ± 0.01	1.67 ± 0.01	1.64 ± 0.01	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet + herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton.
Values bearing different superscripts in a row differ significantly ($P<0.05$). NS: Non-significant.

Similar results were reported by Kathirvelan *et al*. (2013) who found no significant effect on feed conversion ratio in broiler birds on dietary supplementation of natural choline @ 1 kg/ton of feed. Dias *et al*. (2022) also not observed any significant difference in feed conversion ratio in broilers on supplementation of plant source of choline as replacement of 25%, 50% and 100% of choline chloride.

Table 4.8 Analysis of variance mean for average weekly feed conversion ratio (FCR)

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.013	0.004	2.482^{NS}
Error	12	0.022	0.002	-
Total	15	-	-	-

Coefficient of Variation = 2.634 Treatments found to be Non Significant

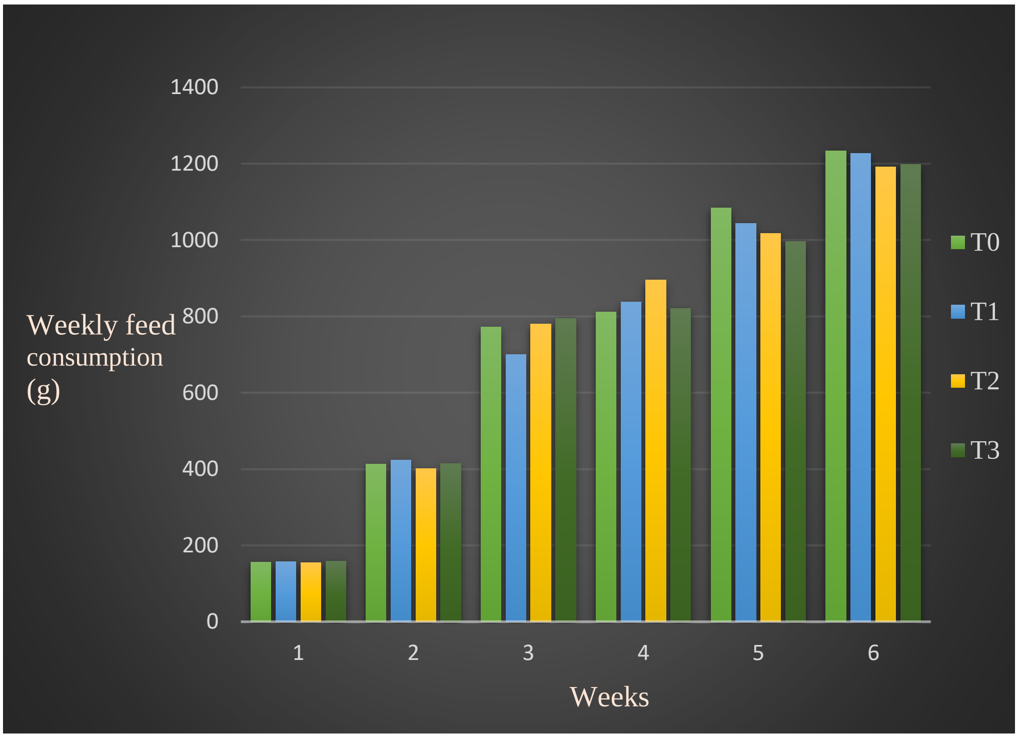


Fig. 4.3 Average weekly feed consumption (g) of birds from various experimental groups.

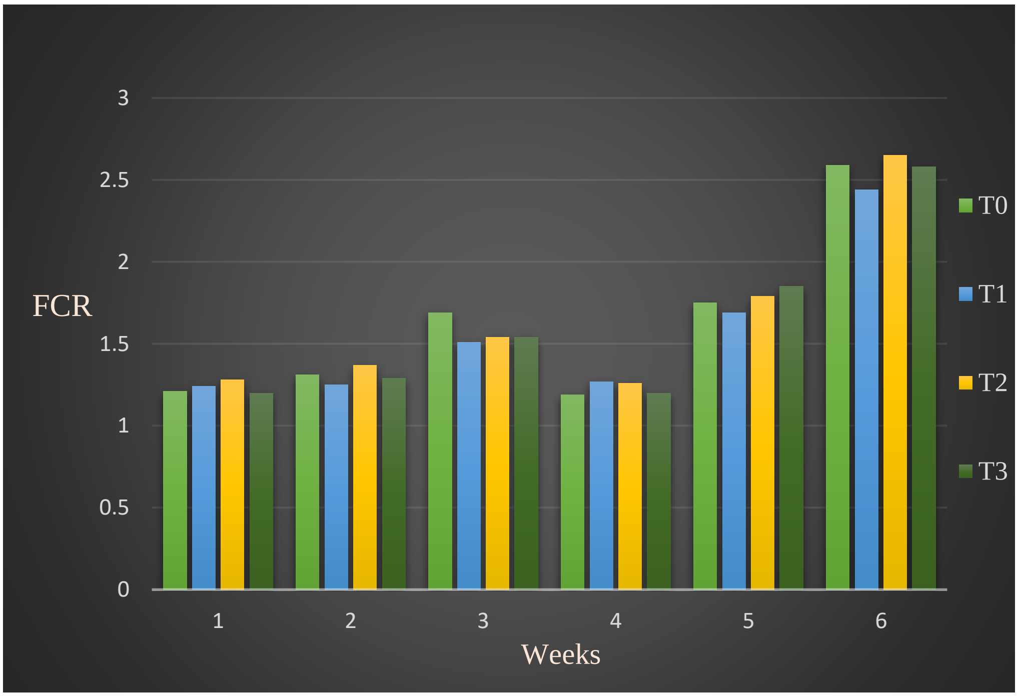


Fig. 4.4 Average weekly feed conversion ratio (FCR) of birds from various experimental groups.

The findings of present study are not inline with, Khose *et al*. (2018) who observed significant ($P<0.05$) improvement in FCR on dietary supplementation of herbal choline @ 0.250, 0.350 and 0.500 kg/ton of broiler feed. Rodelas *et al*. (2011) also found improvement in FCR on dietary supplementation with Biocholine in broiler feed. Similarly, Khosravinia *et al*. (2015) observed improvement in feed conversion ratio on dietary supplementation of Bio choline @ 1 g/kg in broiler diet.

4.1.5 Mortality

Two birds from each group were died during entire experimental period and mortality percentage was 3.33% which was well within permissible limit. No specific lesions were observed during post mortem examination of birds.

4.2 Carcass Traits

The average values of carcass traits are given in Table 4.10. The average live weights of birds selected for carcass evaluation were 2738.47±52.57,

Table 4.9 Effect of dietary supplementation of herbal choline on carcass traits

Carcass traits	Experimental groups				
	T_0	T_1	T_2	T_3	CD
Live wt.(g)	2738.47±52.57	2779.33 ± 6.97	2673.05±33.70	2697.90±41.24	–
Dressed wt. (g)	2021.81±35.58	2063.53±36.45	1953.94±45.88	1985.45±35.41	–
Dressing %	73.84 ± 0.27	74.24 ± 1.19	72.62 ± 1.14	73.59 ± 0.27	NS
Eviscerated wt. (g)	1898.88±32.63	1937.22±37.51	1833.57±42.14	1866.43±34.36	–
Eviscerated yield %	69.35± 0.29	69.70 ± 1.23	68.14 ± 1.05	69.17 ± 0.30	NS
Giblet wt. %	4.49 ± 0.04	4.54 ± 0.10	4.48 ± 0.12	4.42 ± 0.03	NS
Liver wt.%	1.90 ± 0.05	1.88 ± 0.06	1.94 ± 0.02	1.92 ± 0.03	NS
Gizzard wt.%	2.06 ± 0.04	2.11 ± 0.04	1.98 ± 0.06	2.01 ± 0.03	NS
Heart wt.%	0.53 ± 0.05	0.55 ± 0.04	0.56 ± 0.06	0.49 ± 0.02	NS
Breast wt.%	27.34 ± 0.30	27.05 ± 1.16	27.02 ± 0.75	26.62 ± 1.12	NS
Drumstick wt.%	4.35 ± 0.05	4.36 ± 0.09	4.30 ± 0.12	4.27 ± 0.02	NS
Thigh wt. %	9.31 ± 0.07	9.33 ± 0.16	9.37 ± 0.08	9.35 ± 0.02	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet + herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton. ($P<0.005$). NS: Non significant

2779.33±6.97, 2673.05±33.70 and 2697.90±41.24 g in T_0, T_1, T_2 and T_3 groups, respectively and corresponding values for average dressing weight were 2021.81±35.58, 2063.53±36.45, 1953.94±45.88 and 1985.45±35.41 g. The average values for dressing weight percentage were 73.84±0.27, 74.24±1.19, 72.62±1.14 and 73.59±0.27 % in T_0, T_1, T_2 and T_3 groups, respectively. The results revealed slightly higher dressing percentage for group T_1 followed by T_0, T_3 and T_2 groups.

The data for average dressing weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.9. The statistical analysis of data showed non significant effect of feed treatments on average dressing percentage of birds from various experimental groups.

Table 4.10 Analysis of variance for average dressing weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	5.731	1.910	0.663^{NS}
Error	12	34.591	2.883	-
Total	15	-	-	-

Coefficient of Variation = 1.725 Treatments found to be Non Significant

The average values of eviscerated weight were 1898.88 ± 32.63, 1937.22 ± 37.51, 1833.57 ± 42.14 and 1866.43 ± 34.36 g in T_0, T_1, T_2 and T_3 groups, respectively and corresponding values for average eviscerated yield percentage were 69.3 5± 0.29, 69.70 ± 1.23, 68.14 ± 1.05 and 69.17 ± 0.30. The result showed higher eviscerated yield percentage for group T_1 followed by T_0, T_3 and T_2 groups.

The data for average eviscerated yield percentage was subjected to statistical analysis and the results of the same are presented in Table 4.11. The statistical analysis of data showed non significant effect of feed treatments on average eviscerated yield percentage of birds from various experimental groups.

Table 4.11 Analysis of variance for average eviscerated yield percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	5.353	1.784	0.639^{NS}
Error	12	33.522	2.794	-
Total	15	-	-	-

Coefficient of Variation = 2.865 Treatments found to be Non Significant

The average values for giblet weight percentage were 4.49 ± 0.04, 4.54 ± 0.10, 4.48 ± 0.12 and 4.42 ± 0.03 % in T_0, T_1, T_2 and T_3 groups, respectively. The data for average values for giblet weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.12. The statistical analysis of data obtained, revealed that average giblet weight percentage of all the experimental groups was comparable.

Table 4.12 Analysis of variance for average giblet weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.036	0.012	0.408^{NS}
Error	12	0.349	0.029	-
Total	15	-	-	-

Coefficient of Variation = 5.107 Treatments found to be Non Significant

Table 4.13 Analysis of variance for average liver weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.005	0.002	0.276^{NS}
Error	12	0.077	0.006	-
Total	15	-	-	-

Coefficient of Variation = 4.195 Treatments found to be Non Significant

The values for average liver weight percentage in groups T_0, T_1, T_2 and T_3 were 1.90 ± 0.05, 1.88 ± 0.06, 1.94 ± 0.02 and 1.92 ± 0.03 %, respectively. The data for average values for liver weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.13. The statistical analysis of data showed non significant effect of feed treatments on average liver weight percentage of birds from various experimental groups.

Similar results were reported by Khose *et al.* (2018) who revealed non-significant differences in percent of liver weight on supplementation herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed of broilers.

The values for average gizzard weight percentage in groups T_0, T_1, T_2 and T_3 were 2.06±0.04, 2.11±0.04, 1.98±0.06 and 2.01±0.03 %, respectively. The data for average values for gizzard weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.14. The statistical analysis of data obtained, revealed that average gizzard weight percentage of all the experimental groups was comparable.

Table 4.14 Analysis of variance for average gizzard weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.037	0.012	1.429^{NS}
Error	12	0.103	0.009	-
Total	15	-	-	-

Coefficient of Variation = 4.548 Treatments found to be Non Significant

The findings of present study are inline with Khose *et al*. (2018) who revealed non-significant differences in percent gizzard weight on supplementation herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed of broilers.

The values for average heart weight percentage were 0.53 ± 0.05, 0.55 ± 0.04, 0.56 ± 0.06 and 0.49 ± 0.02 %, respectively. The data for average values for heart weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.15. The statistical analysis of data showed non significant effect of feed treatments on average heart weight percentage of birds from various experimental groups.

Similar results were obtained by Khose *et al*. (2018) who revealed non-significant differences in percent of heart weight on supplementation herbal choline @ 0.250, 0.350 and 0.500 kg/ton of feed of broilers.

Table 4.15 Analysis of variance for average heart weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.013	0.004	0.525^{NS}
Error	12	0.100	0.0008	-
Total	15	-	-	-

Coefficient of Variation = 17.232 Treatments found to be Non Significant

The average breast weight percentage in groups T_0, T_1, T_2 and T_3 were 27.34±0.30, 27.05±1.16, 27.02±0.75 and 26.62±1.12 %, respectively. The data for average values for breast weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.16. The statistical analysis of data obtained, revealed that average breast weight percentage of all the experimental groups was comparable.

The average drumstick weight percentage in groups T_0, T_1, T_2 and T_3 were 4.35±0.05, 4.36±0.09, 4.30±0.12, 4.27±0.02 %, respectively. The data for average values for drumstick weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.17. The statistical analysis of data showed non significant effect of feed treatments on average drumstick weight percentage of birds from various experimental groups.

Table 4.16 Analysis of variance for average breast weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	1.046	0.349	0.107^{NS}
Error	12	39.055	3.225	-
Total	15	-	-	-

Coefficient of Variation = 6.680 Treatments found to be Non Significant

Table 4.17 Analysis of variance for average drumstick weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.022	0.007	0.291^{NS}
Error	12	0.301	0.025	-
Total	15	-	-	-

Coefficient of Variation = 3.669 Treatments found to be Non Significant

The average thigh weight percentage in groups T_0, T_1, T_2 and T_3 were 9.31±0.07, 9.33±0.16, 9.37±0.08 and 9.35±0.02 %, respectively. The data for average values for thigh weight percentage was subjected to statistical analysis and the results of the same are presented in Table 4.18. The statistical analysis of data obtained, revealed that average thigh weight percentage of all the experimental groups was comparable.

Table 4.18 Analysis of variance for average thigh weight percentage

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.007	0.002	0.061^{NS}
Error	12	0.439	0.037	-
Total	15	-	-	-

Coefficient of Variation = 2.049 Treatments found to be Non Significant

The result of the present study resembles with Khosravinia *et al.* (2015) who observed no change in carcass yield percentage by supplementation of Bio choline

@ 1 g/kg in moderate and high energy broiler diet. Dias *et al*. (2022) also not observed any significant difference in carcass and cut yields on supplementation of plant source of choline as replacement of 25, 50 and 100% of choline chloride with herbal choline in broilers feed. Likewise, Petrolli *et al*. (2021) also observed no alteration in carcass, cuts yield and organ relative weights on dietary supplementation of vegetable choline @ 100 and 200 mg kg-1 in broilers feed.

4.3 Blood Biochemical Parameters

4.3.1 Serum Protein

The average serum protein values (g/dL) of birds from different groups are presented in Table 4.19. The same is depicted graphically in Fig 4.5. The data related to average serum protein of birds from different groups was statistically analyzed and results of the same are presented in Table 4.20.

The average serum protein for birds in group T_0, T_1, T_2 and T_3 was 3.43±0.05, 3.51±0.08, 3.33±0.03 and 3.38±0.05 g/dL, respectively. The average serum protein value for group T_1 was higher followed by group T_0, T_3 and T_2. On statistical analysis of data, it was found that the difference in average total serum protein among the various experimental groups was non-significant.

Table 4.19 Average blood biochemical parameters of birds from various experimental groups

Parameters	Experimental Groups				CD
	T_0	T_1	T_2	T_3	
Serum Protein (g/dL)	3.43 ± 0.05	3.51 ± 0.08	3.33 ± 0.03	3.38 ± 0.05	NS
Serum Albumin (g/dL)	1.61 ± 0.22	1.63 ± 0.02	1.52 ± 0.02	1.57 ± 0.15	NS
Serum Globulin (g/dL)	1.82 ± 0.26	1.89 ± 0.10	1.81 ± 0.03	1.82 ± 0.17	NS
SGOT (IU/L)	308.93 ± 10.75	291.43 ± 17.06	318.43 ± 3.84	319.33 ± 10.45	NS
SGPT (IU/L)	10.17 ± 1.51	8.40 ± 0.44	10.17 ± 0.44	9.72 ± 2.10	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet + herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton.
NS: Non significant

Findings of present study are matching with Jadhav *et al*. (2009) who observed no significant difference in total serum protein of broiler chicken on dietary inclusion of herbal choline as Repchol (polyherbal formulation) @ 500 g/ton

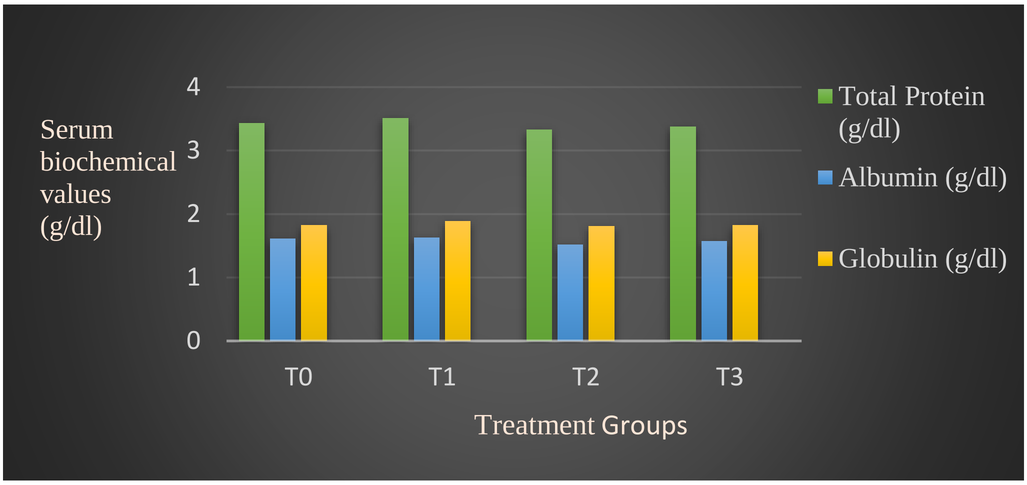


Fig. 4.5 Average blood biochemical parameters (Total protein, Albumin, Globulin) of birds from various experimental groups.

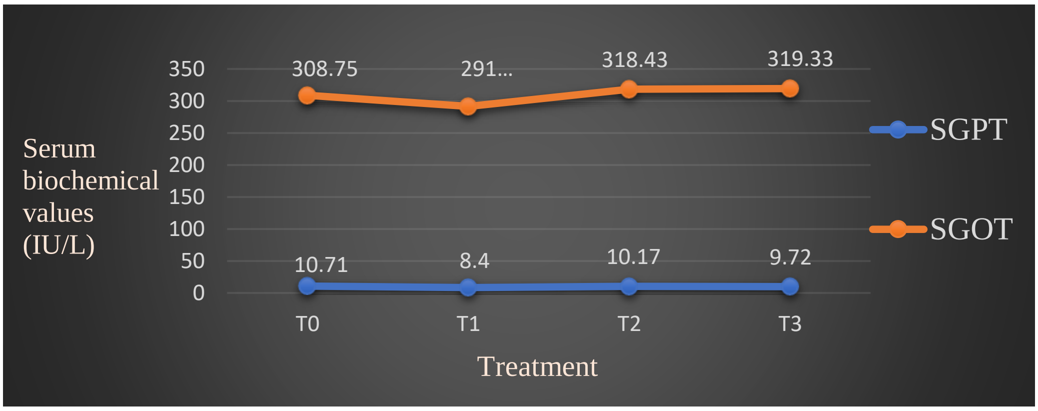


Fig. 4.6 Average blood biochemical parameters (SGOT, SGPT) of birds from various experimental groups.

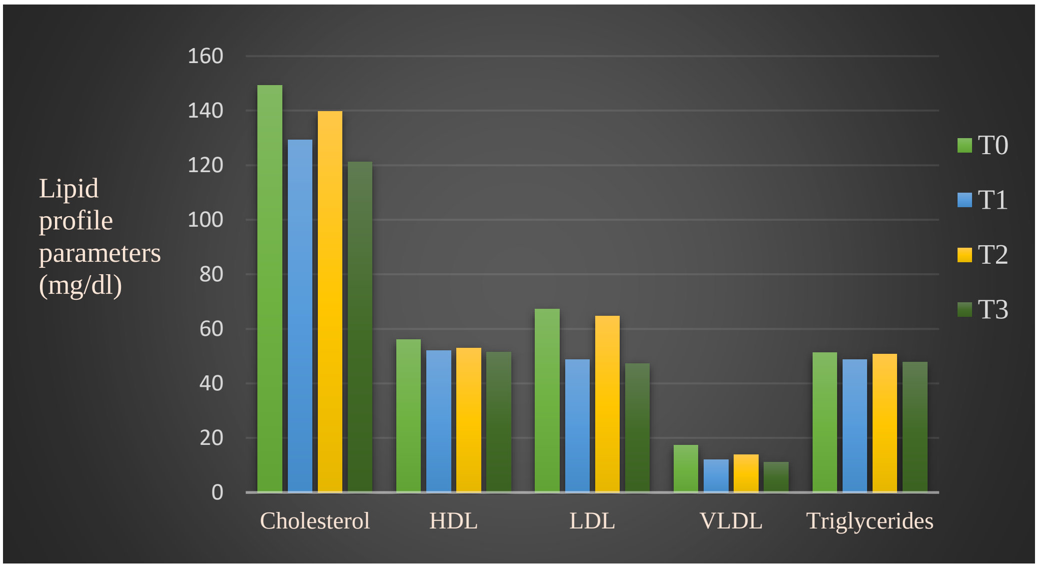


Fig. 4.7 Average Lipid profile parameters (Cholesterol, HDL, LDL, VLDL, Triglycerides) of birds from various experimental groups.

of feed in broiler birds. Aronu *et al*. (2022) also showed non significant difference in total serum protein levels of broiler birds on dietary inclusion of choline chloride @ 0.5, 0.75 and 1 g/kg.

Table 4.20 Analysis of variance for average serum protein

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.073	0.024	2.021^{NS}
Error	12	0.145	0.012	-
Total	15	-	-	-

Coefficient of Variation = 3.226 Treatments found to be Non Significant

On contrary, Khose *et al*. (2017) observed significant difference in serum total protein in broiler birds receiving feed supplement with herbal choline @ 250, 350 and 500 g/ton of feed.

4.3.2 Serum Albumin

The average serum albumin (g/dL) of birds from different groups are presented in Table 4.19. The same is depicted graphically in Fig 4.5. The data related to average serum albumin of birds from different groups was statistically analyzed and results of the same are presented in Table 4.21.

The average serum albumin for groups T_0, T_1, T_2 and T_3 was 1.61±0.22, 1.63±0.02, 1.52±0.02 and 1.57±0.15 g/dL, respectively. It was observed from the Table 4.19, that the average serum albumin among the different experimental groups were statistically non-significant although it was numerically higher for group T_1 followed by group T_0, T_3 and T_2.

Table 4.21 Analysis of variance for average albumin

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.027	0.009	0.124^{NS}
Error	12	0.864	0.072	-
Total	15	-	-	-

Coefficient of Variation = 16.978 Treatments found to be Non Significant

Similar results were obtained by Aronu *et al*. (2022) who found non significant difference in serum albumin on dietary supplementation of choline chloride @ 0.5 g/kg, 0.75 g/kg and 1 g/kg.

Findings of present study are not inline with, Khose *et al*. (2017) who observed significant difference in serum albumin levels of boiler birds received feed containing herbal choline @ 250, 350 and 500 g/ton.

4.3.3 <u>Serum Globulin</u>

The average serum globulin (g/dL) of birds from different groups are presented in Table 4.19. The same is depicted graphically in Fig 4.5. The data related to average serum globulin of birds from different groups was statistically analyzed and results of the same are presented in Table 4.22.

Table 4.22 Analysis of variance for average globulin

Source of variation	DF	SS	MSS	F cal
Treatments	3	0.016	0.005	0.052^{NS}
Error	12	1.231	0.103	-
Total	15	-	-	-

Coefficient of Variation = 17.492 Treatments found to be Non Significant

The average serum globulin for groups T_0, T_1, T_2 and T_3 was 1.82±0.26, 1.89±0.10, 1.81±0.03 and 1.82±0.17 g/dL, respectively. From the Table 4.22 it is evident that difference in average serum globulin among different groups was comparable, although numerically higher values were observed in group T_1 than other three experimental group.

The results of present study corroborated with Khose *et al*. (2017) who found no significant difference in serum globulin values in broiler chicken fed on diet containing herbal choline @ 250, 350 and 500 g/ton.

4.3.4 <u>SGOT</u>

The average SGOT (IU/L) of birds from different groups is presented in Table 4.19. The same is depicted graphically in Fig 4.6. The data related to average SGOT of birds from different groups was statistically analyzed and results of same are presented in Table 4.23.

The average SGOT for groups T_0, T_1, T_2 and T_3 was 308.93±10.75, 291.43±17.06, 318.43±3.84 and 319.33±10.45 IU/L, respectively. It was revealed that the difference in average SGOT of birds from different treatment group was statistically non-significant.

Similar results were obtained by Aronu *et al*. (2022) who found non significant difference in SGOT values on dietary supplementation of choline chloride @ 0.5, 0.75 and 1 g/kg in broiler feed.

On contrary, Muthukumarasamy *et al*. (2004) found significant difference in SGOT levels on dietary addition of biocholine @ 0.5 and 0.75 kg/ton of broiler bird's ration.

The average SGPT (IU/L) of birds from different groups is presented in Table 4.19. The same is depicted graphically in Fig 4.6. The data related to average SGPT of birds from different groups were statistically analyzed and results of same are presented in Table 4.24.

Table 4.23 Analysis of variance for average SGOT

Source of variation	DF	SS	MSS	F cal
Treatments	3	1536.72	512.24	0.965^{NS}
Error	12	6367.97	530.664	-
Total	15	-	-	-

Coefficient of Variation = 7.501 Treatments found to be Non Significant

4.3.5 <u>SGPT</u>

The average SGPT for groups T_0, T_1, T_2 and T_3 were 10.17±1.51, 8.40±0.44, 10.17±0.44 and 9.72±2.10 IU/L, respectively. It was revealed that the difference in average SGPT of birds from different treatment group were statistically non-significant.

Table 4.24 Analysis of variance for average SGPT

Source of variation	DF	SS	MSS	F cal
Treatments	3	8.406	2.802	0.395^{NS}
Error	12	85.168	7.097	-
Total	15	-	-	-

Coefficient of Variation = 27.713 Treatments found to be Non Significant

Similar results were obtained by Aronu *et al*. (2022) who found non significant difference in SGPT values on dietary supplementation of choline chloride @ 0.5, 0.75 and 1 g/kg in feed of broiler birds.

In contrast to the finding of present study, Muthukumarasamy *et al*. (2004) found significant difference in SGPT levels on dietary addition of biocholine @ 0.5 and 0.75 kg/ton in feed of broilers birds.

4.4 Lipid profile parameters

4.4.1 Average Serum Cholesterol

The lipid profile of birds from different groups are presented in Table 4.25. The same is depicted graphically in Fig 4.7. The data related to average serum cholesterol levels (mg/dL) of birds from different groups were statistically analyzed and results of the same are presented in Table 4.26.

The average serum cholesterol levels (mg/dl) were 1.49±2.06, 129.25±1.25, 139.75±1.8 and 121.25±1.70 mg/dL, respectively for birds in experimental group T_0, T_1, T_2 and T_3. The average serum cholesterol level for group T_0 was higher than all other experimental groups received herbal choline supplementation at varying levels. The statistical analysis revealed significantly higher (P<0.01) average serum cholesterol in group T_0 than groups T_1, T_2 and T_3. Further, it is observed that average serum cholesterol levels of birds from group T_1 and T_2 were significantly higher than group T_3, however, average serum cholesterol level of group T_2 was significantly higher than T_1 group.

Table 4.25 Average lipid profile parameters of birds from various experimental groups

Parameters	Experimental Groups				CD
	T_0	T_1	T_2	T_3	
Serum Cholesterol (mg/dL)	$149.25^a \pm 2.06$	$129.25^c \pm 1.25$	$139.75^b \pm 1.8$	$121.25^d \pm 1.70$	5.012
Serum HDL (mg/dL)	52 ± 1.08	56 ± 2.65	51.5 ± 0.65	53 ± 1.87	NS
Serum LDL (mg/dL)	$67.25^a \pm 0.85$	$48.75^b \pm 1.49$	$64.75^a \pm 1.38$	$47.25^b \pm 1.80$	4.381
Serum VLDL (mg/dL)	$17.38^a \pm 1.27$	$11.97^{bc} \pm 0.28$	$13.80^b \pm 0.23$	$11.10^c \pm 0.35$	2.108
Serum Triglycerides (mg/dL)	51.25 ± 0.85	48.75 ± 2.29	50.75 ± 3.45	47.75 ± 2.95	NS

*T_0, Control commercial choline chloride 60% @ 900g/ton; T_1, Standard broiler diet + herbal choline @ 750g/ton; T_2, Standard broiler diet + herbal choline @ 550g/ton; T_3, Standard broiler diet + herbal choline @ 950g/ton.
Values bearing different superscripts in a row differ significantly (P<0.01). NS: Non significant.

Findings of present study are matching with Gangane *et al*. (2010) who observed the significant (P=0.05) reduction in serum cholesterol level on dietary supplementation of herbal source of choline as Repchol @ 500 g/ton of feed in

Table 4.26 Analysis of variance for average serum cholesterol

Source of variation	DF	SS	MSS	F cal
Treatments	3	1790.75	596.917	56.402^{**}
Error	12	127	10.583	-
Total	15	-	-	-

Coefficient of Variation = 2.412 **Treatments found Significant at 1% level
CD (0.05) = 5.012

broilers. Khose *et al*. (2019) also showed the significantly lower levels of serum cholesterol when herbal choline was included @ 0.50 kg/ton of broiler feed. Similarly, Muthukumarasamy *et al*. (2004) observed significant reduction in serum cholesterol after supplementation of biocholine @ 0.5 kg/ton of ration than choline chloride 60% @ 1 kg/ton of broiler ration. Sharma and Ranjan (2015) also found significant lower mean cholesterol levels in treatment group supplemented with Herbal choline as N- Choline @ 500 g than synthetic choline chloride (60%) @ 1000 g/ton of broiler feed.

4.4.2 <u>Average Serum High-Density Lipoprotein (HDL)</u>

The average serum HDL levels (mg/dL) of birds from different groups are presented in Table 4.25. The same is depicted graphically in Fig 4.7. The data related to average serum HDL levels of birds from different groups were statistically analyzed and results of same are presented in Table 4.27.

The average serum HDL levels for groups T_0, T_1, T_2 and T_3 were 52±1.08, 56±2.65, 51.5±0.65 and 53±1.87 mg/dL, respectively. The results revealed that the average serum HDL levels of birds from group T_1 were numerically higher followed by group T_3, T_0 and T_2. Further, on statistical analysis of data it is observed that there was no significant difference in average serum HDL levels among the various experimental groups.

Table 4.27 Analysis of variance for average serum HDL

Source of variation	DF	SS	MSS	F cal
Treatments	3	48.75	16.25	1.345^{NS}
Error	12	145	12.083	-
Total	15	-	-	-

Coefficient of Variation = 6.543 Treatments found to be Non Significant

Similar results were reported by Khose *et al*. (2019) who observed no significant difference in HDL values on supplementation of herbal choline @ 0.50 kg/ton than choline chloride 60% @ 1 kg/ton of broiler bird feed.

4.4.3 Average Serum Low-Density Lipoprotein (LDL)

The average serum LDL levels (mg/dl) of birds from different groups are presented in Table 4.25. The same is depicted graphically in Fig 4.7. The data related to average serum LDL levels of birds from different groups were statistically analyzed and results of same are presented in Table 4.28.

The average serum LDL levels for groups T_0, T_1, T_2 and T_3 were 67.25±0.85, 48.75±1.49, 64.75±1.38 and 47.25±1.80 mg/dL, respectively. The statistical analysis of data revealed significant ($P<0.01$) difference in average serum LDL levels among various experimental groups. The average serum LDL levels of birds from group T_0 and T_2 was significantly ($P<0.01$) higher than T_1 and T_3 groups, however, difference between group T_0 and T_2 and that of group T_1 and T_3 was comparable.

Findings of present study resemble with Khose *et al*. (2019) who found significantly lower levels of serum LDL in broiler birds received feed containing herbal choline @ 0.50 kg/ton than choline chloride-60% @ 1 kg/ton.

Table 4.28 Analysis of variance for average serum LDL

Source of variation	DF	SS	MSS	F cal
Treatments	3	1313	437.667	54.144**
Error	12	97	8.083	-
Total	15	-	-	-

Coefficient of Variation = 4.988 **Treatments found Significant at 1% level
CD (0.05) = 4.381

4.4.4 Average Serum Very Low-Density Lipoprotein (VLDL)

The average serum VLDL levels (mg/dL) of birds from different groups are presented in Table 4.25. The same is depicted graphically in Fig 4.7. The data related to average serum VLDL levels of birds from different groups were statistically analyzed and results of same are presented in Table 4.29.

The average VLDL levels for groups T_0, T_1, T_2 and T_3 were 17.38±1.27, 11.97±0.28, 13.80±0.23 and 11.10±0.35 mg/dl, respectively. The significant

(P<0.01) difference in average serum VLDL levels were observed among various experimental groups. The average serum VLDL levels of birds from group T_0 was significantly (P<0.01) higher than all other experimental groups supplemented with herbal choline and average serum VLDL levels of group T_1 and T_2 and that of group T_1 and T_3 were comparable.

Table 4.29 Analysis of variance for average VLDL

Source of variation	DF	SS	MSS	F cal
Treatments	3	92.776	30.925	16.529**
Error	12	22.451	1.871	-
Total	15	-	-	-

Coefficient of Variation = 10.087 **Treatments found Significant at 1% level
CD (0.05) = 2.108

4.4.5 Average Serum Triglycerides

The average serum triglycerides levels (mg/dl) of birds from different groups are presented in Table 4.25. The same is depicted graphically in Fig 4.7. The data related to average serum triglycerides levels of birds from different groups were statistically analyzed and results of same are presented in Table 4.30.

The average serum triglycerides levels for groups T_0, T_1, T_2 and T_3 were 51.25±0.85, 48.75±2.29, 50.75±3.45 and 47.75±2.95 mg/dl, respectively. On statistical analysis, it was observed that the average serum triglycerides levels showed no significant difference among the various experimental groups although it was numerically higher for group T_0 followed by group T_2, T_1 and T_3.

Table 4.30 Analysis of variance for average triglycerides

Source of variation	DF	SS	MSS	F cal
Treatments	3	32.75	10.917	0.411^{NS}
Error	12	319	26.583	-
Total	15	-	-	-

Coefficient of Variation = 10.390 Treatments found to be Non Significant

The results of the present study corroborated with the findings of Khose *et al*. (2019) who revealed no significant difference in serum triglycerides values on supplementation of herbal choline @ 0.50 kg/ton than choline chloride-60% @ 1 kg/ ton broiler bird feed.

4.5 Liver Histopathological Examination

At the end of experimental period 42 days, the histopathological examination of liver sections of birds from different experimental groups was carried out which revealed no appreciable changes in liver histopathology as no vacuolar changes in hepatocyte and no cell swelling was observed during histopathological studies. Thus, in present experiment efficient metabolism of fat and hepatoprotective effect of herbal choline at all levels of supplementation was clearly seen. (Plate No.3.6)

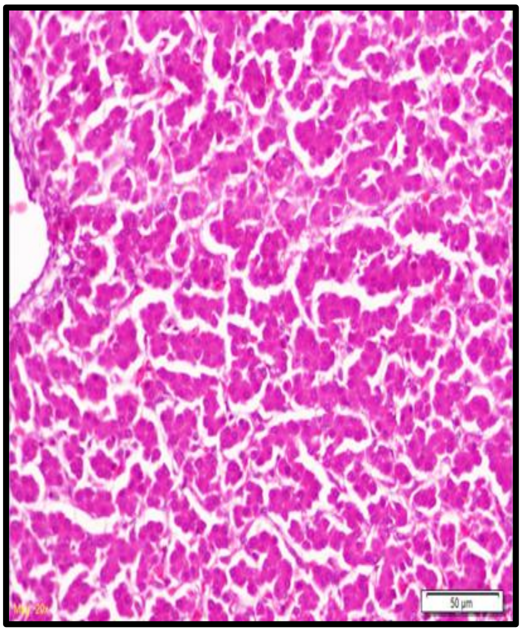
The findings of present experiment were in conformation with Gangane *et al.* (2010) who did not found any appreciable changes in liver on the gross pathological examination of liver of broiler birds, on dietary supplementation of herbal source of choline as Repchol @ 500 g/ton of feed in broilers. Muthukumarasamy *et al*. (2004) also observed no changes in liver colour, appearance and consistency on supplementation of biocholine @ 0.5 kg/ton of broiler feed.

On contrary, Selvam *et al*. (2018) observed reduced cell swelling and mild vacuolar changes in hepatocytes of broiler birds supplemented with synthetic choline chloride or herbal choline as polyherbal formulation @ 400 g/ton of feed.

4.6 Nutrient Intake and Digestibility

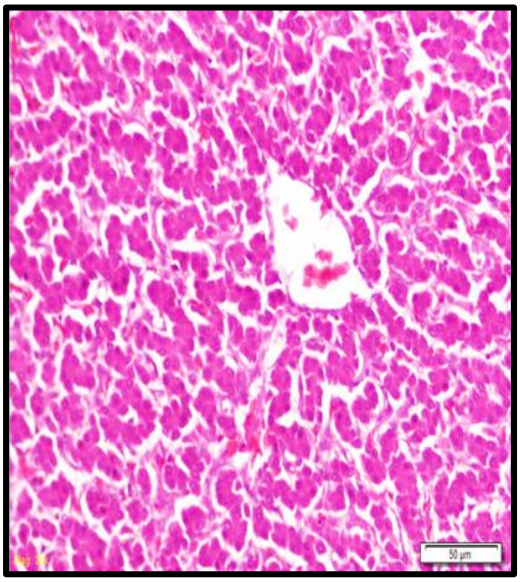
The nutrient digestibility values of different diets provided to broiler birds from various experimental groups are presented in Table 4.31.

For groups T_0, T_1, T_2 and T_3 the digestibility of dry matter was 61.19, 62.36, 60.04, 60.83 % and crude protein 59.09, 59.28, 58.64 and 58.78%, respectively and corresponding values for ether extract were 86.19, 86.24, 85.91, 86.02 % and for nitrogen free extract were 60.84, 61.64, 60.46 and 60.54%. The digestibility percentage of dry matter, crude protein, ether extract and nitrogen free extract was higher in group T_1 followed by control T_0 (control), T_3 and T_2 groups. The digestibility for crude fiber for groups T_0, T_1, T_2 and T_3 was 32.22, 32.53, 31.93 and 32.16%, respectively. No appreciable increase in digestibility of nutrients in choline supplemented groups is evident from Table 4.31.

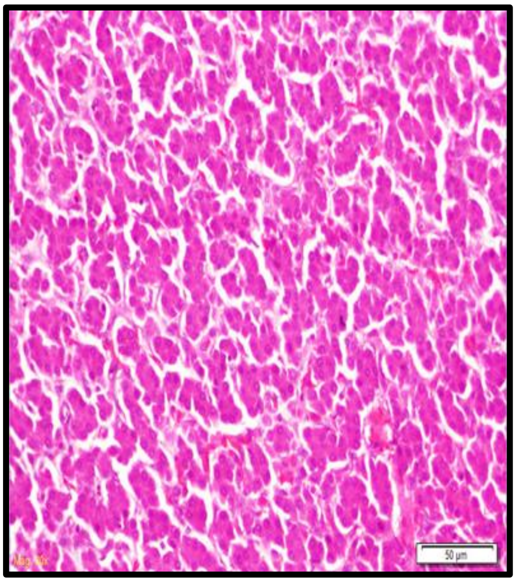


Group T_0

Group T_1



Group T_2



Group T_3

Plate 3.6 Liver Histopathological Examination

Table 4.31 Effect of dietary supplementation of herbal choline on nutrient Digestibility

Nutrients	Experimental groups			
	T_0	T_1	T_2	T_3
Intake (g)				
DM	923.86	942.38	910.28	926.45
CP	186.83	188.65	182.41	187.25
EE	34.74	35.98	34.62	35.42
CF	54.36	56.06	54.03	55.63
NFE	647.7	662.9	646.3	648.2
Outgo (g)				
DM	358.55	354.71	363.75	362.89
CP	76.43	76.82	75.4	77.71
EE	4.80	4.95	4.88	4.95
CF	36.85	37.82	36.78	37.74
NFE	253.64	254.29	255.55	255.78
Digestibility (%)				
DM	61.19	62.36	60.04	60.83
CP	59.09	59.28	58.64	58.78
EE	86.19	86.24	85.91	86.02
CF	32.22	32.53	31.93	32.16
NFE	60.84	61.64	60.46	60.54

4.7 Economics of Broiler Production

The economics of broiler production in present experiment was calculated after completion of six-week period of experiment.

The cost of day old chick, cost of feed, cost of supplement (herbal choline), average total feed consumed per bird, feed consumed per kg live body weight gain, and miscellaneous cost were consider while calculating net profit. For different phases of broilers, such as pre-starter, starter and finisher, the cost of feed was determined on the basis of the cost of feed ingredients used in diet of the experimental groups. The cost of herbal choline was also considered in calculation of feed cost of experimental group supplemented with herbal choline at varying level. The herbal choline was procured from market @ ₹ 95/kg.

It is revealed from the Table 4.32 that, the net cost of production per bird was ₹.211.78, 207.92, 209.82 and 208.07 for T_0, T_1, T_2 and T_3 experimental groups, respectively. The per kg cost of pre-starter feed were ₹.38.01, 37.85, 37.83 and 37.87 for experimental groups T_0, T_1, T_2 and T_3, respectively and corresponding cost of starter feed were ₹ 39.52, 39.36, 39.34 and 39.38 and for finisher feeds were ₹. 39, 38.84, 38.82 and 38.86.

The sale price of birds from different groups was considered as ₹.100/kg on live weight basis. Therefore, the net profit per bird was ₹.62.72, 70.68, 60.68 and 63.73 for groups T_0, T_1, T_2 and T_3 groups, respectively and corresponding net profit/kg body weight of experimental birds was ₹.22.85, 25.37, 22.43 and 23.45,

Table 4.32 Economics of broiler production at the end of six weeks

Parameter	Groups			
	T_0	T_1	T_2	T_3
Chick cost (₹)	27	27	27	27
Feed intake (Kg)				
Pre-starter	0.16	0.16	0.15	0.16
Starter	1.18	1.12	1.18	1.21
Finisher	3.13	3.11	3.11	3.02
Total feed consumed (Kg)	4.47	4.39	4.44	4.39
Feed price per kg (₹)				
Pre-starter	38.01	37.85	37.83	37.87
Starter	39.52	39.36	39.34	39.38
Finisher	39	38.84	38.82	38.86
Cost of Herbal choline/kg	0	95	95	95
Feed cost (₹)/ bird				
Pre-starter	6.08	6.05	5.67	6.06
Starter	46.63	44.08	46.42	47.65
Finisher	122.07	120.79	120.73	117.36
Miscellaneous cost (₹) per bird	10	10	10	10
Net cost of production (₹)	211.78	207.92	209.82	208.07
Body weight at the end of six week(kg)	2.745	2.786	2.705	2.718
Cost of sale /kg live body weight (₹)	100	100	100	100
Return of sale of bird (₹)	274.5	278.6	270.5	271.8
Net profit per bird (₹)	62.72	70.68	60.68	63.73
Net profit / kg live body weight (₹)	22.85	25.37	22.43	23.45
Cost of feed / kg live body weight gain (₹)	63.67	61.34	63.89	62.94

respectively. Cost of feed per kg live body weight gain for groups T_0, T_1, T_2 and T_3 was ₹.63.67, 61.34, 63.89 and 62.94, respectively.

The economics of broiler production of present experiment indicated that net profit per bird and net profit/ kg live body weight was higher in group T_1 followed by T_3, control and T_2 group. The cost of feed per kg body weight gain was lower in group T_1 and T_3 than control group by 2.03 paise and 0.43 paise however, it was lower in control group than group T_2 by 0.52 paise. Thus, economics of broiler production indicated that supplementation of herbal choline @ 0.075% of feed was beneficial in achieving more profit due to improved performance in terms of higher live weight and better feed conversion ratio. Finding of present study also revealed that supplementation of herbal choline @ 0.055% of broiler feed was not cost effective as compared to control.

4.7 Overall performance

The overall performance of birds during entire experimental period of six weeks is presented in Table 4.33. The findings of the present study revealed that the supplementation of herbal choline @ 0.075 % of broiler feed showed numerically higher average gain in weight than other groups, however, average gain in weight of birds from control group was numerically higher than birds from group supplemented with herbal choline @ 0.055% and 0.095% of feed. The average gain in weight of birds from various experimental groups revealed non-significant difference. The average feed conversion ratio of birds from groups supplemented with herbal choline @ 0.075% and 0.095% of feed was better than control group and group supplemented with herbal choline @ 0.055% of feed.

The inclusion of herbal choline @ 0.075% of feed showed slight improvement in carcass traits, Lipid profile parameters and nutrient metabolizability as compared to control and other two experimental groups supplemented with herbal choline. The economics of broiler production also revealed better profit margins in group supplemented with herbal choline @ 0.075% of feed. Further, it was seen that profit margins were also higher for group supplemented with herbal choline @ 0.095% than control, however, it was lower than control in group supplemented with herbal choline @ 0.055 of feed.

Thus, it is concluded that supplementation of herbal choline @ 0.075% of broiler feed providing choline as per standard is subtle beneficial effect in improving weight gain, feed conversion ratio, carcass traits, blood biochemical profile, nutrient metabolizability of birds and such supplementation is also cost effective. Further, it is also concluded that supplementation of herbal choline @ 0.095 % of broiler feed, which provides 25% more choline than standard choline requirement is also cost effective, however supplementation of herbal choline @ 0.055% of broiler feed which provides 25% less choline than standard choline requirement is not cost effective.

Table 4.33 Overall performance of birds at the end of six weeks

Parameters	Groups				p
	T_0	T_1	T_2	T_3	
Growth performance					
Initial body weight (g)	50.88±1.25	46.38±2.47	48.98±1.14	47.55±1.01	NS
Final body weight (g)	2745.97±32.62	2786.33±12.46	2705.80±26.64	2718.66±38.71	NS
Total gain in weight (g)	2695.11±33.33	2740.37±10.03	2656.83±27.50	2671.11±38.83	NS
Total feed consumption (g)	4471.43±48.90	4391.45±3.69	4441.91±10.93	4383.27±48.01	NS
Cumulative FCR	1.66±0.02	1.62±0.01	1.67±0.01	1.64±0.01	NS
Carcass traits (%)					
Dressing	73.84±0.27	74.24±1.19	72.62±1.14	73.59±0.27	NS
Eviscerated wt.	69.35±0.29	69.70±1.23	68.14±1.05	69.17±0.30	NS
Giblet wt.	4.49±0.04	4.54±0.10	4.48±0.12	4.42±0.03	NS
Liver wt.	1.90±0.05	1.88±0.06	1.94±0.02	1.92±0.03	NS
Gizzard wt.	2.06±0.04	2.11±0.04	1.98±0.06	2.01±0.03	NS
Heart wt.	0.53±0.05	0.55±0.04	0.56±0.06	0.49±0.02	NS
Breast wt.	27.34±0.30	27.05±1.16	27.02±0.75	26.62±1.12	NS
Drumstick wt.	4.35±0.05	4.36±0.09	4.30±0.12	4.27±0.02	NS
Thigh wt.	9.31±0.07	9.33±0.16	9.37±0.08	9.35±0.02	NS
Blood Biochemical Parameters					
Serum Protein (g/dL)	3.43±0.05	3.51±0.08	3.33±0.03	3.38±0.05	NS
Serum Albumin (g/dL)	1.61±0.22	1.63±0.02	1.52±0.02	1.57±0.15	NS
Serum Globulin (g/dL)	1.82±0.26	1.89±0.10	1.81±0.03	1.82±0.17	NS
SGOT (IU/L)	308.93±10.75	291.43±17.06	318.43±3.84	319.33±10.45	NS
SGPT (IU/L)	10.17±1.51	8.40±0.44	10.17±0.44	9.72±2.10	NS
Lipid Profile Parameters					
Serum Cholesterol (mg/dl)	149.25^{a}±2.06	129.25^{c}±1.25	139.75^{b}±1.8	121.25^{d}±1.70	5.01
Serum HDL (mg/dl)	52±1.08	56±2.65	51.5±0.65	53±1.87	NS
Serum LDL (mg/dl)	67.25^{a}±0.85	48.75^{b}±1.49	64.75^{a}±1.38	47.25^{b}±1.80	4.38
Serum VLDL (mg/dl)	17.38^{a}±1.27	11.97^{bc}±0.28	13.80^{b}±0.23	11.10^{c}±0.35	2.11
Serum Triglycerides (mg/dl)	51.25±0.85	48.75±2.29	50.75±3.45	47.75±2.95	NS
Retention %					
DM	61.19	62.36	60.04	60.83	-
CP	59.09	59.28	58.64	58.78	-
EE	86.19	86.24	85.91	86.02	-
CF	32.22	32.53	31.93	32.16	-
NFE	60.84	61.64	60.46	60.54	-
Economics of broiler production (₹)					
Net profit/ bird	62.72	70.68	60.68	63.73	-
Net profit/ kg live body wt.	22.85	25.37	22.43	23.45	-
Cost of feed/ kg weight gain	63.67	61.34	63.89	62.94	-

CHAPTER – 5

SUMMARY AND CONCLUSIONS

The present experiment was conducted to evaluate the "Effect of Dietary Supplementation of Herbal Choline on Performance of Broiler Chicken". It was carried out on 240 a day old Vencobb y 430 strain broiler chicks. The 240, broiler chicks were distributed randomly into uniform sized in four treatment groups viz. T_0, T_1, T_2 and T_3, each consisted of 60 chicks with four replicates and 15 experimental chicks in every replicate. Group T_0 (control) received standard broiler diet (BIS, 2007) with commercial choline chloride 60% @ 0.09%, while groups T_1 received standard broiler diet with herbal choline @ 0.075% (Equivalent to synthetic choline), T_2 received standard broiler diet with herbal choline @ 0.055% (25% less than synthetic choline equivalent) and T_3 received standard broiler diet with herbal choline @ 0.095% (25% more than synthetic choline equivalent). The diets of all the experimental groups were iso-caloric and iso-nitrogenous. Each experimental group was fed with standard broiler diet formulated as per BIS (2007).

The experimental birds were raised under standard managemental practices on deep litter system during the experiment for the period of 42 days. Throughout the trial various parameters were recorded to determine the effect of dietary supplementation of herbal choline on performance of broiler chicken such as growth performance (weekly live body weight, weekly body weight gain, weekly feed intake and weekly feed conversion ratio), carcass traits (dressing percentage, eviscerated yield percentage, giblet, heart, liver, gizzard, drumstick, breast, thigh), blood biochemical parameters (total serum protein, serum albumin, serum globulin, SGPT, SGOT and lipid profile), histopathology of hepatic cells and nutrient metabolizability. At the end of the experimental period, the economics of broiler production was also studied.

The average initial live body weights of day old chicks for groups T_0, T_1, T_2 and T_3 were 50.88 ± 1.25, 46.38 ± 2.47, 48.98 ± 1.14 and 47.55 ± 1.01 g, respectively. At the end of six weeks, average live body weights for groups T_0, T_1, T_2 and T_3 were 2745.97 ± 32.62, 2786.33 ± 12.46, 2705.80 ± 26.64 and 2718.66 ±

38.71 g, respectively. The mean average weekly live body weights for group T_0, T_1, T_2 and T_3 were 1189.13 ± 3.83, 1204.75±6.67, 1185.41±11.07 and 1202.69±9.88, respectively. From the data it is seen that group T_1 showed the highest average weekly live weights followed by group T_3, T_0 and T_2. Though there was numerical difference in final average live body weight and mean average weekly live weights of different treatment groups, on statistical analysis of the data it revealed no significant difference among various experimental groups. However, during second week of experiment the average weekly live body weights (g) of birds from group T_1 was higher than control and other two groups. The statistical analysis revealed significantly ($P<0.01$) higher average live body weights of birds from group T_1 than groups T_0, T_2 and T_3. Further, it is observed that average live body weights of birds from group T_0 and T_3 were significantly ($P<0.01$) higher than group T_2, however, difference between group T_0 and T_3 was comparable.

The average weekly body weight gains for groups T_0, T_1, T_2 and T_3 were 449.18±5.55, 456.66±1.67, 442.80±4.58 and 445.18±6.47 g, respectively. At the end of present experiment, the cumulative body weight gain for the groups T_0, T_1, T_2 and T_3 were 2695.11±33.33, 2740.37±10.03, 2656.83±27.50 and 2671.11±38.83 g, respectively. Statistical analysis of the data showed non significant difference in mean and cumulative weight gain among various experimental groups. However, during second and fifth week, the average live weight gain showed significant ($P<0.01$) difference among various experimental groups. During second week of experimental period the average weekly body weight gain of birds from group T_1 was significantly ($P<0.01$) higher than all other experimental groups, however, difference between group T_0 and T_3 was comparable. Further, it is seen that average body weight gain of birds from T_0 and T_3 was significantly higher than group T_2. The data of average weekly body weight gain (g) obtained during fifth week revealed significantly ($P<0.01$) higher body weight gain for control and T_1 group than group T_2 and T_3, however, difference between T_0 and T_3 and that of T_2 and T_3 was non-significant.

The average weekly feed consumption was 745.24±8.15, 731.91±1.15, 740.32±1.82 and 730.54±19.03 g for the groups T_0, T_1, T_2 and T_3, respectively. The cumulative feed consumption at the end of experiment for the groups T_0, T_1, T_2 and

T_3 was 4471.43±48.90, 4391.45±3.69, 4441.91±10.93 and 4338.27±48.01 g, respectively. From the data it is revealed that feed consumption was highest in group T_0 followed by groups T_2, T_1 and T_3. From statistical analysis of the data, it is observed that mean and cumulative feed consumption of various experimental groups was comparable although, significant difference in average feed consumption was observed during third, fourth and fifth week of experiment. During 3rd week, the average feed consumption of birds from T_0, T_2 and T_3 groups was significantly ($P<0.01$) higher than group T_1, however, difference among group T_0, T_2 and T_3 was comparable. In the 4th week of experimental period, the average feed consumption was significantly ($P<0.01$) higher in T_2 group than other three experimental groups, however, difference among group T_0, T_1 and T_2 was non-significant. The fifth week of experiment revealed significantly ($P<0.01$) higher feed consumption in birds from control group than group T_1, T_2 and T_3, however, difference between group T_1 and T_2 and that of group T_2 and T_3 was comparable.

The average weekly feed conversion ratio for birds from groups T_0, T_1, T_2 and T_3 were 1.62±0.03, 1.57±0.01, 1.65±0.01 and 1.61±0.02, respectively. The cumulative FCR of the experimental birds in T_0, T_1, T_2 and T_3 groups were 1.66±0.02, 1.62±0.01, 1.67±0.01 and 1.64±0.01, respectively. No significant difference was found among the experimental groups on the statistical analysis of data of average feed conversion ratio and cumulative feed conversion ratio. The group T_1 was found superior in FCR followed by T_3, T_0 and T_2. Due to similar weight gain and feed intake across all treatment groups in this experiment, a non-significant effect on the feed conversion ratio was observed.

Mortality of two birds from each group was occurred during entire experimental period and mortality percentage was 3.33 % which was well within permissible limit.

For carcass evaluation studies birds were selected and average live weight of selected birds from groups in T_0, T_1, T_2 and T_3 was 2738.47±52.57, 2779.33±6.97, 2673.05±33.70 and 2697.90±41.24 g, respectively. In T_0, T_1, T_2 and T_3 groups the average dressing percentage was 73.84 ± 0.27, 74.24 ± 1.19, 72.62 ± 1.14 and 73.59 ± 0.27 %, respectively. The dressing percentage of group T_1 was higher followed by T_0, T_3 and T_2 groups. The average eviscerated yield percentage was 69.35± 0.29, 69.70 ± 1.23, 68.14 ± 1.05 and 69.17 ± 0.30 % for groups T_0, T_1, T_2 and T_3, respectively. The eviscerated yield percentage of group T_1 was higher followed by groups T_0, T_3 and T_2. From statistical analysis of the data, it is observed that average values of dressing percentage and eviscerated yield percentage were comparable among various experimental groups.

At the end of trial period, no significant difference was noticed within the various experimental groups for the percentage average values of giblet, liver, gizzard, breast, drumstick and thigh weight. The average percentages for giblet weight were 4.49 ± 0.04, 4.54 ± 0.10, 4.48 ± 0.12 and 4.42 ± 0.03 % in T_0, T_1, T_2 and T_3 groups, respectively. The average heart, liver and gizzard weight percentages in groups T_0, T_1, T_2 and T_3 were 0.53±0.05, 0.55±0.04, 0.56±0.06 and 0.49±0.02 %; 1.90±0.05, 1.88±0.06, 1.94±0.02 and 1.92±0.03 % and 2.06±0.04, 2.11±0.04, 1.98±0.06 and 2.01±0.03 %, respectively. In groups T_0, T_1, T_2 and T_3, the average breast weight percentage was 27.34±0.30, 27.05±1.16, 27.02±0.75 and 26.62±1.12 %, respectively. The average drumstick weight percentage in groups T_0, T_1, T_2 and T_3 was 4.35±0.05, 4.36±0.09, 4.30±0.12 and 4.27±0.02 %, respectively. The average thigh weight percentage in groups T_0, T_1, T_2 and T_3 was 9.31±0.07, 9.33±0.16, 9.37±0.08 and 9.35±0.02 %, respectively.

The difference in blood biochemical parameters viz. serum protein, albumin and globulin was found statistically non-significant among various experimental group. The average serum protein for birds in groups T_0, T_1, T_2 and T_3 was 3.43±0.05, 3.51±0.08, 3.33±0.03 and 3.38±0.05 g/dL, respectively. The average serum protein for group T_1 was higher followed by group T_0, T_3 and T_2. The average serum albumin for groups T_0, T_1, T_2 and T_3 was 1.61±0.22, 1.63±0.02, 1.52±0.02, 1.57±0.15 g/dL, respectively. The average serum globulin for groups T_0, T_1, T_2 and T_3 was 1.82±0.26, 1.89±0.10, 1.81±0.03 and 1.82±0.17 g/dL, respectively.

The average SGOT values were 308.93±10.75, 291.43±17.06, 318.43±3.84 and 319.33±10.45 IU/L, respectively for experimental groups T_0, T_1, T_2 and T_3 and values of average SGPT for corresponding treatment groups were 10.17±1.51, 8.40±0.44, 10.17±0.44 and 9.72±2.10 IU/L. No significant difference was observed in average SGOT and SGPT values among the various experimental groups.

Under the lipid profile parameters, the average serum cholesterol levels for experimental groups T_0, T_1, T_2 and T_3 were 149.25±2.06, 129.25±1.25, 139.75±1.8 and 121.25±1.70 mg/dL, respectively. Significant difference ($P<0.01$) was found among the experimental groups on the statistical analysis of data related with average serum cholesterol levels. The average serum cholesterol level of group T_0 was significantly ($P<0.01$) higher than group T_1, T_2 and T_3. Further, it is observed that average serum cholesterol levels of birds from group T_1 and T_2 were significantly ($P<0.01$) higher than group T_3, however, average serum cholesterol level of group T_2 was significantly higher than T_1 group. The average serum triglycerides levels were 51.25±0.85, 48.75±2.29, 50.75±3.45 and 47.75±2.95 mg/dL, for group T_0, T_1, T_2 and T_3, respectively. No significant difference was observed in serum triglycerides levels among the various experimental groups on statistical analysis of the data.

The average serum LDL and VLDL levels for groups T_0, T_1, T_2 and T_3 were 67.25±0.85, 48.75±1.49, 64.75±1.38, 47.25±1.80 mg/dL and 17.38±1.27, 11.97±0.28, 13.80±0.23 and 11.10±0.35 mg/dL, respectively. Statistical analysis of the data showed significant ($P<0.01$) difference in average serum LDL and VLDL levels of various experimental groups. The average serum LDL levels of birds from group T_0 and T_2 were significantly ($P<0.01$) higher than T_1 and T_3 group, however, difference between group T_0 and T_2 and that of group T_1 and T_2 was comparable. The average serum VLDL levels of birds from group T_0 was significantly ($P<0.01$) higher than all other experimental groups and average serum VLDL levels of group T_1 and T_2 and that of T_1 and T_3 group were comparable. The average serum VLDL level for group T_3 was significantly ($P<0.01$) lower than all other experimental groups. The average serum HDL levels for birds in group T_0, T_1, T_2 and T_3 were 52±1.08, 56±2.65, 51.5±0.65, 53±1.87 mg/dL, respectively. No significant difference was found among various experimental groups for the average serum HDL levels.

For groups T_0, T_1, T_2 and T_3 the digestibility of dry matter was 61.19, 62.36, 60.04, 60.83 % and crude protein was 59.09, 59.28, 58.64 and 58.78%, respectively. The digestibility for ether extract and nitrogen free extract for groups T_0, T_1, T_2 and

T_3 was 86.19, 86.24, 85.91, 86.02 % and 60.84, 61.64, 60.46 and 60.54%, respectively. The digestibility percentage of dry matter, crude protein, ether extract and nitrogen free extract was higher in group T_1 followed by T_0, T_3 and T_2 groups. The digestibility for crude fibre for groups T_0, T_1, T_2 and T_3 was 32.22, 32.53, 31.93 and 32.16%, respectively. The digestibility of crude fibre was higher in group T_1 followed by group T_0, T_3 and T_2. The higher digestibility of all nutrients in T_1 group was also reflected in higher body weight gains than other three experimental groups.

The net cost of production per bird was ₹.211.79, 207.74, 209.83 and 207.69 for T_0, T_1, T_2 and T_3 experimental groups, respectively and corresponding net profit per bird was ₹.62.71, 70.86, 60.68 and 63.74. This indicated that net profit per bird and net profit/ kg live body weight was higher in group T_1 followed by T_3, control and T_2 group.

Conclusion

From overall results of the study following conclusions are drawn

1. Supplementation of herbal choline @ 0.075% in broiler feed has subtle beneficial effect on improving weight gain, feed conversion ratio and nutrient metabolizability of birds
2. Dietary supplementation of herbal choline has no effect on blood biochemical parameters and carcass traits but such supplementation is effective in reducing serum cholesterol, LDL and VLDL levels of birds
3. Dietary supplementation of herbal choline reveals no pathological changes in hepatic cells of birds.
4. Herbal choline supplementation @ 0.075 and 0.095% in broiler feed is cost effective than supplementation of broiler feed with commercial choline chloride 60% @ 0.09% of feed.

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THESIS ABSTRACT

a)	Title of thesis (in capital letters)	:	EFFECT OF DIETARY SUPPLEMENTATION OF HERBAL CHOLINE ON PERFORMANCE OF BROILER CHICKEN
a)	Name of student	:	**DHAIGUDE MANGESH VILAS**
b)	Name and address of Major Advisor	:	**Dr. G. M. Gadegaonkar** Associate Professor, Department of Animal Nutrition, COVAS, Udgir.
c)	Degree to be awarded	:	**M.V.Sc**
d)	Year of award of degree	:	**2023**
e)	Major Subject	:	**ANIMAL NUTRITION**
f)	Total No. of pages in thesis	:	**67**
g)	No. of words in the abstract	:	**300**
h)	Signature of Student	:	
i)	Signature, Name, and Address of Forwarding authority (HOD/SH).	:	**Dr. G. M. Gadegaonkar** Head of the Department

ABSTRACT

The experiment involved 240 day-old broiler chicks of "VenCobb-430y" strain distributed among four uniform groups viz. T_0, T_1, T_2 and T_3, with each group having 60 chicks divided in four replicates. Group T_0 (control) received standard broiler diet (BIS, 2007) with commercial choline chloride 60% @ 0.09%, while groups T_1, T_2 and

T_3 received standard broiler diet containing herbal choline @ 0.075% (equivalent to synthetic choline), 0.055% (25% less than synthetic choline equivalent) and 0.095% (25% more than synthetic choline equivalent). All the diets were isonitrogenous and isocaloric. The trial lasted for 42 days. The average live body weight, weight gain, feed consumption and feed conversion ratio among various experimental groups was comparable. The blood biochemical parameters viz. serum total protein, albumin, globulin, SGPT and SGOT did not differ significantly among experimental groups. The average serum cholesterol level of control group was significantly ($P<0.01$) higher than groups T_1, T_2 and T_3. The average serum triglyceride and HDL levels were comparable, however, average LDL levels of birds from group T_0 and T_2 were significantly ($P<0.01$) higher than group T_1 and T_3. Whereas, serum VLDL levels were significantly ($P<0.01$) higher in control group than other groups. Histopathological examination of hepatic cell showed no pathological changes among various experimental groups. The digestibility percentage of DM, CP, CF, NFE and EE was higher in group $T_{1.}$ The carcass traits showed no significant difference among various experiment groups. The net profit per bird and net profit/ kg live body weight was higher in group T_1 followed by T_3, control and T_2 group. Thus, it is concluded that supplementation of herbal choline @ 0.075% in broiler feed is beneficial in improving weight gain, feed conversion ratio, carcass traits, blood biochemical profile and nutrient metabolizability of birds and such supplementation is cost effective. Further, it is also concluded that supplementation of herbal choline @ 0.095% of broiler feed is also cost effective.

प्रबंध सारांश

अ) प्रबंधाचे शिर्षक	:	मांसल कोंबड्यांच्या आहारामध्ये हर्बल कोलीन चा समावेश केल्यास होणारा परिणाम अभ्यासणे
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इ) वर्ष	:	**२०२३**
फ) मुख्य विषय	:	**पशुपोषण आहारशास्त्र**
ग) प्रबंधाची एकूण पाने	:	**७२**
ह) सारांशातील एकूण शब्द	:	**२४५**
ई) विद्यार्थ्याची स्वाक्षरी	:	
ज)विभाग प्रमुखाची स्वाक्षरी	:	

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सारांश

सदर प्रयोगाचे उद्देश हे मांसल कोंबड्यांच्या आहारात हर्बल कोलीनचा अवलंब केल्यास त्यांच्या वाढीवर, पोषणमूल्य चयापचय प्रक्रिया, रक्तातील घटक, त्यांच्या मासांची वैशिष्टे आणि उत्पादन अर्थशास्त्र यांवर होणाऱ्या परिणामांचा अभ्यास करणे हा होता. सदर प्रयोगामध्ये २४० पक्षांचे चार गटामध्ये जसे की टी०, टी१, टी२ व टी३ (प्रत्येकी ६० पक्षी) विभाजन करण्यात आले व त्या नंतर प्रत्येक गटाचे चार उपगटांमध्ये विभाजन करून प्रत्येक उपगटामध्ये १५ पक्षी ठेवण्यात आले होते. टी० गटास मानक खाद्यासोबत ०.०९% कोलीन क्लोराईड (६०%), तर गट टी१, टी२ व टी३ गटास मानक खाद्यासोबत अनुक्रमे ०.०७५%, ०.०५५% आणि ०.०९५% हर्बल कोलीनचा अवलंब करण्यात आला. हा प्रयोग ४२ दिवसांसाठी राबवण्यात आला. सदर प्रयोगाच्या शेवटी असे आढळून आले की, हर्बल कोलीनचा मांसल कोंबड्यांच्या आहारात समावेश केल्यास त्यांच्या सरासरी वजनावर, वजन वाढीवर आणि खाद्याच्या

उपयुक्ततेवर सांख्यिकी दृष्टया विशेष असा कोणताही परिणाम दिसून आला नाही. तसेच रक्तजलातील सरासरी प्रथिने, अल्ब्युमिन आणि ग्लोब्युलिन वर कोणताही परिणाम आढळून आला नाही. परंतु रक्तजलातील सरासरी चरबी आणि कमी व अतिकमी घनता असलेले लिपोप्रोटीन घटक सांख्यिकी दृष्टया कमी आढळून आले. हर्बल कोलीनचा पक्षांच्या पोषणमूल्य चयापचय प्रक्रिया आणि मांसाच्या वैशिष्ट्यांवर सांख्यिकी दृष्टया कोणताही परिणाम दिसून येत नाही. तसेच यकृत सूक्ष्मदर्शनीय उती विकृती अभ्यासात अंतर्गत पेशीमध्ये कोणत्याही विकृती आढळून आल्या नाहीत. हर्बल कोलीनचा मांसल कोंबड्यांच्या आहारात ०.०७५% एवढा अवलंब आर्थिक दृष्टया किफायतशीर आढळून आला. त्या खालोखाल टी३ व टी० गटाचे उत्पादन अर्थशास्त्र अधिक होते. वरील प्रयोगावरून असा अनुमान काढण्यात आले की, हर्बल कोलीनचा ०.०७५% प्रमाणात मांसल कोंबड्यांच्या आहारात समावेश केल्यास त्याचा सरासरी वजनावर, वजनवाढी दरावर, खाद्याच्या उपयुक्ततेवर व पोषणमूल्यांच्या चयापचय प्रक्रियेवर अनुकूल परिणाम आणि किफायतशीर दिसून येतो.