EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL WITH MAIZE GLUTEN MEAL WITH OR WITHOUT SUPPLEMENTATION OF PROTEASE ENZYME ON PERFORMANCE OF BROILERS

T H E S I S

Submitted
In partial fulfilment of the requirements for the Degree of

MASTER OF VETERINARY SCIENCE
IN
ANIMAL NUTRITION

BY
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Enrollment No: -V/17/036

Mumbai Veterinary College, Mumbai

MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY, NAGPUR – 440 001,
(INDIA)

2025

APPENDIX – B

DECLARATION OF THE STUDENT

I hereby declare that the experimental research work and interpretation of the thesis entitled **"EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL WITH MAIZE GLUTEN MEAL WITH OR WITHOUT SUPPLEMENTATION OF PROTEASE ENZYME ON PERFORMANCE OF BROILERS"** 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 or publications of any university or scientific organization. The sources of material used and all assistance received during the course of investigation have been duly acknowledged.

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Place: Mumbai

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Appendix – C (i)

DECLARATION OF ADVISORY COMMITTEE

Miss. BHUJBAL PRITAL RANGNATH has satisfactorily prosecuted her course of research for a period of not less than one semester and that the thesis entitled **"EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL WITH MAIZE GLUTEN MEAL WITH OR WITHOUT SUPPLEMENTATION OF PROTEASE ENZYME ON PERFORMANCE OF BROILERS**" submitted by her is the result of research work is sufficient to warrant its presentation to the examination in 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 of thereof has not been previously submitted by her for a degree of any other university.

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4	Dr. V. H. Shukla	Assistant Professor, Department of Livestock Products Technology	

Appendix – C (ii)

CERTIFICATE

This is to certify that the thesis entitled "**EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL WITH MAIZE GLUTEN MEAL WITH OR WITHOUT SUPPLEMENTATION OF PROTEASE ENZYME ON PERFORMANCE OF BROILERS**" submitted by **Miss. BHUJBAL PRITAL RANGNATH** to the Maharashtra Animal Sciences University, Nagpur, in partial fulfillment of the requirement for the degree of **Master of Veterinary Science (M.V.Sc)** has been approved by the Student's Advisory Committee after examination in collaboration with the External Examiner.

Name & signature of External Examiner

Signature & seal of HOD

Signature of guide/advisor

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Dean /Associate Dean.
Mumbai Veterinary College.

DEDICATED

TO MY

BELOVED

PARENTS

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Date
Place - Mumbai *Bhujbal Prital Rangnath*

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Abbreviations	Long forms
%	Per cent
/	Per
°C	Degree Celsius
<	Less than
>	Greater than
µl	Microliter
ad-lib	ad libitum
ADWG	Average daily weight gain
A:G	Albumin to globulin ratio
AME	Apparent Metabolizable energy
AOAC	Association of official analytical chemist
BCG	Bromocresol Green
BIS	Bureau of Indian Standards
BW	Body weight
BWG	Body weight gain
cal	Calculated
CGM	Corn Gluten Meal
CD	Critical difference
CP	Crude protein
d.	Day
DF	Degrees of freedom
dL	Deciliter
DM	Dry matter
DMB	Dry matter basis
et al.	Ethically all
FCR	Feed conversion ratio
Fig.	Figure
g	Gram
GDP	Gross Domestic Products
IBD	Infectious bursal disease
ICMR	Indian Council of Medical Research
i.e.	Id est (that is)
kcal	Kilocalorie
kg	Kilogram
Mcal	Mega calorie
mg	Milligram
MGM	Maize Gluten Meal
min	Minute

Abbreviations	Long forms
MSS	Mean sum of squares
NFE	Nitrogen free extracts
NPK	Nitrogen Phosphorus Potash
nm	Nanometer
NRC	National Research Council
NS	Non-significant
rpm	Revolutions per minute
Rs.	Rupees
SS	Sum of squares
SV	Source of Variation
UV	Ultra violet

Introduction

1. INTRODUCTION

Livestock is one of the major contributors in improving livelihood and global food security of billions of people. The current world human population is 8.1 billion and it is predicted to increase to 9.7 billion in 2050 (SWP Report 2023, UNFPA) which would definitely face the formidable challenge of feeding with the finite available sources. This scenario may increase the demand of animal protein sources, despite high costs due to their high digestibility, good palatability, and small concentrations of antinutritional compounds as compared with plant origin protein sources (Gottlob *et al*., 2006). Due to the efficiency of eggs and chicken meat as animal protein sources, poultry sector is an integral part and fastest growing sector of livestock economy of India. Poultry sector in India contributes around 1% of national GDP and provides employment opportunities to around 6 million small and medium farmers. Within the poultry sector, broiler and layer segments independently contribute with the monthly turnover of 400 million chicks and 8400 million eggs respectively (ICRA, 2020).

According to Food and Agriculture Organization Corporate Statistical Database production data (FAOSTAT, 2021), India ranks 2nd in Egg production and 5th in Meat production in the world. The consumption of chicken meat is around 4.5 kg per person per annum and the per capita availability of eggs in present is around 101 eggs per annum in India. Whereas, ICMR recommended that the annual consumption of poultry meat should be 10.8 kg and 180 eggs per person. Poultry meat is a rich source of digestible proteins, unsaturated lipids, Vitamin B (thiamine, vitamin B6 and pantothenic acid) and minerals (Iron, Zinc and Copper) with no religious or cultural taboos ensures the nutritional improvement in developing countries. Also, Poultry meat has relatively low and competitive pricing compared to other meat. Owing to these benefits, growing trend in shift of consumption pattern from red meat to white meat, and in order to reduce the disparity between the supply and demand of broiler and layer products, focus should be on increasing production while reducing the cost of the production for the expansion of the poultry industry.

Feed is a major input item for broiler rearing as performance indexes observed in the poultry production is greatly dependent on the quality of feed and proper formulation of diets to provide all the necessary nutrients with their increased bioavailability in the feed (Ajayi, 2015). It accounts for around 75% or sometime more than 75% of the total cost of production (Kamal and Raga, 2014). Thus, poultry diets are frequently formulated on a least cost. The cost of feed is significant factor for its sustainability and profitability. The rising cost of poultry feed ingredients especially protein sources like soybean has resulted in the rise of cost of poultry feed in recent years and ultimately reduced the profit margin in both broiler and layer farming (Purushothaman *et al*., 2015). Hence, to circumvent these economic losses, for ensuring more return and maintaining better health, continuous research for inclusion of new, alternative and novel feed ingredients in ration formulations may replace expensive protein sources like soybean meal and further contribute to sustainable animal diets. However, productive and economic efficiency of alternative feeds should be checked before using in the formulations.

Soybean meal and maize grains are widely used by poultry farmers as main protein and energy source, respectively in poultry diet. But, unfortunately, due to the commodity inflation in the recent past, most of the traditional ingredients including soybean are becoming expensive at farms and many poultry producers are looking for cheaper alternative protein sources to incorporate in the feed formulation. Also, antinutritional factor present in soybean, reduction in nutritional value due to NSPs (Non-starch polysaccharides) and the environmental impact of soybean production are other constraints (Giannenas *et al*., 2017). The search for alternative plant protein source in poultry nutrition has become a focus for several reasons. Corn co-products such as Corn gluten meal, corn dried distilleries grain solubles (DDGS), Crude corn oil (Rochell *et al*., 2011) can be emerged as an absolute protein source in livestock and poultry feeding.

Maize gluten meal is a by-product of corn starch industry and a coproduct of corn wet milling process which yields 5 main products: maize starch, maize germ oil meal, corn gluten meal, corn gluten feed and maize steep liquor. The process starts by steeping maize grains in water with sulfur dioxide (SO_2) for 24-40 hours at 45-55°C, which weakens the gluten matrix by breaking inter and intramolecular disulfide bonds and promotes lactic acid bacteria growth. After steeping, the kernels, containing 45% water, are ground to separate the germ for oil extraction, yielding maize germ oil meal (BeMiller *et al*., 2009). The remaining steep water is condensed into steep liquor. The endosperm is further screened to separate the fiber from the gluten (protein fraction) and starch slurry. The fiber (bran) is then combined with steep liquor and maize germ oil meal to produce corn gluten feed. The fiber-free endosperm is centrifuged to separate the maize gluten meal from starch (Baker *et al*., 2018).

Maize gluten meal comes in three main forms: unprocessed, granulated, and pelletized. Unprocessed maize gluten meal maintains its natural form, and is ideal for consumers looking for whole nutritious and minimal refined options. Granulated maize gluten meal is a byproduct of wet corn milling, functions as a natural pre-emergence herbicide. Whereas, Pelletized corn gluten meal is more often used in feed formulations due to its compact form. Wet-milled corn gluten meal (CGM) typically contains 60% protein, providing slow nitrogen release that nourishes soil and contributes organic matter. Thus, it acts as a natural and excellent nitrogen supplier in the form of fertilizer, commonly having a NPK ratio of 10:0:0. Global corn gluten meal market is growing due to trends in agriculture along with trends in meat consumption, feed efficiency, plant-based proteins and pet food. The corn gluten meal market is projected to reach $10.27 billion by 2028, with a 6% CAGR i.e. compound annual growth rate (Corn Gluten Meal Global Market Report 2024).

The protein profile of maize gluten meal mainly consists of zein (68%), glutelin (27%) and small amounts (1.2%) of globulins (Cha *et al*., 2000). Protein and carbohydrates content of maize gluten meal on dry matter basis is 67–71% proteins (8-10% in maize grain) and 21–26% carbohydrate of which 12–15% is starch (70-75% starch in maize grain). Its crude fat and fiber content ranges from 1-3% and 4-6% respectively (Di Gioia *et al*., 1999; Barley *et al*., 1971; NRC 1994). It is found that the metabolizable energy of Maize Gluten Meal is higher (3.720 Mcal/kg) than that of maize grain (3.350 Mcal/kg) and sometimes also higher than the values reported in NRC (1994) due to the varieties of corn seed and fat content of it (Abdel Raheem *et al*.,2005; Seyedi and Hosseinkhani, 2014). Amino acid content of maize gluten meal is adequate and higher than that of soybean meal and fish meal (Hardwick and Glatz 1989). CGM is a rich source of vitamin E and B complex vitamins with low amounts of phosphorus (Peter *et al*., 2000). It is rich in Methionine (1.5-1.6%), but, its Arginine, lysine (1-1.1%) and tryptophan (0.3-0.36%) contents are comparatively low (Sasse and Baker, 1973; Peter *et al*., 2000; NRC 1994). Furthermore, corn gluten meal is relatively rich in linoleic acid and carotenes-xanthophylls (200–500 mg/kg), especially when made from yellow corn (Livingston *et al*., 1969; Parkhurst and Mountney, 1987).

Maize Gluten Meal could be added as an excellent source of protein in broiler as well as layer diet as it contains 60% crude protein on dry matter basis and highly available sulphur containing amino acids (Sasse *et al*., 1973 and Funaba *et al*., 2005). Moreover, there are some reports on the usefulness of maize gluten meal as a source for xanthophylls added to broiler chicks and layer diets to enhance the colour of skin and yolk pigmentation. Various studies revealed that inclusion of 9-10% maize gluten meal in the broiler ration proved to be the efficient protein rich feed ingredient for optimum broiler growth which significantly improved FCR and weight gain and further increase of the MGM level in the broiler diet did not show any positive or negative effect on production and profitable broiler farming (Ismail *et al*., 2005 ; Abhijeet *et al*., 2021). Whereas, Sushil *et al*., (2024) concluded that 25% dietary CP replacement with MGM could significantly increase the gain in body weight and feed intake in broilers.

Soybean protein is rich in lysine, tryptophan, isoleucine, valine and threonine (Ensminger *et al*., 1990) and is characterized by the highest digestibility for lysine and methionine. Nutritive value of soybean protein is limited by sulfur amino acids. Whereas, most of the protein present in maize gluten is insoluble, and its amino acid content is imbalanced as it contains high leucine, isoleucine and valine (Zheng *et al*., 2012). Unfortunately, a large number of proteins in the MGM cannot be absorbed because of their compositions and structure (Bai *et al*., 2019). Besides its indispensable amino acid profile, arginine, lysine and methionine may act as antinutritional factor to a lesser extent (Peter *et al*., 2000). Moreover, anti-nutritional factors such as mycotoxins, lectins and protease inhibitors might be present in maize gluten meal, limiting the bioavailability of essential nutrients and affecting the overall nutrition profile of maize gluten meal-based products. Maize gluten meal is much richer in cell wall constituents and lower in starch as compared to the maize grain. Thus, it should not be used as the main protein source of nonruminant diet and inclusion level in the diet of poultry should be within limit.

Exogenous enzymes help in making more nutrients available especially when feed ingredients and supplements are with low bioavailability or are of inferior quality (Kocher *et al*., 2001, Cowieson and Adeola, 2008). Enzymes also reduce the amount of nutrients voided in feaces thereby ensuring sustainable feeding by reducing pollutants in the environment (Oxenboll *et al*., 2011). A wide range of endogenous protease enzymes are synthesized and released in the gastrointestinal tract of birds. These are generally considered to be sufficient to optimize the protein and amino acids utilization in feed (Mohammadigheisar and Kim, 2018). However, crude protein and amino acid digestibility reports for poultry indicated that, large amounts of protein pass through the gastrointestinal tract of birds without being completely digested. This offers an opportunity for the use of supplemental exogenous protease in broiler feeds to improve the protein and amino acid digestibility (Lemme *et al*., 2004 ; Angel *et al*., 2011).

The use of exogenous protease enzyme improves amino acid digestibility, feed conversion ratio and intestinal integrity of the broiler chicken (Cowieson *et al*., 2018). Protease effect on the amino acid digestibility may vary according to the level of supplementation as feed ingredient composition directly influences the substrate and modulates the factors affecting digestive enzymes. Also, poor utilization of dietary nutrients due to immaturity of the digestive system of young chicks (Jin *et al*., 1998) and inferior quality of affordable protein sources (Ajayi, 2015) are some of the major issues in protein feeding. Moreover, protein is less digestible (80-85%) compared to starch (90%) in corn-soy based diet which is usually preferred in poultry. Protease enzyme may improve feed efficiency, utilization by the animal, reducing production cost and also lower the total content of nitrogen in manure (Kamel *et al*., 2015) contributing towards sustainable poultry production. Thus, nutritionists can formulate diets with lower levels of dietary protein while maintaining growth performance (Leinonen and Williams, 2015). Positive results of supplementation of 200-250 mg/kg protease enzyme in low protein diet (Kamel *et al*., 2015) and with lower levels of protease enzyme along with non-starch polysaccharide enzyme are also observed (Yuan *et al*., 2017); whereas, further study is required with higher levels of protease enzyme supplementation in broiler diet (Tripathi *et al*., 2020).

Hence, to study the effect of partial replacement of soybean meal with maize gluten meal with or without supplementation of protease enzyme on performance of broilers, the current investigation is planned with following objectives :

1. To study the effect of partial replacement of soybean meal with maize gluten meal with or without supplementation of protease enzyme on growth performance and nutrient utilization of broiler chicken.

2. To study the effect of partial replacement of soybean meal with maize gluten meal with or without supplementation of protease enzyme on carcass traits.

3. To study the effect of partial replacement of soybean meal with maize gluten meal with or without supplementation of protease enzyme on blood biochemical parameters.

4. To study the effect of partial replacement of soybean meal with maize gluten meal with or without supplementation of protease enzyme on economics of broiler production.

Review of Literature

2. REVIEW OF LITERATURE

The review of literature related to "EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL WITH MAIZE GLUTEN MEAL WITH OR WITHOUT SUPPLEMENTATION OF PROTEASE ENZYME ON PERFORMANCE OF BROILERS" are presented in the foregoing pages. The literature has been reviewed on following parameters.

1. Growth performance
2. Carcass traits study
3. Blood biochemical profile
4. Nutrient digestibility
5. Economics of feeding

2.1 Growth Performance

Babidis *et al*., (2002) randomly allocated Sixty day-old broiler chickens to two groups (Control Group and Gluten Group) from a commercial strain cross (Ross Ross). All the birds weighed individually and each group divided into three sub-groups (replicates) of 10 birds each. Control group received a regular compound diet containing herring meal and meat meal as a protein source, whereas birds in the Gluten group were fed with CGM (variety 60% CP) which is replaced by all animal protein sources, i.e. fish and meat meal. Both diets were isocaloric and isonitrogenous. The results suggested that the feeding of broilers with CGM instead of herring meal and meat meal had no significant ($p>0.05$) effect on BW and FCR during the experimental period of 42 days. At the end of the experiment (42nd day), average body weight for Gluten group was 2151 g vs. 2223 of Control group ($p>0.05$), while FCR for the Gluten group was 1.88 vs. 1.95 of Control group ($p>0.05$).

Abdel Raheem *et al*., (2005) designed an experiment by using two plant protein sources, full-fat soybean (FFSB) and corn gluten meal (CGM) as a substitute for soybean meal (SBM) in broiler diet. A total number of 175 day old chicks (Arbor acres) were weighed and randomly distributed into seven groups of 25 chicks each. First trial was bounded to the control group in which three diets was fed, the starter, grower and finisher based on SBM as the main protein source. In other two trials, instead of soybean meal two vegetable protein sources were used i.e. full-fat soybeans and the corn gluten meal. Trial conducted in three groups: First for feeding the FFSB and CGM for all the three feeding phases i.e. starter, grower and finisher; Second for growing- finishing phases and third for finishing period only. Parameters assessed include growth performance, body weight development, weight gain, feed intake and feed conversion efficiency. In the first group of full-fat soybeans trial, slight reduction in growth rate was observed which was about 8%, and eventually slightly low feed conversion efficiency. In the second group, feed intake was high compared to gain in weight which ultimately lowered the feed conversion efficiency and that of third group in which FFSB feeding was limited to the finishing period showed slightly higher growth rate than the control. In third trial, they fed 20% corn gluten meal during the three feeding phases of growth or during growing-finishing periods had adverse effect on the feed intake, body weight, in addition to feed utilization compared to control, whereas CGM feeding to the finishing period was feasible.

Ismail *et al*., (2005) studied the effect of different levels of maize gluten meal (MGM; 60%) on the growth performance of broilers in Sindh Agriculture University between October and November 1999. A total of 300 chicks were divided into six groups: Group A was control group with standard basal diet, while birds in Groups B, C, D, E and F were supplemented with 3, 6, 9, 12 and 15% MGM, respectively. The group C which contains 9% MGM consumed relatively less quantity of feed (4246.51 g) compared to control group and other treatment groups. Also, group C (9% MGM) produced significantly higher weight gain (2063.50 g) and feed conversion ratio (2.05) than any other groups. Thus, it is concluded that inclusion of 9% MGM in the broiler ration proved to be the efficient feed ingredient for optimum broiler growth.

Odetallah ***et al*****., (2005)** conducted a 6-week experimental trial in which day-old male broiler chicks were randomly assigned to 32 pens and fed various diets with or without protease enzyme (Versazyme, VZ). In the first experiment, birds received a control diet, control + 0.1% protease, high-protein (HP) diet, or HP + 0.1% protease. Protease supplementation improved body weight at 21 and 42 days and feed conversion ratio (FCR) at 21 days, with the HP + protease diet showing better cumulative FCR. In the second experiment, birds received HP or HP + 0.1% protease (VZ) from two batches. Protease improved body weight and FCR at 22 days, and overall FCR at 42 days, though no significant body weight difference was observed at 43 days. In conclusion, protease supplementation in starter diets improved broiler growth and FCR.

El-Deek ***et al*****., (2009)** studied the effects of different levels of corn gluten meal (CGM) with or without phytase supplementation (500 FTU/kg) on growth performance of 160 unsexed Hubbard broiler chicks from 7 to 42 days. Broilers were divided into 8 groups with 4 replicates each. CGM substituted 0%, 25%, 50% and 75% of soybean meal protein in iso-nitrogenous, iso-caloric diets. Results showed that body weight and gains were not significantly affected by CGM inclusion or CGM × phytase interaction, but phytase supplementation improved these parameters. Feed consumption was unaffected, though feed conversion ratio showed a slight improvement.

Freitas ***et al*****., (2011)** conducted an experiment to assess the effect of adding exogenous protease to broiler diets based on corn, soybean meal, and meat and bone meal. In the first experiment, 1,764 Ross 308 broilers were fed seven treatments, including positive and negative control diets with varying protease levels (0, 100, 200, 400, 800, and 1,600 ppm). While protease supplementation did not affect body weight, feed efficiency (FE) improved quadratically with increasing protease levels.

Fru-nji ***et al*****., (2011)** examined the effects of a novel serine protease, RONOZYME® ProAct (RPA : supplemented at 15,000 PROT/kg feed) on growth performance of broilers. Trials were arranged in a 2×2 factorial design. Each dietary treatment had 12 replicates. Birds were fed 12.7 MJ ME per kg iso-energetic diets in 2 phases during the first trial. Each diet was fed with or without RPA

(Control+RPA or Control, respectively) to either males or females. In second trial, two diets were fed in four treatments. Diet 1 (211g CP per kg feed in starter and 200g CP per kg feed in grower phases) was fed without or with RPA (NP i.e. Normal protein or NP+RPA, respectively). Diet 2 (200 and 190g CP per kg feed in the starter and grower phases respectively), supplied without or with RPA (LP i.e. Low protein or LP+RPA, respectively). In males, weight gain and FCR was significantly better in C+RPA than C [WG (2,393 g vs. 2,262 g) and FCR (1.60 vs. 1.65)]. In females, FCR (1.37 vs. 1.39) was better in C+RPA treatment group than C in the starter phase. The FCR of LP+RPA was significantly better than LP.

Rabello *et al.*, (2012) evaluated the effect of inclusion of corn gluten meal (CGM21) in the diets of free-range chickens from 32 to 84 days on growth performance. A total of 240 chickens were used in a completely randomized design with four treatments (0, 7, 14, and 21% CGM21) and four replicates. In the growth phase (32-63 days), CGM21 had no effect on performance, but during the total period (32-84 days), feed conversion improved with 9.8% CGM21 inclusion, and maximum weight gain was achieved with 9.05% CGM21.

Kaczmarek *et al.*, (2014) conducted a study to determine the effect of addition of amylase and protease on nutrient digestion during the first 2 weeks of growth of broiler chickens. The diet formulation for the experiment was corn-soybean meal-based and supplemented with either amylase or amylase plus protease. In first experiment, 135 birds were randomly assigned to three dietary treatments consists of 9 replicate cages of 5 birds each. Diet fed of all the three treatment groups was in mash form; first: control, second: control plus amylase (10,000 U/kg) and amyloglucosidase (10 U/kg), and third: control plus amylase, amyloglucosidase, and protease (4,000 U/kg). No effect of enzyme supplementation ($p>0.05$) on growth performance (body weight gain and FCR) was observed.

Seyedi and Hosseinkhani (2014) conducted an experiment to examine the effect of corn gluten meal (CGM) on growth performance of broiler chicks. In the growth assay, 1200-day-old broiler chickens were distributed in a completely randomized design to determine the effect of CGM in four levels (3,6,9 and 12%)

on the growth characteristics. Results for growth performance showed that the all level of CGM had positive effect on broiler performance; while, the group of birds fed with 12% CGM had the best weight gain and FCR.

Ajayi (2015) studied the effect of feeding protease enzyme on growth performance of broilers. Birds were fed ad libitum with low protein diets (with or without protease enzyme) in a 2X3 completely randomized experimental design. The experimental period was 0-42 days divided into two phases: 0-21days and 22-42days. Gain in body weight of birds significantly increased those were on low CP diets with protease supplementation. Feed intake for all birds decreased significantly with inclusion of protease enzyme. Values obtained for decreasing feed intake were from 1530.91g to 1438.6g for the 15.5/12.5%CP diets; from 1475.82g to 1407.84g for the 17.5/14.4%CP diets and from 1555.58g to 1442.56g for the 23/20%CP diets. Feed Conversion Ratio (FCR) for birds on the diets with or without protease was not significantly different.

Kamel *et al.*, (2015) studied the impact of an exogenous mono component dietary protease enzyme on growth performance of broilers with or without matrix values of enzyme. A total of 300 one-day-old chicks (Cobb500) were randomly placed into five treatment groups with three replicates and 20 birds per replicate for five weeks. G1 was control group without any supplementation, G2 served as control group with 200 ppm protease. Birds in G3 received the same protease level as G2 minus whole matrix value of the enzyme. Fortified basal diets after subtracting ME and CP along with Amino acids matrix value of the enzyme were fed to the birds in G4 and G5 respectively. Birds in G2 and G4 significantly ($p \leq 0.05$) improved weight gain, feed intake and FCR compared to all other groups.

Purushothaman *et al.*, (2015) studied the efficacy of protease in broiler chicken rations for a period of six weeks. Total of 900 day-old Vencobb male broiler chicks were divided into 9 treatment groups with 10 replicates and ten birds in each replicate. Treatment group T1 was positive control with standard ration. T2, T3 and T4 were negative control (without protease) and for T6, T7, T8 respectively at 0.5g/kg using 75, 100 and 125% of the amino acid matrix respectively. T5 was a positive control and T1 with protease containing 600000 IU/kg at 0.5g/kg. T9 was formulated with protease at 0.5g/kg using 100% amino acid ME matrix levels (180 kcal/g) of protease. Protease supplemented treatment groups showed higher feed intake and weight gain. Feed efficiency of T9 was poor compared to the positive control.

Giannenas *et al*., (2017) examined the combined effects of reducing the levels of soybean meal (SBM), introducing corn gluten meal (CGM) and adding a dietary protease in the ration of a broilers reared on a commercial Greek farm. 540 chicks were divided into three dietary treatments with six replicates of thirty birds each. The first treatment group (Control) was fed a conventional diet based on corn and SBM, containing 21% w/w crude protein (CP). The second group (Soy-Prot) received a corn and SBM-based diet containing a lower level of CP (20% w/w) and 200 mg of protease RONOZYME® Proact per kg of feed. The third group (Gluten-Prot) was fed a diet without SBM but based on corn and CGM and with CP and protease contents identical to those of the diet of the Soy-Prot group. The growth performance of the second group fed with Soy-Prot diet was similar to the birds fed with Control diet. However, third group fed with Gluten-Prot diet showed a tendency ($p \leq 0.010$) for lower weight gain and feed intake compared to those of the Control diet.

Naik *et al*., (2017) reared 200 one-day-old commercial broiler chicks for a period of 6 weeks under uniform managemental conditions. They were randomly distributed into 4 treatments having 10 replicates and 5 birds per replicate. Diet formulated for these four treatments was corn-soya based and treatment groups were Control, Basal diet (BD) (with 2% less CP compared to control), BD with uncoated protease enzyme and BD with coated protease enzyme. Treatment groups supplemented with either coated or uncoated protease enzyme (6000 IU/ kg) resulted in better FCR, as compared to the control group.

Yuan *et al*., (2017) reared 300 one-day-old male broiler chickens (Ross-308) from 1 to 42 days of age to investigate the effects of exogenous enzymes on growth performance. They were randomly allocated by body weight to five treatments (one control and four supplemented with 150mg/kg NSP enzyme and 0, 40, 60 and 80 mg/kg protease levels), with six replicates of 10 birds each. Average daily feed intake (ADFI) and average daily gain (ADG) of the birds were significantly enhanced by the addition of NSP enzyme in combination with protease supplementation at 40 or 80 mg/kg.

Afrouzi *et al*., (2018) evaluated the effects of different levels of corn gluten meal (4 and 8%) along with both unprocessed and processed 300 mg/L protease enzyme on broiler chicks' growth performance. 200 commercial strains Ross 308 male broiler chicks were assigned to 5 treatments with 10 chicks per replicate, reared for 38 days. During the experiment, feed intake, body weight gain and feed conversion ratio were measured. Results showed that diets with corn gluten meal and protease enzyme increased feed intake, body weight gain, and improved feed conversion ratio ($p < 0.05$) compared to the control diet.

Law *et al*., (2018) conducted an experiment to investigate the effects of dietary crude protein (CP) level and exogenous protease supplementation on growth performance in broiler chickens reared under a tropical climate. A total of 480 day-old male broiler chicks were randomly placed into eight dietary treatments in a 4×2 factorial design. CP level varied from 21.0%, 19.7%, 18.5%, or 17.2% from 1 to 21 days and 19.0%, 17.9%, 16.7%, or 15.6% from 22 to 35 days and protease enzyme supplementation were at two levels 0 ppm or 500 ppm. All experimental diets were fortified with synthetic feed-grade essential amino acids such as lysine, methionine, threonine and tryptophan to satisfy the minimum amino acid recommended levels for Cobb 500. Reducing dietary CP linearly reduced ($p<0.05$) growth performance. Whereas, protease supplementation improved ($p<0.05$) feed conversion ratio and body weight gain.

Wang *et al*., (2018) conducted a study to determine the effects of fermented corn gluten meal (FCGM) with Bacillus natto and Lactobacillus on the growth performance of three-yellow broilers i.e birds with yellow beak, skin and feet. They allocated 450 one-day-old three-yellow male broilers randomly into three dietary treatment groups: control (without FCGM), T1 (5% replacement with FCGM) and T2 (10% replacement with FCGM). Groups are further divided into ten replicates consists of 15 birds each. The results showed that the average daily gain values of T1 and T2 groups were significantly higher ($p< 0.05$) than those of the control group. Whereas, birds fed with 10% FCGM increased more than those that were fed 5% FCGM ($p < 0.05$).

Cardinal *et al.*, (2019) evaluated the effects of a protease enzyme supplementation on the growth performance of broilers. 392 day-old Cobb chicks were reared for 42 days and divided into four treatments, seven replicates of 14 birds each. Treatment groups were given standard diet (SD) without protease, low crude protein and digestible amino acids diet (Low CP & AA) without protease and above diets with protease (1.25g/kg). Birds fed with Low CP & AA had poor feed conversion ratio (FCR). Whereas, addition of the protease enzyme to the Low CP & AA group resulted in improved FCR and body weight gain.

Ndazigaruye *et al.*, (2019) studied the effect of dietary exogenous Protease (bacterial) enzyme on growth performance of broilers for 8–35 days. They used a 2 × 2 factorial design with crude protein (CP), normal diet (NP), low protein (LP) and Protease ENZ. The LP diet contained low in 1% CP and 8–12% amino acids compared to the NP diet. Both NP and LP diets were added without or with (1 g/kg of diet) Protease ENZ. 720 one-week-old Ross 308 male chicks were randomly assigned to one of four treatments with 12 replicates of 15 birds per pen. At 21 days, dietary protease ENZ, but not CP, increased ($p = 0.007$) live body weight. Body weight gain from 8–21 days was affected ($p = 0.006$) by dietary protease ENZ, but was not affected ($p = 0.210$) by CP. The feed conversion ratio was affected by both CP and protease ENZ during the starter period ($p < 0.05$), by protease ENZ ($p = 0.034$) during the finisher period, and by CP ($p < 0.001$) during the entire study period. However, the interaction between CP and protease ENZ did not significantly affect growth performance ($p > 0.05$). Thus, it can be concluded that dietary protease ENZ is more beneficial to younger broilers, independent of CP levels, and that its effect was restricted to body weight and the feed conversion ratio.

Azouz (2020) assessed the effects of hot pepper and corn gluten meal (CGM) on broiler growth performance over 42 days. A total of 180 Ross 308 chicks were randomly assigned to six dietary treatments with three replicates each. The groups included control diets with 5% or 10% CGM, and CGM diets supplemented with 0.1% or 0.2% hot pepper. Results showed that at 6 weeks, the 5% CGM diet significantly improved live body weight, body weight gain, and feed conversion compared to the 10% CGM diet, with no significant difference in feed intake between groups ($P < 0.05$).

De Avila *et al*., (2020) aimed to evaluate the use of corn gluten meal in rooster's diet. 40 roosters of the age of 68 to 72 weeks randomly distributed in two treatments i.e T1 (control) – with corn and soybean meal, and T2 – with 10% of corn gluten meal in the diet. Body weight verification was performed during these 4 weeks. Results showed that there was no significant difference between treatments, in which the 10% of corn gluten meal was incorporated in rooster's diet. They concluded that corn gluten meal can be an alternative diet's input, especially when there is corn or soybean market restriction.

Hafeez *et al*., (2020) designed an experiment which focused on phytogenics and protease enzyme as growth promoter in boilers. Five hundred 40-day-old Cobb broiler chickens were randomly divided in to five treatment groups. First group - control (basal diet only), Second group - basal diet + Ajwan powder (10 mg/kg of feed), Third group - basal diet + Coriander powder (10 mg/kg of feed), Fourth group - basal diet + black Cumin powder (10 mg/kg of feed) and Fifth group - basal diet + protease enzyme (30,000 IU/kg). The results showed that feed intake was significantly ($p < 0.05$) higher in Third (Coriander) and Fifth (Protease) treatment groups at the end of the starter and finisher phases. Body weight was significantly ($p < 0.05$) higher in Protease, Black Cumin and Coriander fed groups compared to the Control. Also, FCR was significantly ($p < 0.05$) improved in fourth (Black Cumin) and fifth (Protease enzyme) treatment groups compared to the control.

Hosseini *et al*., (2020) evaluated the effect of different levels of corn gluten feed on overall growth performance of 500 male Ross 308 broiler chickens. Birds were distributed in a completely randomized design with four treatment groups; each group consists of five replicates and 25 birds per replication. Treatment groups included levels of zero (control), 2.5, 5 and 7.5 percent corn gluten feed in the diet which fed to the birds from 15 to 42 days of age. The results showed that dietary inclusion of corn gluten feed up to 7.5 percent had no significant effect on average weight gain, feed intake and feed conversion ratio when compared with control group.

Park *et al*., (2020) conducted a trial in which 600 broilers were assigned to four dietary treatments (basal diet with 0%, 0.03%, 0.06%, and 0.09% protease) for 35 days to assess the growth performance. Each treatment had 10 replicates (15 birds per replicate). Results showed that protease supplementation increased body weight and average daily gain, while improving feed conversion ratios as protease levels increased ($p < 0.05$).

Saleh *et al*., (2020) examined the effects of adding serine-protease from Bacillus licheniformis on growth performance of broiler chickens under Egyptian condition. A total of 600 one-day-old chicks were randomly assigned to four experimental treatments consists of control diet with 0, 100, 200 and 300 mg/kg serine-protease. Protease supplementation increased ($p<0.05$) body weights (BW). Feed conversion ratio (FCR) was improved ($p<0.05$) due to 200 and 300 mg/kg protease supplementation. In conclusion, exogenous serine-protease could be used as a feed additive in broiler nutrition and supplementing 200~300 mg/kg was sufficient to improve growth performance, probably because of its mechanism to enhance protein digestibility.

Tripathi *et al*., (2020) conducted a trial on diet with reduced crude protein (CP) levels to investigate the effect of dietary supplementation of different levels of protease enzyme on the growth performance of commercial broiler chicken for 6 weeks. Total 160 one-day-old commercial broiler chicks were randomly allocated to five treatments, with four replicates consisting of 14 eight birds in each. The treatment groups were: T1 (Basal diet as per BIS 2007), T2 (1% CP reduction than basal diet with addition of protease enzyme @ 175 g/ton of feed), T3 (2% CP reduction and 175 g/ton of feed protease enzyme), T4 (1% CP reduction and addition of protease enzyme @ 350 g/ton of feed), T5 (2% CP reduction with addition of 350 g/ton of feed protease enzyme). Body weight, gain in body weight, feed consumption and feed conversion ratio were evaluated during the trial. Results suggested that the protease enzyme supplemented treatment groups showed numerically higher body weight and weight gain as compared to the control group.

Abhijeet *et al*., (2021) conducted a feeding trial for 6 weeks to determine the effect of feeding corn gluten meals (CGM) in broilers. Six hundred day old broiler chicks were randomly allocated to the three groups, comprising fifty birds in each treatment group with four replicates. Treatment groups were as follows : T1 birds were fed with composite diet containing 5% corn gluten meal and that of T2 received composite diets comprising of 10% CGM. T3 was a control group with normal broiler diet. Weekly body weight and feed intake were measured and results showed birds of T2 group who received 10% CGM had significantly ($p<0.05$) higher weight gain, lower feed intake and improved FCR than T1 and T3 groups.

Jabbar *et al*., (2021) conducted a trial, with three different dietary crude protein levels (CP: 17%, 19% and 21%) and two levels of exogenous protease (0 and 30,000 IU/kg) on 540 two- week old broilers (Ross-308). They were randomly allocated to 36 floor pens (15 birds/pen) over 15–28 days of age. Results showed that gain in body weight was significantly ($p < 0.05$) higher and improved FCR ($p<0.05$) was observed in birds fed CP-19% (1114.7 g) and CP-21% (1108.8 g) with enzyme supplementation. Protease supplemented treatment group of CP-17% had higher Feed intake ($p<0.05$) than CP-19% with supplementation of the protease enzyme.

Shad *et al*., (2022) evaluated the effects of a novel protease supplemented with low crude protein corn distiller dried grain with solubles (cDDGS) based diets on growth performance of broilers. An experimental trial conducted on 160 one-day-old chicks by randomly allocating them to 4 dietary treatments. Each dietary treatment had four replicates, with 10 birds in each replicate. Two basal diets i.e. starter (1-21 days) and finisher (22-35 days) were formulated which then divided as a positive control (PC) in which a corn soybean meal based diet given and another one is negative control (NC) consists of 5% cDGGS with 5% reduction in CP compared with positive control diet. The negative control diet was further subdivided into 3 parts such as One part was without enzyme supplementation, while the other two parts were supplemented with a novel protease (PROT1) and a commercial protease (PROT2), respectively

Jia *et al*., (2023) conducted an experiment to investigate the effects of replacement of soybean meal with corn gluten meal at different levels on growth performance of yellow feet broilers. A total of 1280 nineteen-days-old yellow feet broilers were selected and randomly divided into 4 groups with 8 replicates (40 birds per replicate). Broilers in group A (control group) were fed a basal diet and other three treatment groups fed with corn gluten meal (CGM). Group B (1.3% CGM), Group C (2.6% CGM) and Group D (3.8% CGM) replaced the soybean meal with corn gluten meal. Duration of experiment was 38 days, which divided into 2 stages: 19 to 36 days of age and 37 to 56 days of age. The results showed that there were no significant differences in growth performance at both stages.

Sushil *et al*., (2024) studied the effect of replacing dietary crude protein of vegetable source with that of Maize Gluten Meal on the growth performances of Vencobb broilers. Total of 200 day-old Vencobb broiler chicks were individually weighed, wing-tagged and randomly distributed into five groups (40 birds per group) further divided in 16 to 4 replicates of 10 birds each. Five treatment groups viz. CON (control / basal diet containing no maize gluten meal), MGM25 (Soybean meal and Groundnut cake was replaced with CP of maize gluten meal at 25%), MGM50 (SBM and GNC replaced with CP of 50% MGM), MGM75 (75% maize gluten meal based on CP), MGM100 (100% replacement of SBM and GNC with MGM on protein basis). All the diets were isonitrogenous. The average body weight and average feed intake of each group were recorded fortnightly. Results showed that the MGM25 had significantly ($p<0.05$) higher gain in body weight and feed intake in comparison to other treatment groups. However, replacement with 75% MGM or above led to significant ($p<0.05$) drop in body weight gain. FCR was best (1.69) in MGM25.

2.2 Carcass traits study

Babidis *et al*., (2002) allocated 60 day-old Ross broiler chickens into two groups: Control (regular diet with herring and meat meal) and Gluten (diet with 60% CP Corn Gluten Meal, replacing all animal proteins). Each group was divided into three sub-groups of 10 birds. Both diets were isocaloric and isonitrogenous. Carcass yield and chemical composition of muscles were analysed. No significant differences were found in body weight, carcass yield or muscle composition (protein, fat, ash) between groups.

Rabello *et al.*, (2012) studied the carcass parameters of free range broilers by including corn gluten meal (CGM21) in their diets. The study was carried in semi-intensive system from 32 to 63 and 32 to 84 days in which 240 birds were reared. Four treatment groups were consisted with the inclusion of 0,7,14 and 21% CGM21. Upon carcass evaluation, it was found that the maximum yield of liver, thigh weight, drumstick yield and abdominal fat yield obtained at the inclusion level of CGM21 of 9.64, 8.60, 11.65 and 13.65 respectively. In conclusion, CGM21 can be added up to 10% starting from the growth phase as maximum carcass percentage values were obtained at 10.75%.

Rada *et al.*, (2013) investigated the effect of adding exogenous protease (monocomponent serine protease from *Bacillus licheniformis*) to broiler grower diets on carcass parameters. The study involved 990 one-day-old ROSS 308 broilers, divided into three treatments with 9 experimental units of 110 chicks. The experiment ran from day 10 to day 35, with a basal diet based on corn, wheat and soybean meal. The treatments included a positive control (PC) diet with normal protein (207 g CP/kg) and two low protein (LP) diets (4% reduced CP) with and without protease supplementation (LP0 and LP1, with 15,000 PROT PRO g feed). Results showed that the protease supplementation had no significant effect on carcass weight, yield or abdominal fat content.

Seyedi and Hosseinkhani (2014) studied the effects of corn gluten meal (CGM) at four levels (3%, 6%, 9%, and 12%) on the carcass characteristics of 1200-day-old broiler chickens. The results showed that CGM inclusion up to 6% had no significant effect on performance or carcass traits. However, a 12% CGM inclusion improved empty body weight and breast weight ($p < 0.01$) and numerically reduced abdominal fat.

Ajayi (2015) carried out a study to investigate the effect of protease supplementation on the carcass yield of broiler chickens from 0-42 days. Results indicated that protease supplementation significantly improved dressed weight and breast meat yield in birds fed low-protein diets, with values of 73.1% and 23.62%

for the 15.5/12.5% CP diet, and 73.68% and 24.33% for the 17.5/14.4% CP diet. However, protease had no significant effect on organ weights (drumstick, liver, kidney, gizzard).

Kamel *et al*., (2015) examined the effect of an exogenous mono component dietary protease enzyme on carcass parameters of broilers. A total of 300 one-day-old Cobb500 chicks were divided into five groups, with 20 birds per replicate, over five weeks. Group 1 (G1) served as the control, while Group 2 (G2) received 200 ppm protease. Group 3 (G3) received the same protease level as G2, minus its full matrix value. Groups 4 (G4) and 5 (G5) were fed diets with the protease matrix values subtracted for ME and CP/amino acids, respectively. At the end of the experimental period, five birds from each replicate of control and treatment groups were randomly chosen to evaluate the carcass parameters such as dressing yield% and breast meat yield%. Results revealed that all enzyme supplemented groups had a higher dressing yield% and breast meat yield% compared to control.

Naik *et al*., (2017) reared 200 one-day-old commercial broiler chicks for a period of 6 weeks, dividing them randomly into four groups with 10 replicates of 5 birds each. Diet formulated for these four treatments was corn-soya based and treatment groups were Control, Basal diet (BD) (with 2% less CP compared to control), BD with uncoated protease enzyme and BD with coated protease enzyme. At the end of the study, 2 birds per replicate were slaughtered to assess carcass parameters such as dressing percentage, heart, gizzard, liver and abdominal fat weight. Supplementation of uncoated and coated protease enzymes did not show any significant difference in carcass parameters in terms of dressing percentage, giblet weight percentage and abdominal fat percentage as compared to control and basal diet.

Afrouzi *et al*., (2018) conducted an experiment to investigate the effect of using 2% untreated and treated corn gluten meal with protease enzyme (300 mg/kg) at three different times (120, 180 and 240 minutes) on carcass characteristics of broilers. 240 commercial strains Ross 308 male broiler chicks with five treatments including: 1. control diet, 2. control diet containing 2% corn gluten, 3, 4, and 5. control diet containing 2% corn gluten meal processed with protease enzyme at 120, 180 and 240 minutes, respectively, four replicates and 12 chicks in each replicate in a completely randomized design were reared for 38 days. To evaluate carcass components at 38 d, eight birds from each treatment were selected and weights of carcass, thighs, breast, abdominal fat, liver, pancreas, spleen and Bursa Fabricius were determined. In this experiment, the experimental diets had not significant effect on carcass components and blood parameters.

Law *et al*., (2018) conducted an experimental trial to study the effects of dietary crude protein (CP) level and exogenous protease supplementation on carcass characteristics in broiler chickens reared under a tropical climate. A total of 480 male chicks were assigned to eight dietary treatments in a 4×2 factorial design, with CP levels ranging from 17.2% to 21.0% and protease supplementation at 0 ppm or 500 ppm. On day 36, two birds per pen were slaughtered to assess carcass parameters. Reducing CP levels decreased dressing ($p<0.001$) and breast meat yields ($p=0.049$) and increased abdominal fat ($p<0.001$). Protease supplementation improved carcass yield ($p=0.042$) but had no significant effect on breast meat yield or abdominal fat. CP × protease interactions were not significant.

Hussain *et al*., (2019) assessed the effects of exogenous enzyme supplementation in high-protein (Hi-Pro) corn DDGS-based diets on broiler carcass parameters. A total of 200 broiler chicks were divided into four groups with five replicates of 10 birds each. The control group (CON) received a basal diet with 20% crude protein (CP), including 15% CP from Hi-Pro corn DDGS. Three experimental diets were supplemented with protease (CON-P), a mannanase-xylanase mix (CON-MX) and all three enzymes protease with mannanase-xylanase mix. On day 35, carcass parameters like yield and organ weights were evaluated. Supplementation with exogenous enzymes did not significantly affect these parameters.

Azouz (2020) conducted an experiment to evaluate the carcass parameters of broilers which were reared for 42 days. 180 unsexed one day-old Ross 308 broiler chicks were randomly allocated to six dietary treatments of three replicates each and 10 birds per replicate. Two treatment groups consisted of 5% and 10% corn gluten meal without hot pepper served as controls. Third and fourth treatment groups were including 5% CGM with 0.1 and 0.2 % hot pepper, respectively. Last two groups (5^{th} and 6^{th}) supplemented with 10% CGM along with 0.1 and 0.2 % hot pepper, respectively. The results indicated that the control diet with 5% CGM significantly ($P < 0.05$) improved live body weight, dressing %, and abdominal fat % compared to the 10% CGM diet, with no significant differences in liver %, gizzard %, and heart %. Chicks fed diets with hot pepper (0.1% with 5% CGM), hot pepper (0.2% with 5% CGM), and hot pepper (0.2% with 10% CGM) had significantly lower body weight ($P < 0.01$) than those on the 10% CGM control diet. No significant differences in liver %, gizzard %, and heart % were found between chicks on hot pepper and control diets.

Saleh *et al*., (2020) investigated the impact of adding serine-protease from *Bacillus licheniformis* on carcass characteristics of broiler chickens. A total of 600 one-day-old chicks were randomly assigned to four experimental treatments consists of control diet with 0, 100, 200 and 300 mg/kg protease. Carcass evaluation showed that hot carcass and breast weights were significantly higher ($P < 0.05$) in all protease-supplemented groups compared to the control. Thigh muscle weight increased only with 200 and 300 mg of protease. Additionally, abdominal fat decreased significantly with the inclusion of 300 mg of protease. For internal organ weights, liver weight increased with 200 and 300 mg protease supplementation, while heart and gizzard weights were unaffected.

Tripathi *et al*., (2020) investigated the effect of protease enzyme supplementation on broiler performance with reduced crude protein (CP) levels. A total of 160 chicks were reared for 6 weeks. They were divided into five treatments, each with four replicates of 14 birds. The treatments included a basal diet (T1), diets with 1% or 2% CP reduction and protease supplementation at 175 g/ton (T2, T3) or 350 g/ton (T4, T5). Carcass parameters like pre-slaughter weight, dressed weight, dressing percentage and giblet percentage were measured. Results showed no significant effects of protease supplementation or CP reduction on these carcass characteristics.

2.3 Blood biochemical profile

Abdel Raheem *et al*., (2005) carried out three experimental trials on 175 broiler chicks (Arbor acres) to check the value of plant proteins, full-fat soybean (FFSB) and corn gluten meal (CGM), as substitutes for soybean meal (SBM) in broiler diets. Chicks were randomly allocated to seven groups of 25 chicks each. First trial provided with SBM diet tended to be a control. In remaining two trials, SBM diet was replaced by FSB and CGM in different feeding phases. Blood biochemical analysis of serum total protein, albumin and globulin were performed. No significant differences were found in serum total protein and albumin levels between the trial groups. In the FFSB trial, globulin levels were lowest (0.32 g/dl) during the finishing period compared to the control group (0.80 g/dl). In the CGM trial, the lowest globulin value was observed in the first group fed the CGM diet throughout the entire experiment.

Kamel *et al*., (2015) performed serum biochemical parameters to determine the effect of protease enzyme on broilers. A total of 300 one-day-old chicks (Cobb500) were divided into five treatment groups with three replicates of 20 birds each. G1 was control group without any supplementation, G2 served as G1 with 200 ppm protease. Group 3 (G3) received the same protease level as G2, minus its full matrix value. Groups 4 (G4) and 5 (G5) were fed diets with the protease matrix values subtracted for ME and CP/amino acids, respectively. Blood samples were collected randomly from nine birds in each group on the 27th day. Results of serum parameters revealed that enzyme supplementation had no significant effect on serum total protein, albumin, globulin, Albumin/Globulin ratio, AST, ALT, uric acid and creatinine.

Naik *et al*., (2017) studied the effect of coated and uncoated protease on performance of broilers by determining various parameters including serum biochemical evaluation. Diet formulated for the four treatments was corn-soya based and treatment groups were Control, Basal diet (BD) (with 2% less CP compared to control), BD with uncoated protease enzyme and BD with coated protease enzyme. Blood samples were collected for estimation of serum biochemical constituent i.e. total protein. It has been found that serum total protein level was improved by addition of both coated and uncoated protease as compared to the control group.

Afrouzi *et al*., (2018) investigated the effect of 2% untreated and treated corn gluten meal with protease enzyme (300 mg/kg) at three time intervals (120, 180, and 240 minutes) on blood biochemical parameters of broilers. A total of 240 Ross 308 male broiler chicks were divided into five treatments: 1) control diet, 2) control diet with 2% corn gluten, and 3-5) control diet with 2% corn gluten treated with protease for 120, 180, and 240 minutes. The experiment lasted 38 days. At the end of the experimental trial, eight birds per treatment were selected for blood sample collection to measure glucose, total protein, albumin, globulin, triglycerides, cholesterol, HDL-C, LDL-C and VLDL-C. Results showed that there was no significant effect on blood biochemical parameters between control and treatment groups.

Law *et al*., (2018) aimed to investigate the effects of dietary crude protein (CP) level and exogenous protease supplementation on serum metabolites in broiler chickens. The main effects were observed for CP level (21.0%, 19.7%, 18.5%, or 17.2% from 1 to 21 days and 19.0%, 17.9%, 16.7%, or 15.6% from 22 to 35 days) and protease enzyme supplementation (0 ppm or 500 ppm) on 480 day-old broiler chicks. Results revealed that CP × protease interactions were significant for serum levels of albumin, triglycerides and uric acid but not for serum total protein. Irrespective of protease supplementation, reducing the CP level linearly decreased ($p < 0.001$) serum total protein.

Wang *et al*., (2018) studied the effects of fermented corn gluten meal (FCGM) with Bacillus natto and Lactobacillus on the blood biochemistry of three-yellow broilers. A total of 450 male chicks were randomly assigned to three groups: control (no FCGM), T1 (5% FCGM) and T2 (10% FCGM), with 10 replicates of 15 birds each. Serum parameters such as total protein, albumin, globulin and blood urea nitrogen (BUN) were analysed. During the starter phase, birds fed 10% FCGM had lower BUN and higher total protein. In the finishing phase, FCGM-fed birds had lower BUN and those on 10% FCGM had higher total protein, albumin and globulin compared to the control group, with 10% FCGM showing the highest globulin.

Hafeez *et al*., (2020) conducted an experiment to study the effects of phytogenics and protease enzyme as growth promoters in broilers. A total of 540, 40-day-old Cobb broiler chickens were divided into five groups: control (basal diet), Ajwan powder (10 mg/kg), coriander powder (10 mg/kg), black cumin powder (10 mg/kg), and protease enzyme (30,000 IU/kg). Serum biochemistry parameters such as HDL, LDL, cholesterol, triglycerides, total protein, and blood glucose were measured. No significant differences were observed in these parameters between the control and treatment groups.

Amer *et al*., (2021) examined the effects of different feeding regimens, with or without protease supplementation, on the blood biochemistry of broilers. A total of 300 Ross 308 chicks were assigned to a 3×2 factorial design with three feeding regimens: FR1 (recommended protein SBM diet), FR2 (low-protein SBM diet), and FR3 (low-protein diet with 5% DDGS and 5% SFM), with or without protease (250 mg/kg). Protease supplementation or feeding regimens did not significantly affect serum total protein, albumin, globulin, albumin/globulin ratio. However, the FR3 group showed higher serum ALP and increased IgM levels, with protease supplementation also raising IgM levels ($p=0.04$).

2.4 Nutrient digestibility

Cruz *et al*., (2005) studied ileal digestibility and the ability of broilers to select feed to meet their protein and energy requirements. Six diets were tested: R+S (rice bran and soybean meal), C+G (corn and corn gluten meal), R+G (rice bran and corn gluten meal), C+S (corn and soybean meal), R+C+G+S (rice bran, corn, corn gluten meal, and soybean meal), and BD (basal diet of corn and soybean meal). Results showed that the C+G diet had the highest digestibility of dry matter and crude protein, while C+S had the highest digestibility of ether extract and nitrogen-free extract.

Freitas *et al*., (2011) conducted an experiment which involved 8 treatments and 11 replicates (22 male Cobb 500 broilers per replicate). Birds were fed diets with varying protein (7% difference), energy (3% difference), and protease (0 or 200 ppm) levels. High-protein, high-energy diets improved performance, and protease supplementation enhanced fat and crude protein digestibility ($P \leq 0.01$), regardless of protein or energy content. Overall, protease improved digestibility and metabolizable energy values.

Naik *et al.*, (2017) evaluated the effect of coated and uncoated protease on broiler performance by assessing various parameters, including nutrient digestibility determination. The diets for the four treatment groups were corn-soya based and included: Control, Basal diet (BD) with 2% less crude protein (CP) compared to the control, BD with uncoated protease enzyme, and BD with coated protease enzyme. A metabolic trial was conducted for three days after 42 days of trial by keeping 2 birds in each replicate. Faecal samples were collected daily carefully by separating feathers, scales and traces of feed. All the faecal samples were oven dried and weighed to analyze the crude protein and gross energy of dried excreta. Results showed no significant difference on dry matter and retention of energy but protease enzyme supplementation improved retention of protein compared to basal diet.

Yuan *et al.*, (2017) examined the effect of exogenous protease on nutrient digestibility in Ross 308 broilers. A total of 301-day-old male broiler chickens were fed corn-soybean basal diets supplemented with non-starch polysaccharide (NSP) enzyme and varying levels of acid protease from 1 to 42 days of age to investigate the effects. Results showed that the supplementation with NSP enzyme or NSP enzyme combined with 40 or 160 mg/kg protease significantly improved the apparent digestibility of crude protein (ADCP) ($p<0.05$). The highest ADCP increase (7.26%) was observed with NSP enzyme and 160 mg/kg protease. Between days 35-37, the combination of NSP enzyme and 40 mg/kg protease resulted in the greatest ADCP increase (12.66%).

Saleh *et al.*, (2020) studied the effect of protease enzyme on nutrient digestibility in broiler chickens. Results revealed that the digestibility of dry matter was significantly improved ($P < 0.05$) with the addition of 200 and 300 mg/kg protease compared to the control, showing a linear response. Similarly, crude protein digestibility increased linearly with higher protease levels. Ether extract digestibility was highest with 100 mg/kg protease supplementation.

Jabbar *et al*., (2021) studied the effects of different crude protein (CP) levels (17%, 19%, 21%) and exogenous protease (0 or 30,000 IU/kg) on 540 two-week-old Ross-308 broilers. Birds were allocated to 36 pens from days 15–28. On 25^{th} day of the experiment, five birds per replicate were selected and separated in metallic cages till the end of the trial. At the end of the experiment i.e on 28^{th} day, excreta samples were collected to determine the total tract N retention. Results showed that birds fed with 17% CP without protease had lower nitrogen retention and gross energy (GE), and higher abdominal fat compared to other groups. The 19% CP diet with protease improved nutrient digestibility in broilers during this period.

2.5 Economics of feeding

Abdel Raheem *et al*., (2005) aimed to evaluate the use of full-fat soybean (FFSB) and corn gluten meal (CGM) as substitutes for soybean meal (SBM) in broiler diets. A total of 175 day-old Arbor Acres chicks were randomly assigned to seven groups of 25 chicks each. The first trial served as a control with SBM-based diets for all phases (starter, grower, finisher). In the other two trials, FFSB and CGM replaced SBM, with diets tested across three phases: 1) all phases (starter, grower, finisher), 2) grower-finisher phases, and 3) finisher phase only. Economical evaluation carried out for both FFSB and CGM trials. Results showed that feeding of full-fat soybean reduced economical feed efficiency by 16% during the entire trial period when compared with control group. On the other hand, replacement of finisher broiler diet with 20% CGM decreased economical feed efficiency by around 15.5% compared with control. Thus, it has been concluded that grower-finisher broiler diet could be replaced with 20% full-fat properly cooked whole soybeans. Whereas, corn gluten meal could replace 20% of the finisher broiler diet.

El-Deek *et al*., (2009) conducted a study to evaluate the effects of different levels of corn gluten meal (CGM) with or without phytase supplementation (500 FTU/kg) on economic performance in Hubbard broiler chicks (n=160) from 7 to 42 days of age. Chicks were divided into 8 groups with 4 replicates each, with CGM replacing 0, 25, 50, or 75% of soybean meal protein in iso-nitrogenous and iso-caloric diets. Results showed that the best economic efficiency was observed with 25% CGM inclusion, with or without phytase supplementation.

Giannenas *et al*., (2017) conducted an experimental trial to evaluate the combined effects on economic performance of reducing soybean meal (SBM), adding corn gluten meal (CGM), and including protease in broiler diets on a commercial Greek farm. 540 chicks were divided into three dietary treatments with six replicates of thirty birds each. The treatments were: 1) Control – conventional corn and SBM diet with 21% crude protein (CP), 2) Soy-Prot – corn and SBM diet with 20% CP and 200 mg/kg protease (RONOZYME® Proact), and 3) Gluten-Prot – corn and CGM diet with similar CP and protease levels as Soy-Prot. Comparing the feed costs of the Control group and the Soy-Prot group, it can be concluded that protease inclusion reduced the feed cost per kg, as the enzyme's additional cost was offset by the reduction in total crude protein in the feed. Substituting SBM with CGM had a marginal impact on feed cost per kg. However, the overall feed cost per unit of weight gain was similar across all groups.

Tripathi *et al*., (2020) evaluated the effect of dietary protease supplementation on the economics of broiler production with reduced crude protein (CP) levels over 6 weeks. A total of 160 one-day-old commercial broiler chicks were randomly assigned to five treatments with four replicates of 14 birds each. The treatments were: T1) Basal diet (BIS 2007), T2) 1% CP reduction with protease (175 g/ton), T3) 2% CP reduction with protease (175 g/ton), T4) 1% CP reduction with protease (350 g/ton), and T5) 2% CP reduction with protease (350 g/ton). Results clearly showed that the protease enzyme supplementation @ 350 g/ton of feed with 2% reduction in CP level (T5) and 175 g/ton of feed with 1% reduction in CP level (T2) in broiler diet (pre-starter, starter and finisher) received higher economic return (Rs./bird) in terms of ROFC as compared to other treatment groups and control diet.

Amer *et al*., (2021) assessed the economic impacts of various feeding regimens with or without protease supplementation on broiler rations. A total of 300 Ross 308 chicks were assigned to a 3 × 2 factorial design with three feeding regimens: 1) recommended protein SBM diet (FR1), 2) low-protein SBM diet (FR2), and 3) low-protein diet with 5% DDGS and 5% SFM (FR3), with or without 250 mg/kg protease supplementation. Economic measures such as total costs, variable costs, total return, and net profit were calculated. Results showed no significant effect of the feeding regimens or protease supplementation on economic efficiency.

Thus, from the above cited literature it is evident that maize gluten meal is a novel feed which can be used in poultry diet as a partial replacement of protein, also, the supplementation of protease enzyme is responsible for further improvement in broiler production.

Material and Methods

3. MATERIAL AND METHODS

The present experiment was carried out at the Department of Animal Nutrition and Department of Poultry Science, Mumbai Veterinary College, Parel, Mumbai – 400012.

3.1 Maize Gluten Meal and Protease enzyme

Maize gluten meal is used in the present study because of its high crude protein value. Whereas, the protease enzyme (SynerZyme-Pro) is the mixture of proteolytic enzyme in powder form used in poultry diet.

Table 3.1 Proximate Compositions (%DMB) of Maize Gluten Meal

Nutrients	Composition
Crude protein %	61.70
Ether extract %	4.03
Crude Fiber %	1.61
Total ash %	3.12
Nitrogen free extract %	29.54
Metabolizable Energy (kcal/kg) (calculated)	3750

3.2 Plan and Design of Experiment

The experiment was conducted on the Two hundred and twenty five day old commercial broiler chicks (Cobb-400) for 42 days. The chicks were purchased from the Venkateshwara Hatcheries Pvt Ltd., Panvel. On the arrival of broiler chicks, they were weighed for body weight. Then, the chicks were randomly divided into five equal groups viz. T_0, T_1, T_2, T_3 and T_4 with each group having 45 chicks. The individual groups were further subdivided into three replicates and each replicate consists of 15 chicks. The trial was conducted at the broiler unit of Department of Poultry Science, Mumbai Veterinary College, Mumbai.

3.3 Feed Treatment

The birds received pre-starter (up to 7 days), starter (8 to 21 days) and finisher diet (22 to 42 days) in the mash form as per BIS (2007). The required quantities of feed ingredients were mixed in a horizontal mixer. The pre-starter, starter and finisher diets for all the groups were formulated as shown in tables 3.3, 3.4 and 3.5, respectively. The chicks were randomly distributed into five groups of 45 chicks, in which each group consists of three replicates having 15 chicks in each replicate and each group was allotted to one of the following treatments.

Chicks from control group (T_0) were fed with corn-soy based mash as per the standard diet. Whereas T_1, T_2, T_3 and T_4 groups fed with different levels of Maize Gluten Meal by replacing Soybean meal on crude protein basis with or without addition of Protease enzyme at two different levels. Treatment group T_1 and T_3 replaced 10% and 20% crude protein respectively, from soybean meal with maize gluten meal without protease enzyme. Treatment group T_2 received 10% crude protein replacement from soybean meal with maize gluten meal with 200mg/kg protease enzyme. Whereas, birds of treatment group T_4 fed with 20% crude protein replacement from soybean meal with maize gluten meal with 300mg/kg protease enzyme. All the diets were made isocaloric and isonitrogenous.

Plate 1. Maize Gluten Meal

Plate 2. Protease enzyme powder

Table 3.2 Feed treatments

Treatment	Pre-starter Experimental diet	Starter Experimental diet	Finisher Experimental diet
T_0	Standard Broiler Diet (BIS 2007) including soybean meal	Standard Broiler Diet (BIS 2007) including soybean meal	Standard Broiler Diet (BIS 2007) including soybean meal
T_1	10% replacement of crude protein from soybean meal with Maize Gluten Meal without protease enzyme	10% replacement of crude protein from soybean meal with Maize Gluten Meal without protease enzyme	10% replacement of crude protein from soybean meal with Maize Gluten Meal without protease enzyme
T_2	10% replacement of crude protein from soybean meal with Maize Gluten Meal with 200mg/kg protease enzyme	10% replacement of crude protein from soybean meal with Maize Gluten Meal with 200mg/kg protease enzyme	10% replacement of crude protein from soybean meal with Maize Gluten Meal with 200mg/kg protease enzyme
T_3	20% replacement of crude protein from soybean meal with Maize Gluten Meal without protease enzyme	20% replacement of crude protein from soybean meal with Maize Gluten Meal without protease enzyme	20% replacement of crude protein from soybean meal with Maize Gluten Meal without protease enzyme
T_4	20% replacement of crude protein from soybean meal with Maize Gluten Meal with 300mg/kg protease enzyme	20% replacement of crude protein from soybean meal with Maize Gluten Meal with 300mg/kg protease enzyme	20% replacement of crude protein from soybean meal with Maize Gluten Meal with 300mg/kg protease enzyme

3.4 Proximate analysis and Formulation for experimental diet

The Proximate analysis of feed samples was done as per AOAC (2005) in the Department of Animal Nutrition, Mumbai Veterinary College Parel, Mumbai – 400012. The percent proximate composition of pre-starter, starter and finisher diets are presented in tables 3.3, 3.5 and 3.7, respectively. Different broiler diet formulations were developed to align with the nutritional standards specified by BIS in 2007 and are presented in table 3.4, 3.6 and 3.8.

Table 3.3 Proximate compositions (% DMB) of pre-starter diet

Nutrients	T0	T1	T2	T3	T4
Crude protein %	23.05	23.02	23.06	23.11	23.09
Ether extract %	6.30	5.97	5.92	6.21	6.28
Crude Fiber %	4.06	4.16	4.18	4.09	4.14
Total ash %	8.58	8.19	8.22	8.07	8.12
Nitrogen free extract %	58.01	58.66	58.62	58.52	58.37
Metabolizable Energy (kcal/kg) (calculated)	3001.35	3049.49	3050.22	3061.40	3059.00

Table 3.4 Formulation for pre-starter diets

Ingredients (%)	T_0	T_1	T_2	T_3	T_4
Maize	49.92	50.95	50.90	52.83	52.83
Soyabean meal	41.14	37.02	37.02	32.90	32.90
Maize Gluten Meal		3.08	3.08	6.17	6.17
Vegetable oil	4.81	4.76	4.79	3.86	3.83
Mineral mixture	0.10	0.10	0.10	0.10	0.10
Vitamin Mixture	0.05	0.05	0.05	0.05	0.05
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone powder	1.20	1.20	1.20	1.20	1.20
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.08	0.16	0.16	0.24	0.24
Methionine	0.14	0.12	0.12	0.09	0.09
Choline chloride	0.11	0.11	0.11	0.11	0.11
Toxin binder	0.10	0.10	0.10	0.10	0.10
Salinomycin	0.05	0.05	0.05	0.05	0.05
Protease enzyme			0.02		0.03
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Table 3.5 Proximate compositions (% DMB) of starter diet

Nutrients	T0	T1	T2	T3	T4
Crude protein %	22.05	22.22	22.17	22.20	22.22
Ether extract %	8.07	8.02	8.00	8.06	8.08
Crude Fiber %	4.03	3.98	4.01	3.91	3.93
Total ash %	8.28	8.33	8.28	8.13	8.10
Nitrogen free extract %	57.57	57.45	57.54	57.70	57.67
Metabolizable Energy (kcal/kg) (calculated)	3102.50	3128.90	3127.30	3117.62	3122.22

Table 3.6 Formulation for starter diets

Ingredients (%)	T_0	T_1	T_2	T_3	T_4
Maize	49.96	51.35	51.35	52.48	52.48
Soyabean meal	39.25	35.32	35.32	31.40	31.40
Maize Gluten Meal		2.94	2.94	5.88	5.88
Vegetable oil	6.60	6.14	6.12	5.94	5.91
Mineral mixture	0.10	0.10	0.10	0.10	0.10
Vitamin Mixture	0.05	0.05	0.05	0.05	0.05
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone powder	1.20	1.20	1.20	1.20	1.20
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.13	0.21	0.21	0.28	0.28
Methionine	0.15	0.13	0.13	0.11	0.11
Choline chloride	0.11	0.11	0.11	0.11	0.11
Toxin binder	0.10	0.10	0.10	0.10	0.10
Salinomycin	0.05	0.05	0.05	0.05	0.05
Protease enzyme			0.02		0.03
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

Table 3.7 Proximate compositions (% DMB) of finisher diet

Nutrients	T0	T1	T2	T3	T4
Crude protein %	20.00	20.08	20.09	20.20	20.17
Ether extract %	8.88	8.95	8.94	8.81	8.86
Crude Fiber %	3.63	3.98	3.93	4.04	3.98
Total ash %	7.03	7.38	7.40	7.19	7.24
Nitrogen free extract %	60.46	59.61	59.64	59.76	59.75
Metabolizable Energy (kcal/kg) (calculated)	3201.30	3204.64	3213.97	3214.94	3217.94

Table 3.8 Formulation for finisher diets

Ingredients (%)	T_0	T_1	T_2	T_3	T_4
Maize	54.49	55.55	55.53	56.84	56.81
Soyabean meal	33.55	30.18	30.18	26.82	26.82
Maize Gluten Meal		2.51	2.51	5.03	5.03
Vegetable oil	7.60	7.35	7.35	6.85	6.85
Mineral mixture	0.10	0.10	0.10	0.10	0.10
Vitamin Mixture	0.05	0.05	0.05	0.05	0.05
Dicalcium phosphate	2.00	2.00	2.00	2.00	2.00
Limestone powder	1.20	1.20	1.20	1.20	1.20
Salt	0.30	0.30	0.30	0.30	0.30
Lysine	0.27	0.34	0.34	0.41	0.41
Methionine	0.18	0.16	0.16	0.14	0.14
Choline chloride	0.11	0.11	0.11	0.11	0.11
Toxin binder	0.10	0.10	0.10	0.10	0.10
Salinomycin	0.05	0.05	0.05	0.05	0.05
Protease enzyme			0.02		0.03
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**

3.5 Housing and Management:

The birds were reared on deep litter system of housing. Similar environmental and managemental conditions were provided to all the groups throughout the period of experiment. An identical and adequate feeding and watering space was provided to all the birds throughout the experimental period. The electric tube heating was employed for the initial two weeks during the brooding phase.

The group feeding practice was followed throughout the experimental period. The feed quantity provided to each replicate within the group was measured and recorded. The feed refusal was weighed in the next morning to arrive at actual daily feed consumption of each group. The free access of fresh, clean and wholesome drinking water was given to the birds during 6 weeks of experimental period.

3.6 Medication and Vaccination

Immediately after the arrival of chicks, antistress vitamins were provided with drinking water. Birds were immunized against Ranikhet Disease (B1 strain) and Infectious Bursal Disease (IBD-Intermediate strain) on 7th day and 14th day respectively followed by the booster dose on 21st day (B1) and 28th day (IBD) through drinking water.

3.7 Parameters studied during the experiment

A. Growth Performance

a) Weekly feed intake

b) Weekly body weight gain

c) Weekly feed conversion ratio (FCR)

B. Carcass traits study

a) Dressing percentage

b) Giblet weight

c) Edible meat percentage

d) Breast meat weight

C. Blood biochemical profile

a) Total protein

b) Albumin

c) Globulin

d) A:G ratio

D. Nutrient digestibility

Nutrient digestibility involves the estimation of dry matter, crude protein (by calculating total tract N retention), ether extract, crude fibre, nitrogen free extract and total ash of the fecal samples.

E. Economics of feeding

Factors such as feed cost, day-old chick expenses, medication, vaccination, labour, miscellaneous expenses, and returns from the sale of broiler birds are considered while assessing the economics of feeding.

PARAMETER STUDIED:

3.7.1 Growth Performance

1. Weekly feed intake

The daily feed consumption of birds was recorded replicate wise based on the quantity of feed which was offered every day and the feed refusal. The offered feed (ad-lib) and feed refusal was weighed every day in the morning to calculate the actual feed intake. Records were maintained for daily feed consumption used to arrive at average feed consumptions by various groups.

2. **Weekly gain in body weight**

The birds were weighed individually on weekly basis to know the body weight gain of broilers till six weeks of age. Weekly gain in body weight was calculated by subtracting the live body weight of previous week from that of current week and recorded in grams.

Weekly gain in body weight (g) = Current week weight – Previous week weight

3. **Weekly Feed conversion Ratio (FCR)**

Consumption of feed and body weight gain in each week of each replicate group were recorded separately. Feed conversion ratio was calculated by using following formula,

$$\text{Feed Conversion Ratio} = \frac{\text{Quantity of feed consumed (g) during the week}}{\text{Gain in body weight (g) during the week}}$$

3.7.2 Carcass traits

1. **Dressing percentage:**

At the end of the experimental period, one bird from each replicate was slaughtered and evaluated for the carcass yield. The dressed weight of each bird was obtained separately after complete bleeding and removal of feathers and nonedible viscera. The dressing percentage was calculated by using the formula,

$$\text{Dressing Percentage} = \frac{\text{Weight of dressed chicken}}{\text{Live body weight}} \times 100$$

2. **Giblet weight:**

From each replicate one bird was slaughtered at the end of the experimental period i.e. on 42nd day and evaluated for giblet yield. Heart without pericardium, liver without gall bladder and empty gizzard without mucus membrane were also weighed individually and weights of these organs were recorded for the respective groups.

3. **Edible meat percentage:**

At the end of the experimental period one bird from each replicate was slaughtered and evaluated for edible meat yield.

4. **Breast meat weight:**

One bird from each replicate was slaughtered at the end of the experimental period and breast meat was evaluated.

3.7.3. Blood biochemical profile

Two birds from each replicate were selected in 5th week for blood samples collection. Blood was collected using syringes from the wing vein. Serum was separated by centrifugation at 2000 rpm for 10 min, aspirated in serum vials and stored at -20°C until testing. Evaluation of serum samples was done with the help of Transasia semi-automated biochemistry analyser. All the blood biochemical tests were performed by using analysing kit Erba Mannheim- XL.

a) Serum total protein

Serum total protein was estimated by Biuret method. 10µl of blood serum was added in 500µl of working reagent and incubated for 10 minutes at 37°C. After incubation period the absorbance was measured in 546 nm under UV spectrophotometer and the values were expressed in g/dL. Before conducting an internal calibration, a volume of 500µl of working reagent was utilized with 10µl of distilled water serving as the blank and 10µl of protein standard added to 500µl of working reagent for the standard.

b) Serum albumin

Serum albumin was estimated by BCG end point method. 5µl of serum was added in 500µl of working reagent and incubated for 1 minute at 37°C temperature. After incubation period two absorbance was measured at 630nm under UV spectrophotometer and the values were expressed in g/dL. Prior to analysis, an internal calibration process was executed, employing 500µl of working reagent and 5µl distilled water as the blank and augmenting 500µl of working reagent with an additional 5µl of albumin standard to serve as the standard.

c) Serum globulin

Serum globulin was calculated by subtracting the serum albumin from the serum total protein. The results were expressed in g/dL.

d) A : G ratio

The Albumin : Globulin ratio can be calculated by dividing serum albumin by serum globulin by the formula,

$$\text{A:G ratio} = \frac{\text{Serum albumin}}{\text{Serum globulin}}$$

3.7.4. Nutrient Digestibility

The metabolism trial was conducted at the end of 5th week of experimental period. From each treatment group two birds were transferred to metabolic cages for digestibility trial. The birds were housed separately in individual cages with the facility for feeding, drinking, and faecal collection. Weighed feed was given to the birds for 7 days and faeces were collected separately daily at 9 am for each bird. The excreta were removed at every 2 hours interval and put in polyethylene sample collection container with the lid and total amount of excreta obtained in 24 hours period was weighed. The faecal sample used for protein estimation was collected daily in the separate container in sulphuric acid solution. The collected faecal samples were oven dried at 60°C for 24 hrs and weighed to record the faecal output on dry matter basis. Further, samples were subjected for proximate composition according to AOAC (2005).

3.7.5. <u>Economics of broiler production</u>

The economics of rearing the broiler chicks for complete experimental period was calculated by taking into consideration the cost of production. Net profit per bird was calculated from the sale of birds in the local market on live body weight basis. Similarly net profit (rupees) per kg body weight was calculated.

3.8 <u>Statistical Analysis:</u>

All the data obtained were subjected to statistical analysis as per Snedecor and Cochran (1994), by using completely randomized design.

Results and Discussion

4. RESULTS AND DISCUSSION

The present research trial was conducted on Two hundred and twenty five day-old broiler chicks for 42 days to evaluate the effect of partial replacement of soybean meal protein with maize gluten meal, with or without supplementation of protease enzyme on performance of broilers.

The study assessed several parameters, including growth performance (weekly live body weight, body weight gain, weekly feed intake and feed conversion ratio), carcass traits (dressing percentage, giblet weight, edible meat percentage and breast meat weight), blood biochemical profile (serum total protein, albumin, globulin, and albumin : globulin ratio), nutrient digestibility, and the economic aspects of broiler feeding. The collected data was then subjected to statistical analysis for result interpretation.

4.1 Growth performance

Growth performance of the experimental birds was evaluated on the basis of average weekly and total live body weight, average weekly and total body weight gain, average weekly and total feed consumption and average weekly and total feed conversion ratio.

4.1.1 Average weekly live body weight

The data related to the average weekly live body weights (g) of birds from day-old to six weeks (42^{nd} day) of age across different groups are summarized in Table 4.1 and graphically illustrated in Figure 4.1.

The Table 4.1 explains that the average initial live body weight of day-old chicks for groups T_0, T_1, T_2, T_3 and T_4 was 44.84 ± 0.84, 44.75 ± 0.19, 44.80 ± 0.03, 43.63 ± 0.87 and 45.32 ± 0.38 g, respectively. The differences between the average day-old body weights of chicks among treatment groups were non-significant which creates the suitable condition to check the effect of replacement of maize

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

Weeks	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
0	44.84 ± 0.84	44.75 ± 0.19	44.80 ± 0.03	43.63 ± 0.87	45.32 ± 0.38	NS
1	129.67^{ab} ± 1.06	126.30^{abc} ± 0.35	133.91^{a} ± 0.30	122.48^{bc} ± 1.19	121.61^{c} ± 4.43	7.69
2	317.40^{b} ± 1.06	312.27^{bc} ± 0.43	325.19^{a} ± 0.28	305.57^{d} ± 0.99	309.36^{cd} ± 3.78	6.64
3	669.96^{b} ± 1.24	667.54^{b} ± 1.88	681.51^{a} ± 1.22	656.33^{c} ± 4.46	665.70^{bc} ± 2.67	9.43
4	1078.60^{ab} ± 5.48	1070.35^{b} ± 2.17	1088.82^{a} ± 4.89	1064.10^{b} ± 2.86	1075.80^{ab} ± 4.26	14.99
5	1509.03^{ab} ± 5.26	1497.80^{bc} ± 1.67	1518.25^{a} ± 4.29	1493.59^{c} ± 2.81	1501.03^{bc} ± 3.88	13.78
6	2031.15^{bc} ± 15.66	2010.68^{c} ± 6.12	2078.83^{a} ± 10.14	2015.78^{c} ± 12.22	2063.06^{ab} ± 13.00	43.16
Mean	825.81^{b} ± 282.88	818.53^{bc} ± 280.44	838.76^{a} ± 287.75	814.50^{c} ± 281.31	825.98^{b} ± 286.47	8.40

Means marked with at least one common superscript (a,b,c) in the same row do not differ significantly. NS – Non significant

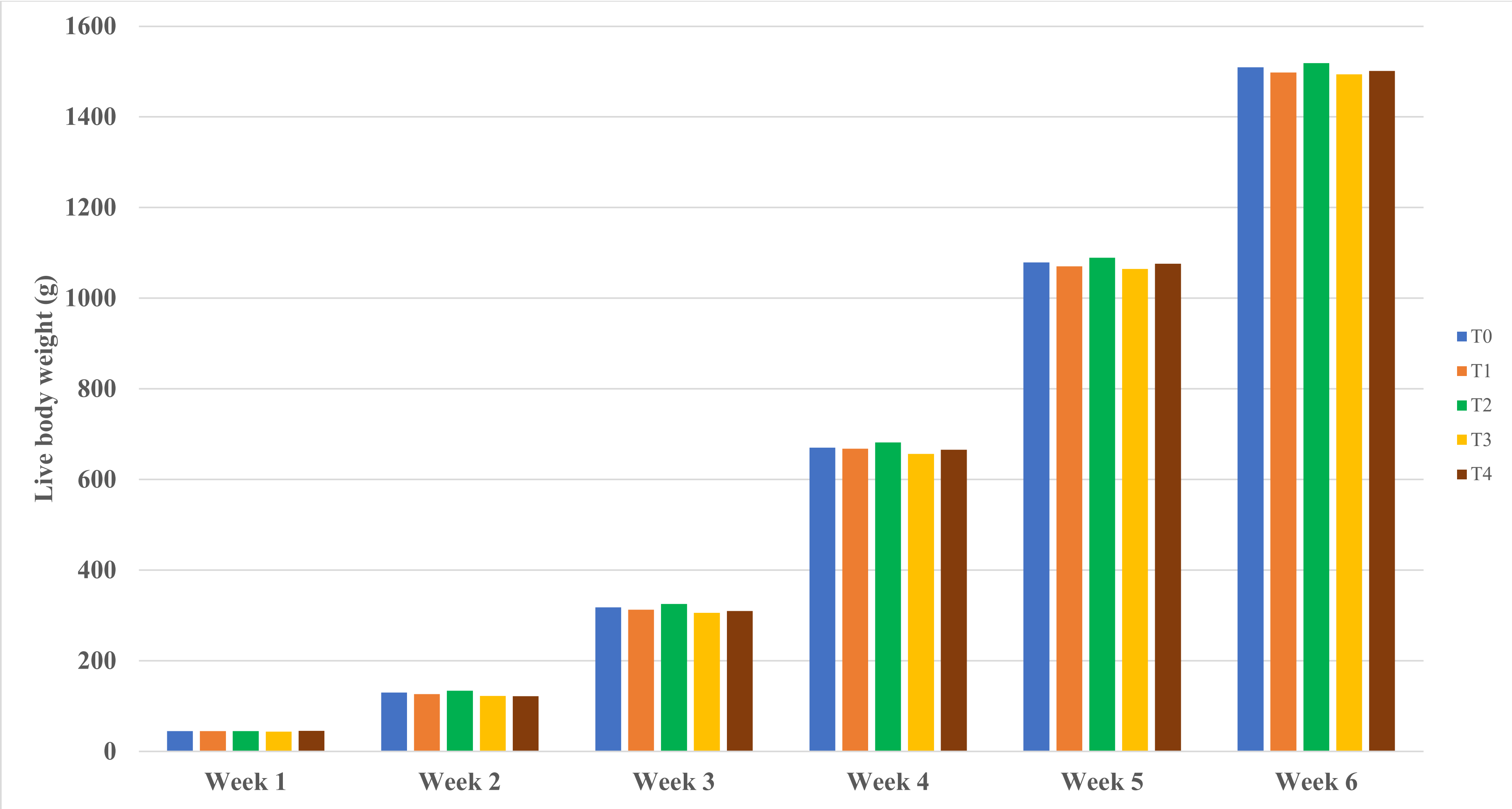

Figure 4.1 Average weekly live body weight (g) of birds

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

SV	DF	SS	MSS	(F cal)
Treatments	4	1028.266	257.066	12.032
Error	10	213.644	21.364	-
Total	14	-	-	-

Coefficient of variation = 0.560

gluten meal with or without protease enzyme as dietary treatments during the subsequent periods of prestarter, starter and finisher phase.

Correspondingly, the average live body weights at the end of six weeks were 2031.15 ± 15.66, 2010.68 ± 6.12, 2078.83 ± 10.14, 2015.78 ± 12.22 and 2063.06 ± 13 g for groups T_0, T_1, T_2, T_3 and T_4 respectively.

On statistical analysis, the highest total live body weight of birds was shown by group T_2, receiving 10% replacement with maize gluten meal with protease enzyme followed by groups T_4, T_0, T_3 and T_1. Similarly, consistent significant ($P<0.05$) increase in average live body weight of group T_2 was recorded followed by group T_4, T_0, T_1 and T_3, Also, treatments T_2 and T_4 both receiving maize gluten meal with protease enzyme showed highest body weights which suggest the use of protease enzyme with MGM had improved the body weight gain of experimental birds.

These findings are in accordance with the Azouz (2020) and Abhijeet *et al.*, (2021) who have also reported significant ($P<0.05$) difference in live body weight of the experimental birds at 2,4,5 and 6 weeks of age when basal diet was replaced with MGM protein. Results obtained by Odetallah *et al.*, (2005), Hafeez *et al.*, (2020) and Saleh *et al.*, (2020) are in line with the present study's findings as protease enzyme supplementation significantly ($P<0.05$) improved the live body weight at the end of the experiment. This might be due to the increased availability of amino acids and enhanced digestibility of crude protein and dry matter on inclusion of protease enzyme (Hafeez *et al.,* 2020; Saleh *et al*., 2020). Moreover, protease neutralizes the anti-nutritional factors and improves the hydrolysis of protein (Ghazi *et al*., 2002).

It was also found that body weights of birds from control group, group T_1 and group T_3 was comparable which suggested that MGM can be included in the broiler diet up to 20% protein replacement level without affecting the growth of the birds. This was also concluded by Jia *et al*., (2023) who observed no significant (P>0.05) difference with the replacement of soybean meal with corn gluten meal up to 3.8% with no adverse effects on growth performance.

In contrast, Babidis *et al*., (2002) and Giannenas *et al*., (2017) were noted lower body weights of MGM containing groups than control group. Also, Cardinal *et al*., (2019) and Tripathi *et al*., (2020) reported protease enzyme supplementation in broilers, statistically non-significant (P>0.05) but numerically higher differences compared to control.

This indicated that MGM can be used in the diet of poultry up to 20% level of protein replacement without affecting body weight of the birds, however inclusion of MGM with protease enzyme had showed significantly better performance with regards to body weight of birds.

4.1.2 Average weekly body weight gain

Table 4.3 represents the data regarding the average weekly gain in body weights of birds among different treatment groups during the experimental period. The data is depicted graphically in Figure 4.2. The total body weight gain at the end of experiment for groups T_0, T_1, T_2, T_3 and T4 was 1986.33 ± 16.55, 1965.87 ± 6.14, 2034.03 ± 10.12, 1975.17 ± 9.66 and 2017.73 ± 12.62 g, respectively.

The above data was subjected to statistical analysis that showed the average weekly body weight gain of birds from group T_2 and T_4 was highest, followed by group T_0, T_3 and T_1. The difference in mean weakly body weight gain was statistically significant (P<0.05).

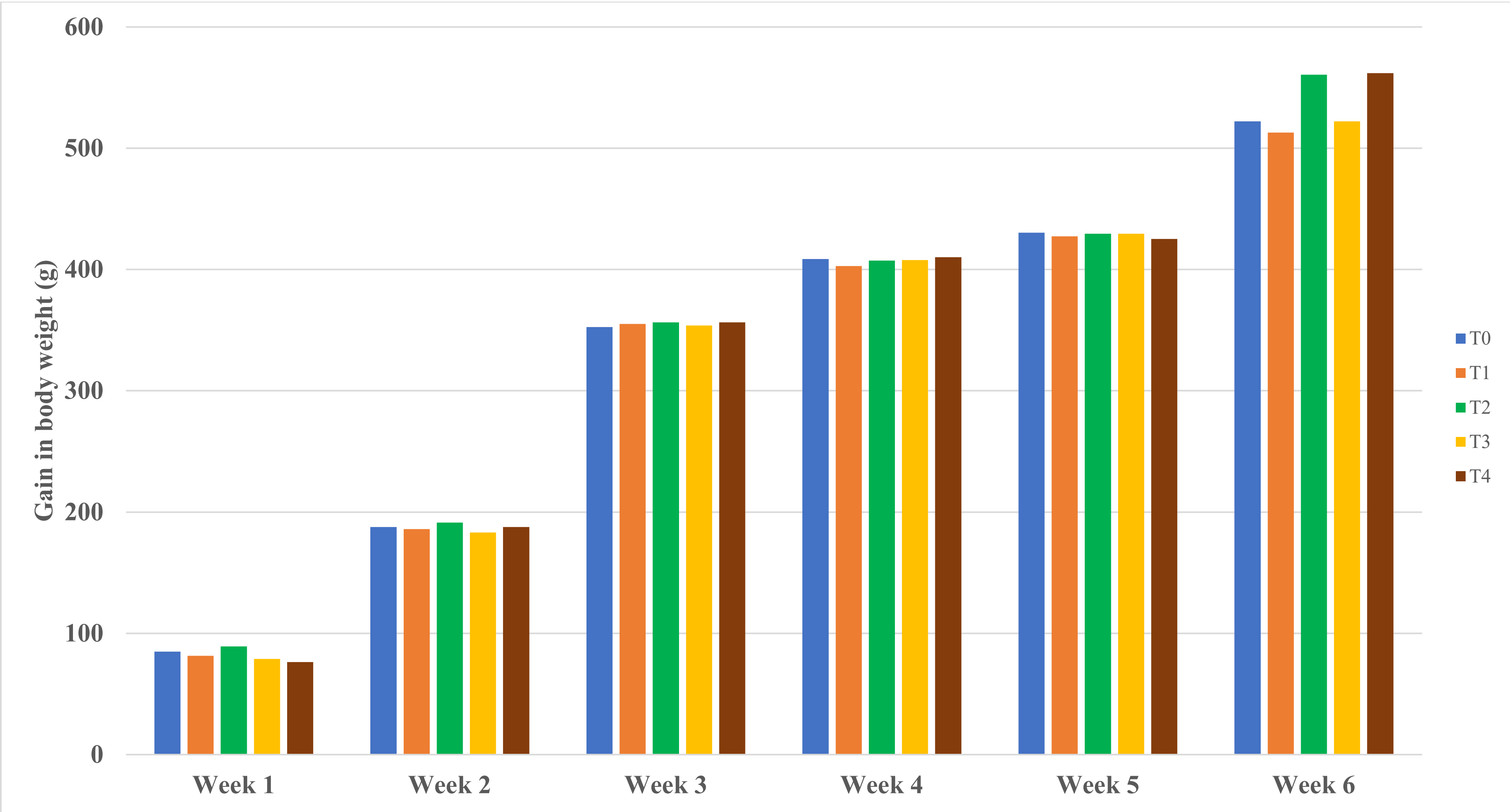

Figure 4.2 Average weekly body weight gain (g) of birds

It is also evident that group T_0, T_1 and T_3 had statistically similar average body weight gain which suggested inclusion of MGM from 10% to 20% replacement of protein in the broiler diet had no deleterious effect on performance of broilers. Babidis *et al*., (2002) has also found no significant difference in body weight gain of the broilers on inclusion of MGM at 8% level in their diet.

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

Weeks	Experimental groups					
	T_0	T_1	T_2	T_3	T_4	CD
1	$84.83^{ab} \pm 0.28$	$81.55^{bc} \pm 0.23$	$89.11^{a} \pm 0.32$	$78.84^{bc} \pm 1.31$	$76.29^{c} \pm 4.20$	7.20
2	$187.77^{ab} \pm 2.12$	$185.98^{bc} \pm 0.12$	$191.27^{a} \pm 0.38$	$183.09^{c} \pm 0.21$	$187.75^{ab} \pm 0.84$	3.77
3	352.56 ± 0.26	355.23 ± 1.84	356.32 ± 1.05	353.76 ± 2.92	356.34 ± 2.42	NS
4	408.64 ± 6.46	402.81 ± 3.55	407.31 ± 5.65	407.76 ± 2.91	410.10 ± 4.53	NS
5	$430.43^{a} \pm 0.42$	$427.45^{b} \pm 0.58$	$429.43^{a} \pm 0.60$	$429.49^{a} \pm 0.40$	$425.23^{c} \pm 0.39$	1.76
6	522.12 ± 17.38	512.88 ± 4.87	560.58 ± 14.42	522.19 ± 13.31	562.03 ± 13.74	NS
Mean	$331.06^{bc} \pm 66.84$	$327.65^{c} \pm 66.16$	$339.00^{a} \pm 69.88$	$329.19^{c} \pm 67.85$	$336.29^{ab} \pm 71.74$	6.99
Total Body weight gain	$1986.33^{bc} \pm 16.55$	$1965.87^{c} \pm 6.14$	$2034.03^{a} \pm 10.12$	$1975.17^{c} \pm 9.66$	$2017.73^{ab} \pm 12.62$	42.01

Means marked with at least one common superscript (a,b,c) in the same row do not differ significantly. NS – Non significant

Table 4.4 Analysis of variance of average total body weight gain (g)

SV	DF	SS	MSS	(F cal)
Treatments	4	10062.636	2515.659	4.716
Error	10	5334.133	533.413	-
Total	14	-	-	-

Coefficient of Variation = 1.157

The above findings are in support with Afrouzi *et al*. (2018), who studied the combined effect of corn gluten meal with protease enzyme which showed significant (P<0.05) difference in the weight gain of broilers. Likewise, significantly (P<0.01) better gain in body weight was noted by Seyedi and Hosseinkhani (2014) and Azouz (2020) at inclusion of 12% and 10% MGM in the broiler diet, respectively. Kamel *et al*., (2015), Hafeez *et al*., (2020) and Jabbar *et al*., (2020) had also revealed that protease enzyme supplementation improved gain in body weight of broilers.

In distinction to the findings of current study, Ismail *et al*., (2005) reported that inclusion of MGM in broilers' diet beyond 9% level adversely affected growth performance and also observed reduced body weight gain.

Angel *et al*., (2011), Freitas *et al.* (2011) and Naik *et al.* (2017) observed no significant (P<0.05) increase in average weekly body weight gain in protease enzyme supplemented groups.

Hence, it can be concluded that the replacement of protein up to 20% level through maize gluten meal with 300 mg/kg protease enzyme had significantly (P>0.05) improved the gain in body weight of broilers. It can also be concluded from current investigation that up to 20% of replacement of protein by MGM without supplementation of protease enzyme had not adversely affected the body weight gain of broiler birds as compared to corn-soya based broiler diet.

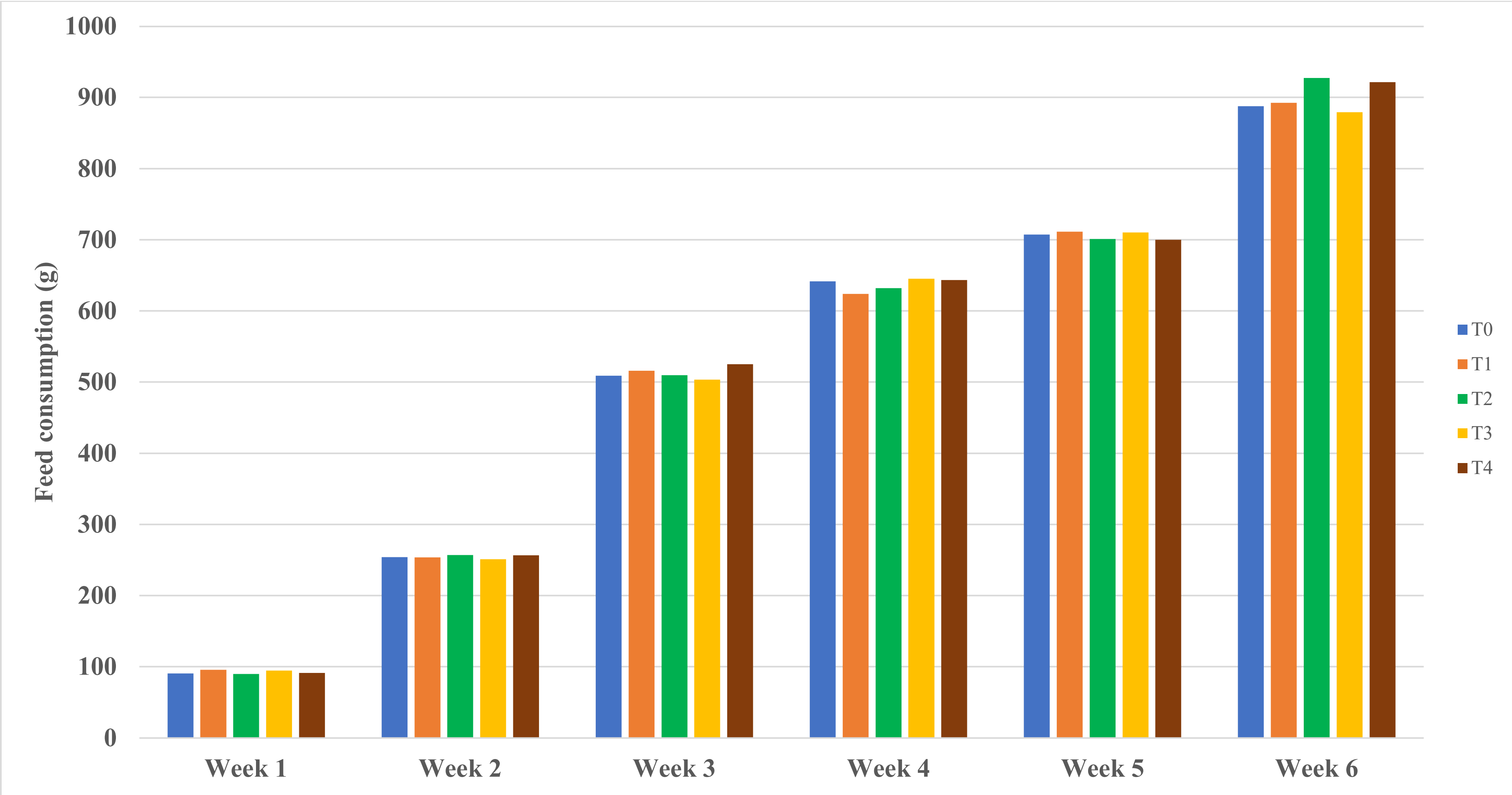

Figure 4.3 Average weekly feed consumption (g) of birds

4.1.3 Average weekly feed intake

Table 4.5 Average weekly feed intake (g) of experimental birds from different groups

Weeks	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
1	90.64 ± 1.73	95.60 ± 0.56	89.94 ± 0.48	94.73 ± 1.24	91.42 ± 1.77	NS
2	254.19 ± 2.53	253.53 ± 0.56	257.02 ± 0.67	251.06 ± 0.43	256.74 ± 1.64	NS
3	509.00^{bc} ± 1.05	515.99^{ab} ± 2.28	509.56^{bc} ± 2.68	503.52^{c} ± 5.17	525.06^{a} ± 3.19	11.55
4	641.51 ± 9.78	623.94 ± 5.56	632.17 ± 9.66	645.17 ± 3.53	643.55 ± 5.28	NS
5	707.26^{a} ± 1.43	711.41^{a} ± 3.04	701.06^{b} ± 0.73	710.37^{a} ± 0.37	699.82^{b} ± 1.44	6.09
6	887.57 ± 11.88	892.36 ± 1.84	927.27 ± 10.40	879.11 ± 10.55	921.64 ± 18.15	NS
Mean	515.03^{b} ± 121.09	515.47^{b} ± 120.71	519.50^{ab} ± 124.56	513.99^{b} ± 120.29	523.04^{a} ± 124.08	6.13
Total feed consumption	3090.18^{b} ± 11.72	3092.83^{b} ± 11.57	3117.03^{ab} ± 13.84	3086.95^{b} ± 18.25	3138.23^{a} ± 15.55	34.99

Means marked with at least one common superscript (a,b,c) in the same row do not differ significantly. NS – Non significant

It is seen from the Table 4.5 that average total feed intake of birds from treatment groups T_0, T_1, T_2, T_3 and T_4 was 3090.18 ± 11.72, 3092.83 ± 11.57, 3117.03 ± 13.84, 3086.95 ± 18.25 and 3138.23 ± 15.55 g, respectively.

Records and observations of average weekly feed intake of different treatment groups from first to sixth week of age are outlined in Table 4.5 and represented graphically in Figure 4.3.

Table 4.6 Analysis of variance of mean of average total weekly feed intake

SV	DF	SS	MSS	(F cal)
Treatments	4	5828.185	1457.056	3.938
Error	10	3699.578	369.958	-
Total	14	-	-	-

Coefficient of Variation = 0.619

The data was subjected to statistical analysis and the results of the same presented in Table 4.6. The difference in the average weekly feed intake was significantly ($P>0.05$) higher in group T_2 and T_4 receiving MGM with corresponding levels of protease enzyme than groups T_0, T_1 and T_3. Group T_2 and T_4 were at par with regards to feed intake. The average weekly feed intake by the birds from groups T_0, T_1 and T_3 was comparable.

These results are in accordance with Babidis *et al*., (2001) and Wang *et al*., (2018) who observed no significant ($P>0.05$) difference in feed consumption due to inclusion of maize gluten meal. Similarly, Hafeez *et al*., (2020) noted significantly ($P<0.05$) higher feed intake by the broilers in protease supplemented dietary treatments.

On the contrary, Giannenas *et al*., (2017) found no significant ($P<0.10$) difference in feed intake among treatments when maize gluten meal with protease enzyme was added in the broiler diet. Yuan *et al*., (2017) and Saleh *et al*., (2020) observed no significant (P<0.05) difference in feed intake of the experimental birds on protease supplementation.

In difference to present study, findings of experimental trials conducted by Azouz, (2020) and Sushil *et al*., (2024) showed significantly (P<0.05) higher feed intake in MGM supplemented treatments than MGM non-supplemented groups irrespective of levels of MGM. However, Seyedi and Hosseinkhani (2014) and Abhijeet *et al*., (2021) noticed the significant (P<0.05) decrease in feed consumption due to supplementation of maize gluten meal at 12% and 10% level in the diet, respectively.

Thus, it can be concluded that protease supplementation had significant (P>0.05) effect on average total weekly feed intake by the experimental birds. In the present study, increased feed intake was observed in protease supplemented groups, was may be due to improved digestion and more effective nutrient utilization of protein because of inclusion of protease enzyme. However, feed intake from groups supplemented with MGM and control was comparable which indicated that palatability of diet was not adversely affected due to inclusion of MGM up to 20% protein replacement level (Azouz *et al.*, 2020; Babidis *et al.*, 2002; Rose *et al.*, 2003).

4.1.4 <u>Average weekly feed conversion ratio (FCR)</u>

Feed conversion ratios (FCR) represent the feed intake required per unit gain in body weight which is detailed in Table 4.7 and the same data is graphically depicted in Figure 4.4.

The feed conversion ratio at the end of the experimental period for different treatment groups was 1.556 ± 0.007, 1.573 ± 0.005, 1.533 ± 0.007, 1.565 ± 0.006 and 1.555 ± 0.003 in T_0, T_1, T_2, T_3 and T_4 group, respectively.

Following statistical analysis, group T_2 receiving 10% protein replacement with MGM and protease supplementation at 200mg/kg of diet consistently showed significantly (P<0.05) improved FCR as compared to remaining treatment groups and control group. All other treatment groups viz, T_0, T_1, T_3 and T_4 showed comparable feed conversion ratios.

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

Week	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
1	$1.069^{b} \pm 0.022$	$1.172^{a} \pm 0.010$	$1.009^{b} \pm 0.002$	$1.202^{a} \pm 0.005$	$1.205^{a} \pm 0.045$	0.083
2	$1.360^{b} \pm 0.002$	$1.363^{a} \pm 0.002$	$1.344^{c} \pm 0.001$	$1.371^{a} \pm 0.004$	$1.368^{a} \pm 0.003$	0.009
3	$1.444^{bc} \pm 0.002$	$1.453^{b} \pm 0.002$	$1.430^{c} \pm 0.004$	$1.435^{bc} \pm 0.002$	$1.474^{a} \pm 0.010$	0.018
4	$1.570^{a} \pm 0.002$	$1.549^{b} \pm 0.002$	$1.552^{b} \pm 0.003$	$1.582^{a} \pm 0.003$	$1.570^{a} \pm 0.009$	0.016
5	$1.643^{b} \pm 0.002$	$1.664^{a} \pm 0.005$	$1.632^{c} \pm 0.004$	$1.654^{ab} \pm 0.002$	$1.646^{bc} \pm 0.004$	0.014
6	1.731 ± 0.014	1.697 ± 0.051	1.656 ± 0.025	1.691 ± 0.027	1.641 ± 0.008	NS
Mean FCR	$1.470^{b} \pm 0.097$	$1.483^{a} \pm 0.081$	$1.437^{c} \pm 0.099$	$1.489^{a} \pm 0.077$	$1.484^{a} \pm 0.071$	0.019
Total FCR	$1.556^{a} \pm 0.007$	$1.573^{a} \pm 0.005$	$1.533^{b} \pm 0.007$	$1.565^{a} \pm 0.006$	$1.555^{a} \pm 0.003$	0.021

Means marked with at least one common superscript (a,b,c) in the same row do not differ significantly. NS – Non significant

Table 4.8 Analysis of variance of mean of average weekly feed conversion ratio

SV	DF	SS	MSS	(F cal)
Treatments	4	0.003	0.001	5.058
Error	10	0.001	0.000	-
Total	14	-	-	-

Coefficient of Variation = 0.753

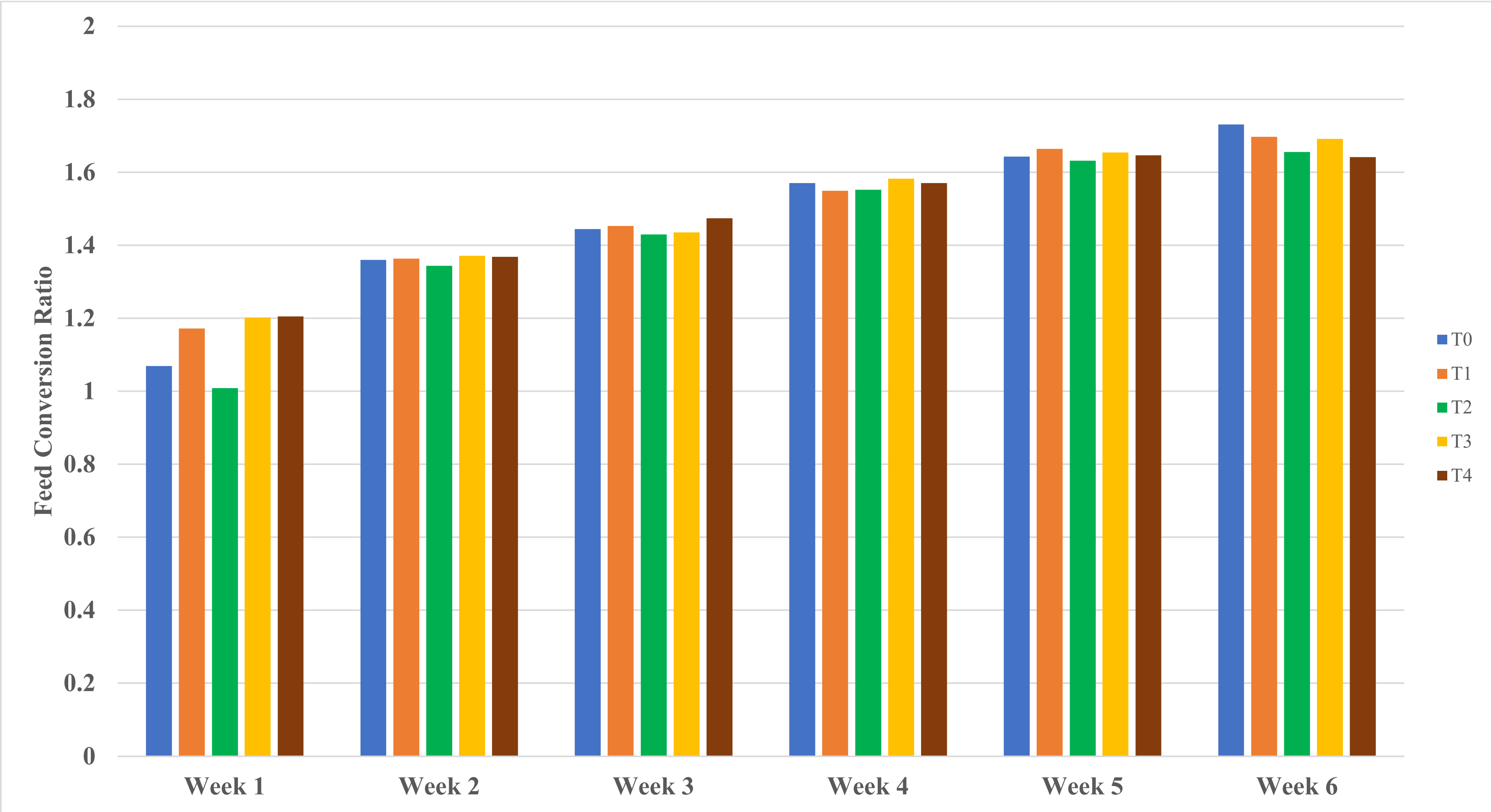

Figure 4.4 Average weekly feed conversion ratio of birds

These findings of the present study are in line with Odetallah *et al*., (2005), Fru-Nji *et al*., (2011), Kamel *et al*., (2015), Naik *et al*., (2017), Yuan *et al*., (2017), Hafeez *et al*., (2020), Saleh *et al*., (2020) and Tripathi *et al*., (2020) who noticed protease supplementation had significantly ($P<0.05$) improved the FCR.

With respect to the inclusion of maize gluten meal without protease enzyme, Babidis *et al*., (2002) and Wang *et al*., (2018) observed no significant difference in FCR values during the experimental period when compared with corn-soya based diet. These results are in corroboration with the findings of current investigation.

On difference, results of the experimental trial conducted by Angel *et al*., (2011), Freitas *et al*., (2011), Law *et al*., (2018), Cardinal *et al*., (2019) and Amer *et al*., (2021) revealed no significant ($P<0.05$) difference in total feed conversion ratio in broilers on inclusion of protease enzyme. Seyedi and Hosseinkhani (2014) and Abhijeet *et al*., (2021) recorded improvement in feed conversion ratios when diet was incorporated with maize gluten meal without protease enzyme.

This showed that 10% replacement of soybean meal protein with maize gluten meal protein with addition of 200 mg/kg protease enzyme produced consistently better feed conversion ratios in broilers. These results indicated that, protease enzyme enhanced the digestibility and feed utilization for better growth of broilers with optimum feed intake.

4.2 **<u>Carcass traits</u>**

Composition of diet influences carcass characteristics in broilers. Hence, carcass evaluation was undertaken in the present study. At the end of experimental trial, carcass was evaluated for various parameters such as dressed weight, dressing percentage, edible meat percentage, giblet weight percentage (including individual liver%, heart% and Gizzard%) and breast meat percentage and details of the same are extracted in the Table 4.9.

Table 4.9 Average carcass traits evaluation of birds of various experimental groups

Carcass traits	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
Dressed weight (g)	1618.50^{bc} ± 10.40	1586.13^{c} ± 6.19	1757.17^{a} ± 14.80	1596.67^{c} ± 12.10	1642.33^{b} ± 4.80	37.62
Dressing %	75.91 ± 1.19	76.44 ± 0.47	76.98 ± 0.48	76.54 ± 0.62	74.14 ± 0.45	NS
Edible meat (g)	1462 ± 31.28	1406.33 ± 16.36	1568 ± 31.37	1448.33 ± 26.39	1515.67 ± 43.11	NS
Edible meat %	68.53 ± 0.86	67.77 ± 0.77	68.71 ± 1.50	69.43 ± 1.24	68.40 ± 1.57	NS
Breast meat (g)	541.50 ± 5.63	548.00 ± 4.18	566.50 ± 4.26	547.00 ± 8.84	552.17 ± 7.38	NS
Breast meat %	25.40 ± 0.42	26.41 ± 0.14	24.82 ± 0.11	26.23 ± 0.64	24.93 ± 0.40	NS
Giblet weight	118.00 ± 6.56	119.67 ± 6.83	100.67 ± 4.25	102.33 ± 5.06	123.00 ± 9.54	NS
Giblet weight %	5.53 ± 0.34	5.75 ± 0.36	4.41 ± 0.19	4.90 ± 0.23	5.55 ± 0.46	NS
Liver weight %	2.19 ± 0.09	2.26 ± 0.16	1.65 ± 0.04	1.91 ± 0.09	2.25 ± 0.27	NS
Gizzard weight %	2.87 ± 0.22	3.04 ± 0.22	2.36 ± 0.11	2.39 ± 0.08	3.01 ± 0.25	NS
Heart weight %	0.46 ± 0.03	0.45 ± 0.02	0.50 ± 0.04	0.52 ± 0.05	0.48 ± 0.03	NS

Means marked with at least one common superscript (a,b,c) in the same row do not differ significantly. NS – Non significant

4.2.1 <u>Dressed weight</u>

It is seen from the table 4.9 that average dressed weight (g) of the experimental dietary treatments were 1618.50 ± 10.40, 1586.13 ± 6.19, 1757.17 ± 14.80, 1596.67 ± 12.10 and 1642.33 ± 4.80, respectively. This data was analysed for statistical analysis and results of the same was presented in Table 4.10.

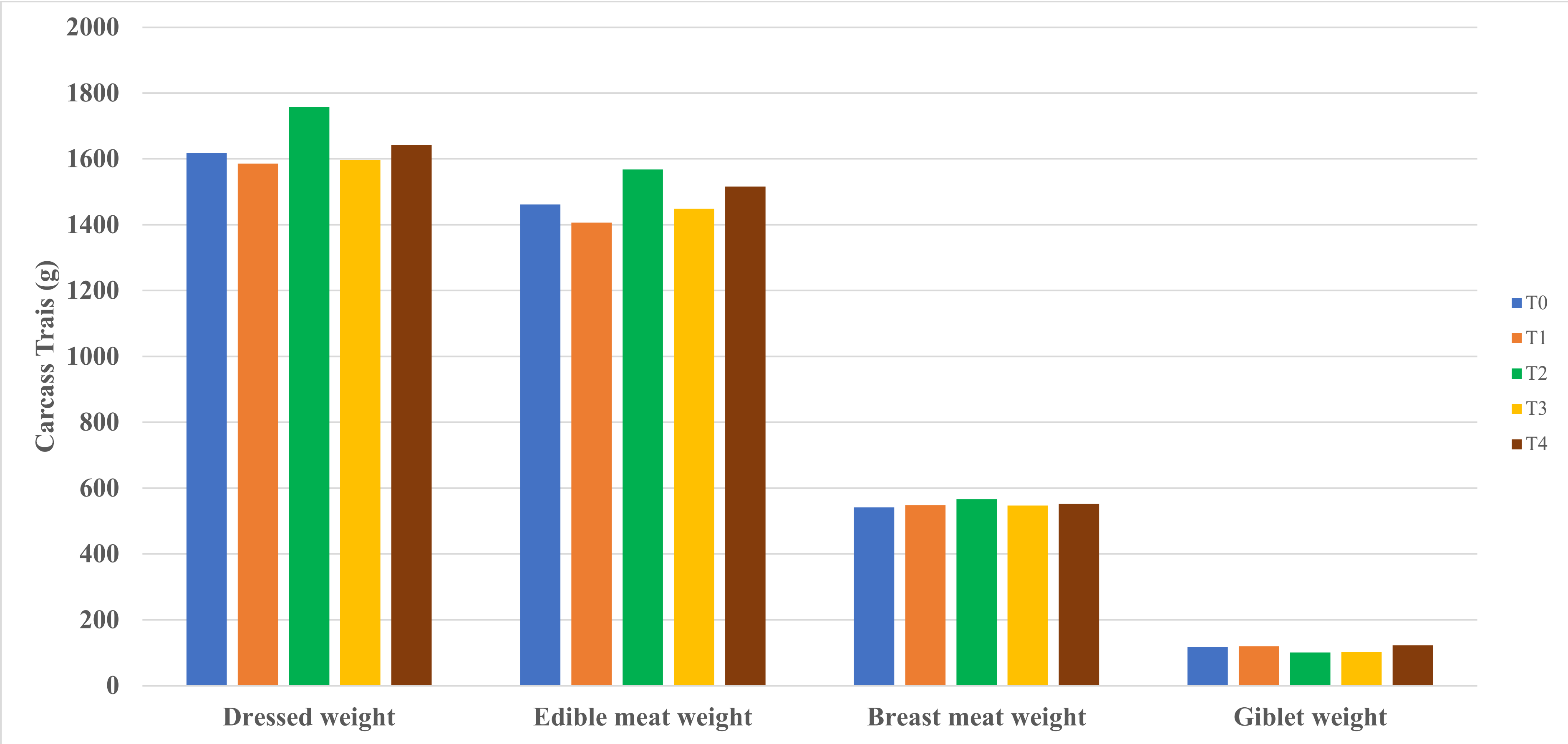

Figure 4.5 Average carcass traits weight (g) of birds

Table 4.10 Analysis of variance of average dressed weight

SV	DF	SS	MSS	(F cal)
Treatments	4	56924.969	14231.242	33.273
Error	10	4277.107	427.711	-
Total	14	-	-	-

Coefficient of Variation = 1.261

Findings of the statistical analysis unveiled significant (P<0.05) difference on dressed weight (g) among the various treatment groups. The highest dressed weight was observed in the treatment group T_2 followed by groups T_4, T_0, T_3 and T_1. The difference was comparable between group T_0 and T_4. Dressed weight when compared between T_1 and T_3 were at par. The significant difference observed in the dressed weight was due to significant difference in the live body weights at the end of 6^{th} week.

These findings of the present study are consistent with Seyedi and Hosseinkhani (2014), Ajayi *et al.*, (2015) and Kamel *et al.*, (2015). Seyedi and Hosseinkhani (2014) observed significant (P<0.01) difference of inclusion of maize gluten meal than control group on dressed weight. Ajayi *et al.*, (2015) and Kamel *et al.*, (2015) noted significant effect on carcass yield on protease supplementation.

In disparity to these results, Babidis *et al.*, (2002) did not find any significant (P>0.05) effect on dressed weight or carcass yield with the replacement of protein source by maize gluten meal. Studies conducted by Rada *et al.*, (2013) and Tripathi *et al.*, (2020) also showed no significant differences across experimental groups supplemented with protease enzyme.

This implied that birds who received 10% protein replacement by maize gluten meal along with protease enzyme significantly (P<0.05) improved dressed weight which can be corelated with the final body weight achieved by the birds in the group.

4.2.2 Dressing percentage

The Dressing percentages of birds reflect the amount of edible meat and bones in relation to the live wight of the birds. The data regarding dressing % for various dietary treatments is summed up in Table 4.9 and visually represented in Figure 4.5. The average dressing percentages for groups T_0, T_1, T_2, T_3 and T_4 were 75.91 ± 1.19, 76.44 ± 0.47, 76.98 ± 0.48, 76.54 ± 0.62 and 74.14 ± 0.45, respectively.

Table 4.11 Analysis of variance of average dressing percentage

SV	DF	SS	MSS	(F cal)
Treatments	4	14.735	3.684	1.880
Error	10	19.599	1.960	-
Total	14	-	-	-

Coefficient of Variation = 1.842

Results of the statistical analysis for data pertaining to the dressing percentages of experimental birds across different treatment groups are outlined in Table 4.10. The statistical analysis revealed non-significant (P<0.05) difference in the average dressing percentage of birds for various treatments.

Similar findings were observed by Rada *et al*., (2013), Law *et al*., (2018) and Tripathi *et al*., (2020). No significant (P<0.05) difference on dressing percentage was noted on protease enzyme supplementation. Babidis *et al*., (2002) concluded non-significant (P>0.05) difference on dressing percentage with the inclusion of MGM in diet.

On contrary, Ajayi (2015) and Kamel *et al*., (2015) found significant effect on dressing percentage of the birds supplemented with protease enzyme. Azouz (2020) studied the effect of MGM on two different level (5% and 10%), and found

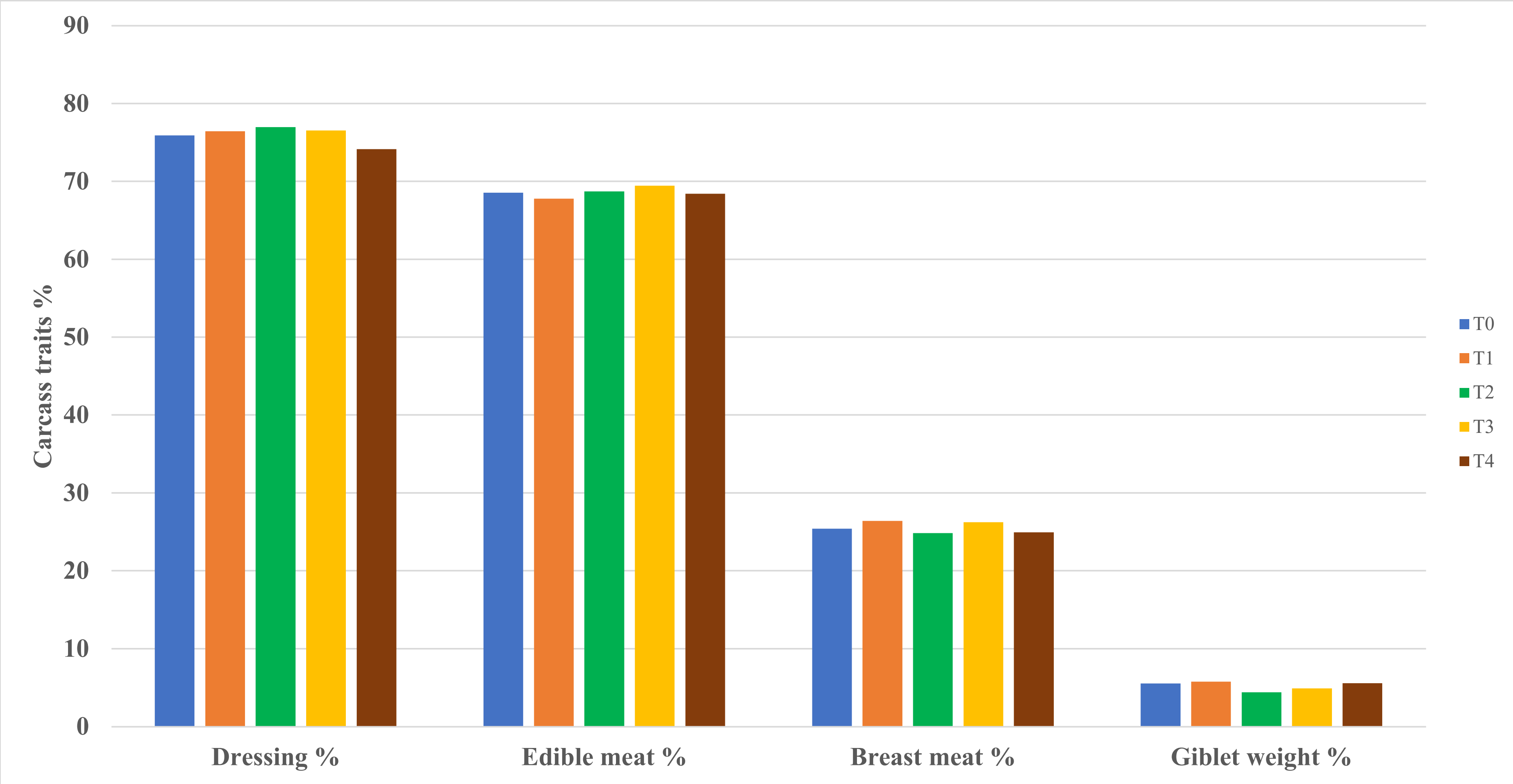

Figure 4.6 Average carcass traits percentage of birds

significantly (P<0.01) higher dressing percentage in 5% MGM incorporated dietary treatment.

This highlighted that partial replacement (10-20%) of soybean meal protein with maize gluten meal protein with or without protease enzyme supplementation has non-significant (P>0.05) difference on dressing percentage of broilers.

4.2.3 Edible meat percentage

The dressing percentages were further considered on the basis of edible meat yield and their percentage. Average edible meat percentage values for experimental birds across different groups are featured in Table 4.9 and visually represented in Figure 4.5.

On a live weight basis, the average percentages of edible meat for groups T_0, T_1, T_2, T_3 and T_4 were 68.53 ± 0.86, 67.77 ± 0.77, 68.71 ± 1.50, 69.43 ± 1.24 and 68.40 ± 1.57, respectively. The data of edible meat percentage were analysed statistically with respect to treatments and the results are presented in Table 4.11.

Statistical analysis revealed non-significant (P>0.05) difference in the edible meat percentage among experimental broiler chickens across different treatments.

Table 4.12 Analysis of variance of average edible meat percentage

SV	DF	SS	MSS	(F cal)
Treatments	4	4.277	1.069	0.176
Error	10	60.719	6.072	-
Total	14	-	-	-

Coefficient of Variation = 3.594

4.2.4 <u>Breast meat percentage</u>

The average breast weight of birds in different experimental groups are detailed in Table 4.9 and visually depicted in Figure 4.6. The average breast meat percentage for groups T_0, T_1, T_2, T_3 and T_4 on a live weight basis were found to be 25.40 ± 0.42, 26.41 ± 0.14, 24.82 ± 0.11, 26.23 ± 0.64 and 24.93 ± 0.40, respectively. Statistical analysis was conducted to explore the variability in breast meat percentage of different treatment groups presented in Table 4.12.

On statistical analysis, no significant (P>0.05) difference was found in different dietary treatment groups for breast meat weight percentage. These findings align with Law *et al*., (2018) who stated that breast meat percentage displayed non-significant (P=0.049) difference on protease supplementation. Afrouzi *et al*., (2018) also noted non-significant (P<0.05) difference on breast meat percentage of broilers on inclusion of 2% corn gluten meal with 300mg/kg protease enzyme.

In difference, Ajayi *et al*., (2015), Kamel *et al*., (2015) and Saleh *et al*., (2020) were noticed significantly (P≤0.05) higher difference on breast meat percentage with the inclusion of protease enzyme in the diet. Also, Seyedi and Hosseinkhani (2014) concluded significant (P<0.01) difference on breast meat percentage at different levels of maize gluten meal compared to control.

This suggested that replacement with MGM at 10 or 20% protein level with or without protease enzyme addition exhibited non-significant (P>0.05) difference on breast meat percentage among experimental treatment groups.

Table 4.13 Analysis of variance of average breast meat percentage

SV	DF	SS	MSS	(F cal)
Treatments	4	6.431	1.608	3.063
Error	10	5.249	0.525	-
Total	14	-	-	-

Coefficient of Variation = 2.835

4.2.5 <u>Giblet weight percentage</u>

The data regarding average giblet weight percentage of birds across different groups are detailed in Table 4.9 and visually represented in Figure 4.5.

The average giblet weight percentages for groups T_0, T_1, T_2, T_3 and T_4 were varied from 5.53 ± 0.34, 5.75 ± 0.36, 4.41 ± 0.19, 4.90 ± 0.23 and 5.55 ± 0.46, respectively. This data was analysed statistically with respect to treatments and its results are spotlighted in Table 4.14.

Results of statistical analysis showed non-significant ($P>0.05$) difference on percentage of giblet weight. Similar findings were noted by Tripathi *et al*. (2020) where no statistically significant ($P>0.05$) difference in treatment groups and control group was reported in giblet weight % by supplementation of protease enzyme at different levels.

This denoted that protein replacement by MGM with or without protease enzyme could not alter the giblet weight percentage.

Table 4.14 Analysis of variance of average giblet weight percentage

SV	**DF**	**SS**	**MSS**	**(F cal)**
Treatments	4	3.772	0.943	2.163
Error	10	4.359	0.436	-
Total	14	-	-	-

Coefficient of Variation = 12.629

4.2.6 Liver weight percentage

The treatment wise data of liver weight percentage of experimental birds depicted in Table 4.9 and graphically demonstrated in Figure 4.7. Details of statistical data expressed in Table 4.15.

The average liver weight percentages were 2.19 ± 0.09, 2.26 ± 0.16, 1.65 ± 0.04, 1.91 ± 0.09 and 2.25 ± 0.27 for treatment groups T_0, T_1, T_2, T_3 and T_4. Statistical analysis exhibited non-significant ($P>0.05$) difference in liver weight percentage across the various experimental groups. These results are in support with the findings of the experimental trial conducted by Naik *et al*., (2017), Azouz (2020) and Tripathi *et al*., (2020) as they did not come across with significant ($P<0.05$) differences among the treatment groups supplemented with protease enzyme and control group. Azouz (2020) revealed non-significant ($P<0.05$) difference on liver weight % on incorporation of MGM in different treatment groups.

However, Ajayi *et al*., (2015) and Saleh *et al*., (2020) marked a significant difference in the relative liver weight percentage of the birds who received 200 and 300 mg/kg protease enzyme supplementation in the diet.

Table 4.15 Analysis of variance of average liver weight percentage

SV	DF	SS	MSS	(F cal)
Treatments	4	0.862	0.216	2.296
Error	10	0.939	0.094	-
Total	14	-	-	-

Coefficient of Variation = 14.940

4.2.7 Gizzard weight percentage

Values for the average gizzard weight percentage of birds across different experimental groups are traced in Table 4.9 and visually represented in Figure 4.7. The average gizzard weight percentage was calculated on the basis of live body

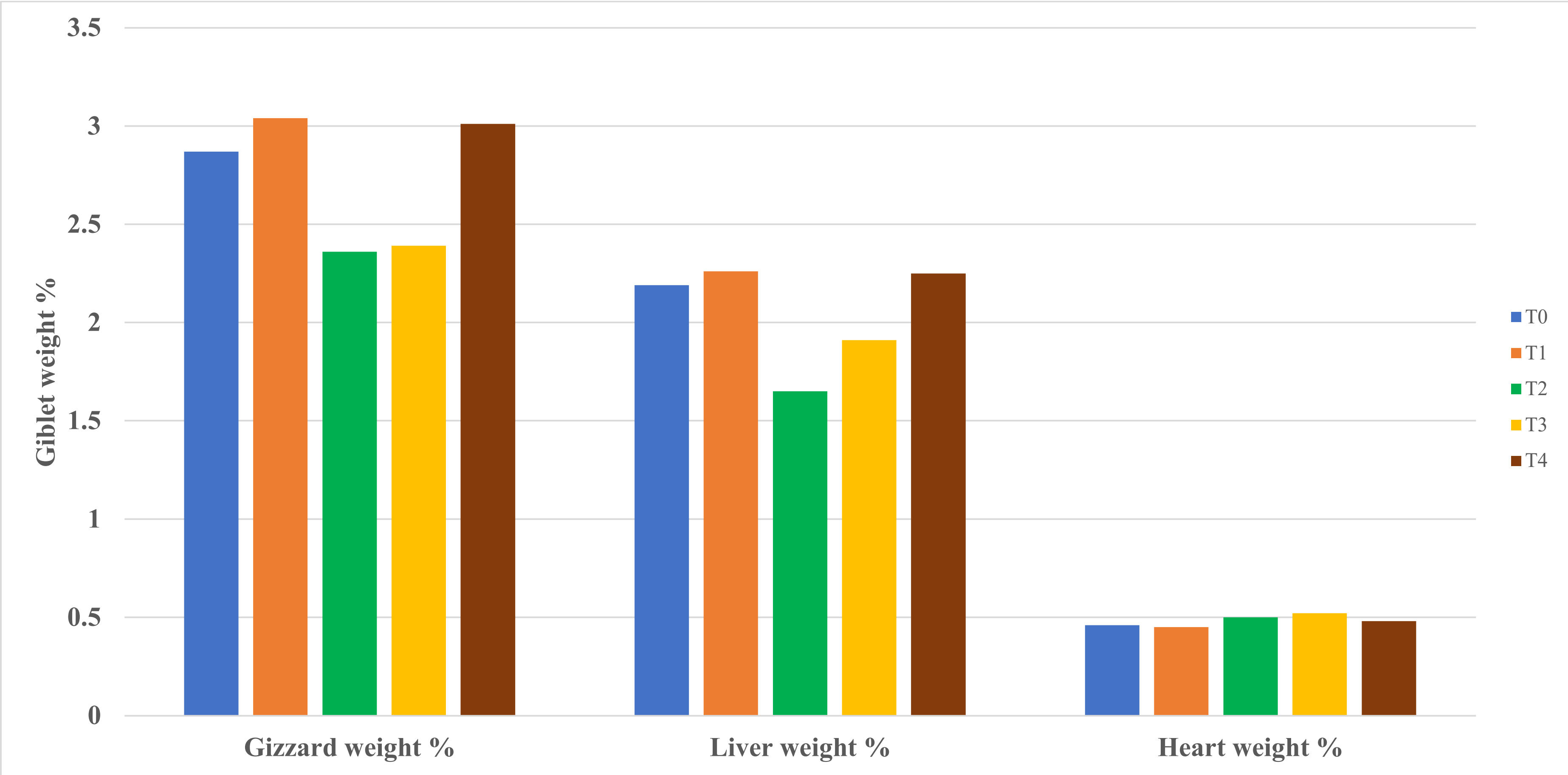

Figure 4.7 Average giblet weight percentage of birds

weight were 2.87 ± 0.22, 3.04 ± 0.22, 2.36 ± 0.11, 2.39 ± 0.08 and 3.01 ± 0.25 for treatment groups T_0, T_1, T_2, T_3 and T_4 respectively. Statistical analysis pointed out non-significant (P>0.05) difference for the data regarding gizzard weight percentage among the various experimental groups.

Similar findings were recorded by Naik *et al*., (2017), Azouz (2020) and Saleh *et al*., (2020). Naik *et al*., (2017) and Saleh *et al*., (2020) did not notice significant (P<0.05) difference on gizzard weight percentage with the inclusion of protease enzyme in the diet at different levels. Azouz (2020) observed non-significant (P<0.05) difference on gizzard weight percentage by providing 5% and 10% MGM in the broilers diet. Whereas, Ajayi (2015) commented that protease enzyme supplementation significantly (P=0.05) increased the gizzard weight percentage.

Table 4.16 Analysis of variance of average gizzard weight percentage

SV	DF	SS	MSS	(F cal)
Treatments	4	1.334	0.334	2.325
Error	10	1.434	0.143	-
Total	14	-	-	-

Coefficient of Variation = 13.840

4.2.8 Heart weight percentage

The average heart weight percentage values in different experimental treatment groups are highlighted in Table 4.9, its visual presentation portrayed in Figure 4.7 and statistical analysis showed in table no. 4.17. The average percentages of heart weight for groups T_0, T_1, T_2, T_3 and T_4 were 0.46 ± 0.03, 0.45 ± 0.02, 0.50 ± 0.04, 0.52 ± 0.05 and 0.48 ± 0.03, respectively.

On statistical analysis, treatments exhibited non-significant (P>0.05) difference for heart weight percentage. These findings are in agreement with Naik *et al*., (2017) and Saleh *et al*., (2020) who conducted an experimental trial on protease enzyme supplementation in the broilers diet and recorded non-significant (P<0.05) difference for heart weight percentage. Azouz (2020) studied the incorporation of two levels of MGM (5% and 10%) in the broiler diet and did not find significant (P<0.05) difference on heart weight percentage.

Table 4.17 Analysis of variance of average heart weight percentage

SV	**DF**	**SS**	**MSS**	**(F cal)**
Treatments	4	0.010	0.003	0.479
Error	10	0.052	0.005	-
Total	14	-	-	-

Coefficient of Variation = 14.947

4.3 Blood biochemical profile

Blood biochemical profile of the present study involves examination of serum total protein, serum albumin, serum globulin and A:G ratio. Data obtained regarding these parameters during the experimental period is summarised in table 4.18.

4.3.1 Serum total protein

The serum total protein values of birds across different dietary treatment groups are outlined in Table 4.18 and visually shown in Figure 4.8.

The average serum total protein values recorded were 3.45 ± 0.25, 3.74 ± 0.17, 4.53 ± 0.24, 3.93 ± 0.23 and 4.53 ± 0.32 g/dL for experimental groups T_0, T_1, T_2, T_3 and T_4, respectively.

Table 4.18 Average blood biochemical profile of experimental birds from different groups

Parameters	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
Serum Total Protein (g/dl)	3.45 ± 0.25	3.74 ± 0.17	4.53 ± 0.24	3.93 ± 0.23	4.53 ± 0.32	NS
Serum Albumin (g/dl)	1.83 ± 0.13	2.08 ± 0.22	2.61 ± 0.18	2.27 ± 0.24	2.54 ± 0.24	NS
Serum Globulin (g/dl)	1.62 ± 0.12	1.66 ± 0.07	1.93 ± 0.10	1.67 ± 0.02	1.99 ± 0.09	NS
A: G	1.13 ± 0.04	1.26 ± 0.18	1.35 ± 0.10	1.36 ± 0.15	1.27 ± 0.09	NS

NS – Non significant

Table 4.19 Analysis of variance of average serum total protein

SV	DF	SS	MSS	(F cal)
Treatments	4	2.793	0.698	2.885
Error	10	2.420	0.242	-
Total	14	-	-	-

Coefficient of Variation = 12.184

Following statistical analysis, it was found that experimental treatments showed non-significant (P>0.05) difference for serum total protein. Treatment groups T_2 and T_4, received MGM (10 and 20% respectively) along with protease enzyme showed numerically similar and higher values for serum total protein compared to other groups but were statistically non-significant.

These results of the present study are in consonant with Abdel-Raheem *et al*., (2005), Kamel *et al*., (2015), Law *et al*., (2018), Hafeez *et al*., (2020) and Amer *et al*., (2021). Abdel-Raheem *et al*., (2005) reported no significant difference on serum total protein in different experimental groups fed with maize gluten meal, full fat soybean meal and basal diet. Whereas, Kamel *et al*., (2015), Law *et al*., (2018), Hafeez *et al*., (2020) and Amer *et al*., (2021) mentioned that serum total protein values remain unaffected, displayed non-significant ($P<0.05$) difference at different levels of protease incorporation in the broilers diet.

In disparity to the current findings, Naik *et al*., (2017), Wang *et al*., (2018) and Saleh *et al*., (2020) observed significant ($P<0.05$) differences among the treatment groups for serum total protein. Wang *et al*., (2018) stated that birds fed 10% fermented corn gluten meal in both starter and finisher period showed significantly ($P<0.05$) higher total protein content in serum than control and 5% corn gluten meal group. Naik *et al*., (2017) and Saleh *et al*., (2020) denoted significantly ($P<0.05$) elevated levels of serum total protein on protease enzyme supplementation which was then supported by the fact protease performed crucial role in retention of protein.

Thus, present findings of this study implied that incorporation of MGM at 10% or 20% protein level with or without protease enzyme in the broilers diet showed non-significant difference on serum total protein content in experimental birds. The values of serum total protein level were found to be within normal range (Akinduro, 2021).

4.3.2 <u>Albumin</u>

It is seen from the Table 4.18 that average serum albumin values of birds for groups T_0, T_1, T_2, T_3 and T_4 were 1.83 ± 0.13, 2.08 ± 0.22, 2.61 ± 0.18, 2.27 ± 0.24 and 2.54 ± 0.24 g/dL, respectively. Data regarding statistical analysis presented in

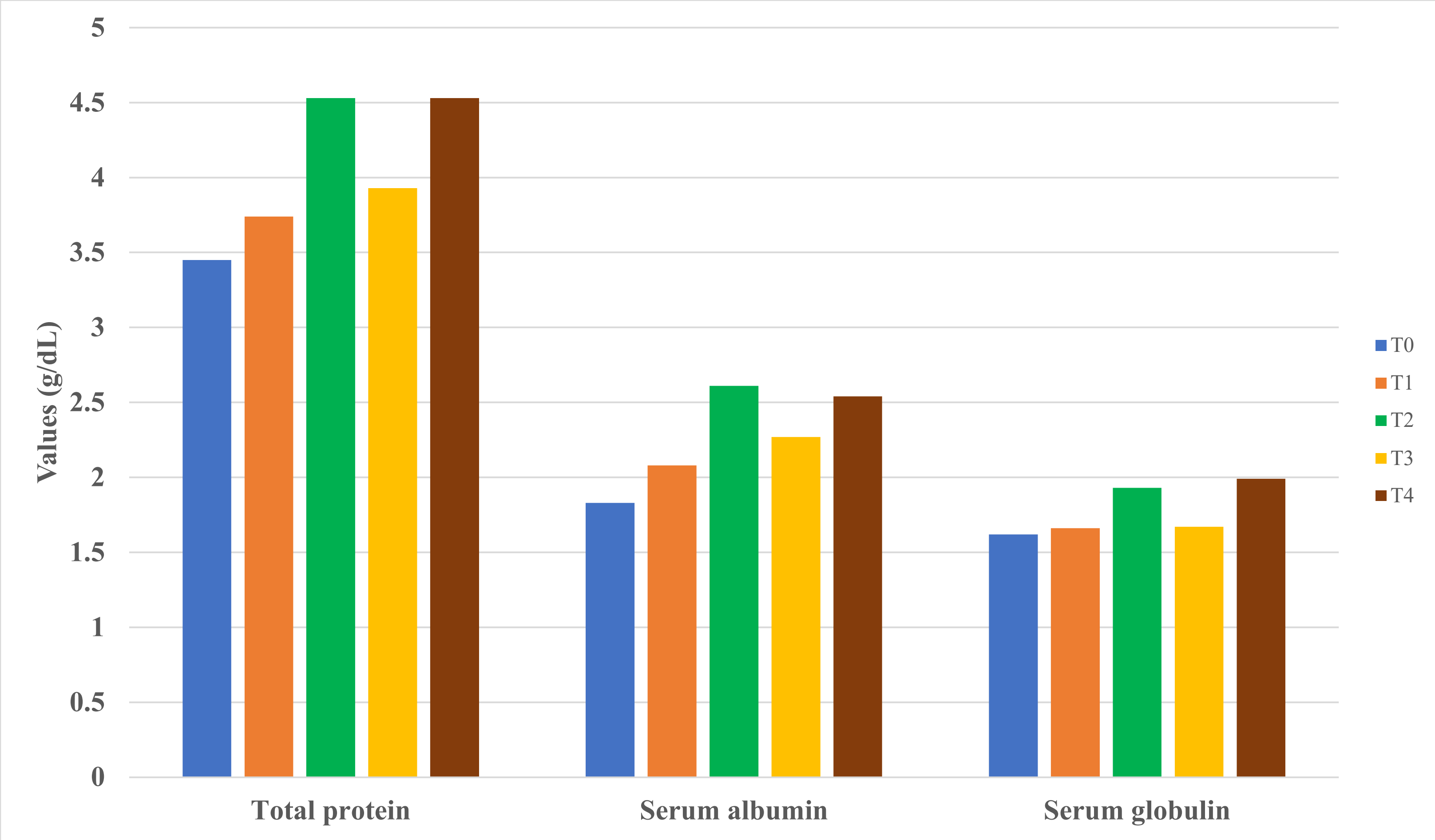

Figure 4.8 Average blood biochemical profile of birds

Table 4.20. which revealed non-significant (P>0.05) difference for serum albumin levels of experimental birds of different treatment groups.

Abdel-Raheem *et al*., (2005) and Wang *et al*., (2018) also came across with similar findings. Abdel-Raheem *et al*., (2005) found non-significant difference for serum albumin among the different dietary treatments provided with maize gluten meal, full fat soybean and basal diet. Wang *et al*., (2018) marked no significant (P>0.05) difference in the serum albumin content at two different levels (5% and 10%) of maize gluten meal.

However, Law *et al*., (2018) and Saleh *et al*., (2020) indicated significant (P<0.05) difference in serum albumin levels with inclusion of protease enzyme in the broilers diet.

Table 4.20 Analysis of variance of average serum albumin

SV	DF	SS	MSS	(F cal)
Treatments	4	1.253	0.313	1.856
Error	10	1.688	0.169	-
Total	14	-	-	-

Coefficient of Variation = 18.137

4.3.3 Globulin

Table 4.18 represents the serum globulin values of birds across different groups which are also illustrated in Figure 4.8. The average values for serum globulin levels for groups T_0, T_1, T_2,T_3 and T_4 were observed as 1.62 ± 0.12, 1.66 ± 0.07, 1.93 ± 0.10, 1.67 ± 0.02 and 1.99 ± 0.09 g/dl, respectively. Statistical analysis was conducted for this serum globulin parameter detailed in Table 4.21.

Results of statistical analysis concluded that the average serum globulin showed non-significant (P>0.05) difference among the average serum globulin levels of various treatment groups. Similar results were obtained by Kamel *et al.*, (2015), Wang *et al.*, (2018) and Amer *et al.*, (2021) for serum globulin content. Wang *et al.*, (2018) reported non-significant (P>0.05) difference in the serum globulin content at both 5% and 10% replacement level of maize gluten meal. Kamel *et al.*, (2015) and Amer *et al.*, (2021) evaluated the serum biochemical parameters and did not find significant (P<0.05) difference in serum globulin levels on protease enzyme supplementation.

On contrary, Abdel-Raheem *et al.*, (2005) recorded significantly lower values for serum globulins in the dietary treatments (fed with maize gluten meal and full fat soybean) compared with control group throughout the experimental period (starter and finisher phase).

Table 4.21 Analysis of variance of average serum globulin

SV	DF	SS	MSS	(F cal)
Treatments	4	0.348	0.087	2.758
Error	10	0.315	0.032	-
Total	14	-	-	-

Coefficient of Variation = 10.020

4.3.4 A:G ratio

Albumin : Globulin ratios of experimental birds across different groups are outlined in Table 4.18. Statistical analysis for the same displayed in Table 4.22 which highlights the non-significant (P>0.05) difference in albumin and globulin ratio among the different dietary treatment groups.

The average albumin and globulin ratio values for groups T_0, T_1, T_2, T_3 and T_4 were 1.13 ± 0.04, 1.26 ± 0.18, 1.35 ± 0.10, 1.36 ± 0.15 and 1.27 ± 0.09, respectively. On comparison, treatment group T_2 exhibited the maximum A:G ratio than other treatment groups. Kamel *et al.*, (2015) and Amer *et al.*, (2021) also observed non-significant (P<0.05) difference in A:G ratio on protease enzyme supplementation.

Table 4.22 Analysis of variance of albumin to globulin ratio

SV	DF	SS	MSS	(F cal)
Treatments	4	0.105	0.026	0.446
Error	10	0.589	0.059	-
Total	14	-	-	-

Coefficient of Variation = 19.037

4.7 <u>Nutrient digestibility</u>

Table 4.23 Average nutrient digestibility (%) of experimental birds from different groups

Digestibility %	Experimental groups				
	T_0	T_1	T_2	T_3	T_4
Dry matter	70.61 ± 0.50	69.66 ± 0.45	72.28 ± 0.23	71.67 ± 0.55	72.28 ± 0.51
Crude protein	67.89 ± 0.23	67.89 ± 0.70	72.02 ± 0.05	69.29 ± 0.56	70.88 ± 0.41
Ether extract	81.20 ± 0.17	82.17 ± 0.60	82.03 ± 0.65	81.47 ± 0.41	82.72 ± 0.33
Crude fibre	43.21 ± 0.25	42.38 ± 0.22	43.69 ± 0.22	42.58 ± 0.33	41.87 ± 0.26
Nitrogen free extract	61.86 ± 0.54	63.58 ± 0.09	63.26 ± 0.19	64.57 ± 0.01	63.75 ± 0.18

Table 4.23 and Fig. 4.9 describes the data related to nutrient digestibility of various dietary treatments received by the experimental birds across different groups.

4.7.1 Dry matter

Dry matter digestibility percentage for groups T_0, T_1, T_2, T_3 and T_4 were recorded as 70.61 ± 0.50, 69.66 ± 0.45, 72.28 ± 0.23, 71.67 ± 0.55 and 72.28 ± 0.51, respectively. Table 4.23 highlights the values obtained for dry matter digestibility which were numerically higher for the treatments T_2 and T_4 receiving 10 and 20% protein replacement through MGM, respectively along with protease enzyme.

The present findings corroborate with Cruz *et al*., (2004), Naik *et al*., (2017) and Saleh *et al*., (2020) for dry matter digestibility. Cruz *et al*., (2004) found significantly ($P>0.05$) higher dry matter digestibility of birds fed with dietary treatments incorporating maize gluten meal. Moreover, Naik *et al*., (2017) and Saleh *et al*., (2020) also observed increase in dry matter digestibility due to inclusion of protease enzyme in the diet.

4.7.2 Crude protein

The digestibility percentages of crude protein obtained were 67.89 ± 0.23, 67.89 ± 0.70, 72.02 ± 0.05, 69.29 ± 0.56 and 70.88 ± 0.41 for groups T_0, T_1, T_2, T_3 and T_4, respectively. It is observed from the Table 4.23 that group T_2 showed highest CP digestibility followed by, T_4, T_3, T_0 and T_1. Treatments T_2 and T_4 received MGM along with protease enzyme which ultimately reflected in the CP digestibility of the birds from these groups. Treatment T_3 provided with 20% MGM replacement which increased the CP digestibility of birds compared to treatment T_1 (10% MGM) and control group.

Findings of the present study are in support with Cruz *et al*., (2004), Freitas *et al*., (2011), Naik *et al*., (2017) and Saleh *et al*., (2020). Cruz *et al*., (2004) determined highest digestibility coefficients of crude protein in maize gluten meal incorporated diet. Freitas *et al*., (2011), Naik *et al*., (2017) and Saleh *et al*., (2020) found improvement in CP digestibility of birds on addition of protease enzyme.

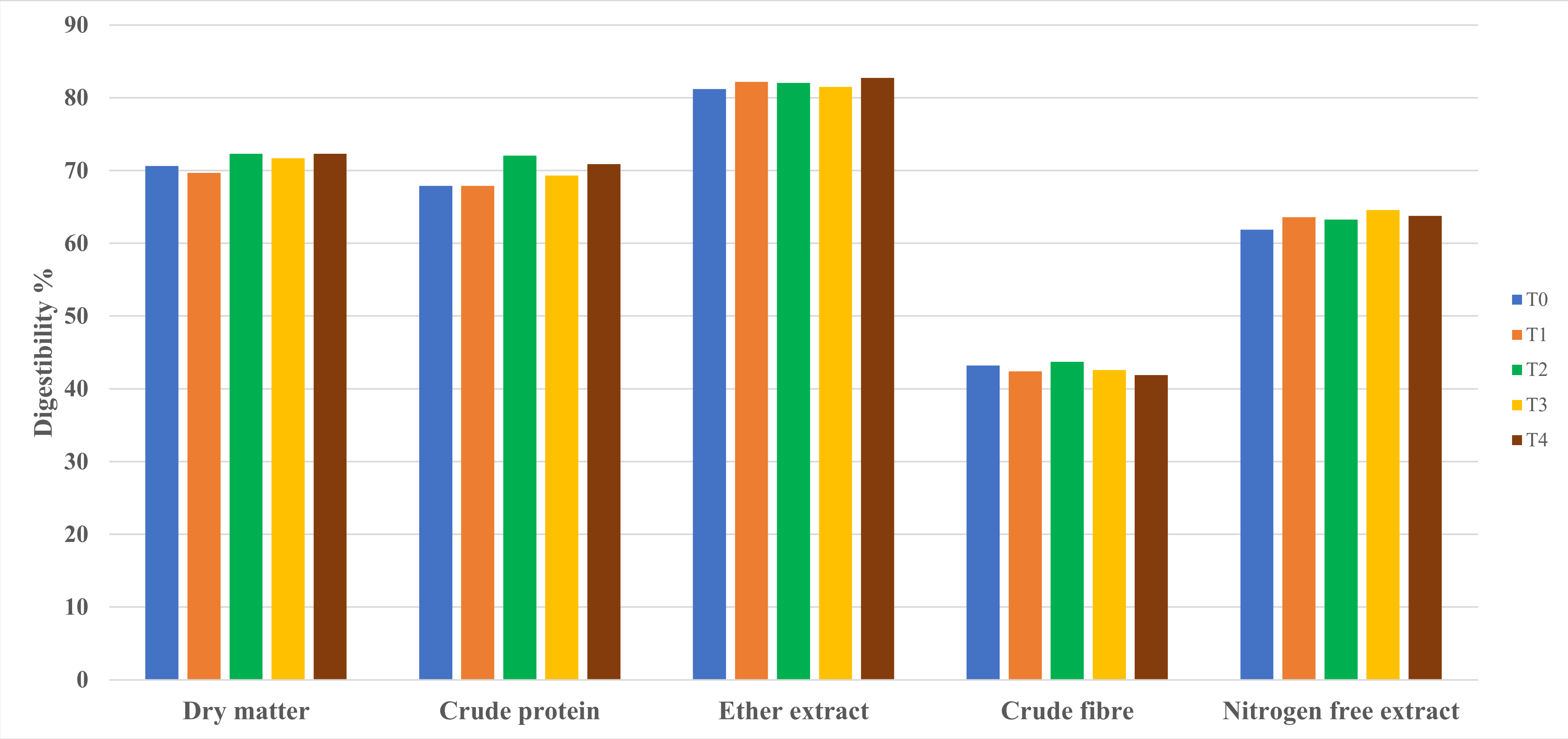

Figure 4.9 Average nutrient digestibility % of experimental birds from different groups

Thus, it can be concluded that both protease enzyme and maize gluten meal improved the CP digestibility of the experimental birds. It is also reflected in body weight gain and feed conversion ratio as protease supplemented group outperformed throughout the entire experimental period.

4.7.3 Ether extract

The data regarding digestibility percentages of ether extract for groups T_0, T_1, T_2, T_3 and T_4 were 81.20 ± 0.17, 82.17 ± 0.60, 82.03 ± 0.65, 81.47 ± 0.41 and 82.72 ± 0.33 respectively. Although, ether extract digestibility was highest in group T_4 (20% MGM + protease), it was found to be nearly similar across different treatment groups. However, Cruz *et al*., (2004) and Saleh *et al*., (2020) denoted significant effect in digestibility percent of ether extract with the inclusion of maize gluten meal and protease enzyme respectively.

4.7.4 Crude fibre

The digestibility percentages of crude fibre for groups T_0, T_1, T_2, T_3 and T_4 were 43.21 ± 0.25, 42.38 ± 0.22, 43.69 ± 0.22, 42.58 ± 0.33 and 41.87 ± 0.26 respectively. Numerically, the digestibility of crude fibre was marked to be higher in group T_2 followed by group T_0, T_4, T_3 and T_1.

4.7.5 Nitrogen free extract

The digestibility percentages of nitrogen free extract across the groups T_0, T_1, T_2, T_3 and T_4 were 61.86 ± 0.54, 63.58 ± 0.09, 63.26 ± 0.19, 64.57 ± 0.01 and 63.75 ± 0.18, respectively. The digestibility of nitrogen free extract was reported higher in group T_3 followed by T_4, T_1, T_2 and the control group.

4.8. Economics of broiler production

The economic analysis of broiler production for the present study was based on the prevailing market prices for inputs, as outlined in Table 4.24. To evaluate the profitability of broiler production which was one of the objectives of this study, key factors such as day-old chick expenses, feed costs, vaccination and medication, labour, miscellaneous costs, and revenue from the sale of broiler birds were consistently considered across all experimental groups.

Apart from standard basal diet of control group, other dietary treatment groups received Maize gluten meal and Protease enzyme which were priced at Rs. 52/kg and Rs. 320/kg, respectively. Cost of soybean meal was Rs. 37/kg. Feed cost of different experimental groups varied slightly from each other due to different inclusion levels of maize gluten meal and protease enzyme. Total feed cost per bird was varied for pre-starter, starter and finisher due to big differences in their feed intake.

Net production cost (including chick cost, total feed cost and miscellaneous cost) per bird by the end of experimental trial (42^{nd} day) was Rs. 191.33, Rs. 191.40 Rs. 192.68, Rs. 190.46 and Rs. 193.22 for the experimental groups T_0, T_1, T_2, T_3 and T_4 respectively.

Considering a selling price of Rs. 150 per kg on a live weight basis, per bird net profit was calculated for treatment groups T_0, T_1, T_2, T_3 and T_4 which resulted in Rs. 113.32, Rs. 110.10, Rs. 119.02, Rs. 111.79 and Rs. 116.23, respectively which shows that highest net profit observed in group T_2. While, profitability analysis considering net profit/kg live body weight produced the figures such as Rs. 55.79, Rs. 54.77, Rs. 57.27, Rs. 55.47 and Rs. 56.34 for the groups T_0, T_1, T_2, T_3 and T_4, respectively which determined the maximum net profit/kg live body weight was noticed in group T_2, followed by T_4, T_3, T_0 and T_1.

The percent profit per kg live body weight when compared with corn-soya based control diet, it was found 2.65% increase in group T_2, followed by T_4 (0.98%), both receiving MGM protein at 10% and 20% replacement level with 200 and 300 mg/kg protease enzyme, respectively.

This indicates that the 10% protein replacement by maize gluten meal with inclusion of protease enzyme in broilers diet was economically beneficial as compared to the control group, 10% replacement and treatments groups without protease. Furthermore, incorporating maize gluten meal along with protease enzyme improved performance of broilers and increased the profitability as well.

The outcomes of this economic assessment are in consistent with the study carried out by El-Deek *et al.* (2009), who commented that the best economic efficiency was observed with inclusion of 25% MGM by replacing soybean meal protein compared to 0, 75 and 100% inclusion, with or without phytase supplementation in the broilers diet. Giannenas *et al*., (2017) and Tripathi *et al*., (2020) pointed out that protease inclusion reduced the feed cost and gave higher economic returns compared to other treatment groups. Whereas, Amer *et al*., (2021) recorded non-significant effect of protease supplementation on economic efficiency.

Table 4.24 Economics of broiler production at the end of six weeks

Parameter	Groups				
	T0	T1	T2	T3	T4
Chick cost (Rs.)	44.00	44.00	44.00	44.00	44.00
Feed Intake (kg)					
Pre-starter	0.090	0.095	0.089	0.094	0.091
Starter	0.763	0.769	0.766	0.754	0.781
Finisher	2.236	2.227	2.260	2.234	2.265
Total feed consumed (kg)	3.089	3.091	3.115	3.082	3.137
Feed price per kg (Rs.)					
Pre-starter	42.22	42.54	42.61	41.88	41.98
Starter	44.07	43.92	43.99	44.05	44.15
Finisher	44.69	44.72	44.78	44.46	44.56
Feed cost (Rs.) / Bird					

Pre-starter	3.79	4.04	3.79	3.93	3.82
Starter	33.62	33.77	33.69	33.21	34.48
Finisher	99.92	99.59	101.20	99.32	100.92
Total feed cost per bird (Rs.)	137.33	137.40	138.68	136.46	139.22
Miscellaneous cost (Rs.) per bird	10	10	10	10	10
Net cost of production (Rs.) per bird	191.33	191.40	192.68	190.46	193.22
Body weight at the end of six week (g)	2031.15	2010.68	2078.83	2015.78	2063.00
Body weight gain at the end of 6 week (g)	1986.33	1965.87	2034.03	1975.17	2017.73
Cost of retail sale / kg live body weight (Rs.)	150	150	150	150	150
Return of sale of bird (Rs.)	304.65	301.50	311.70	302.25	309.45
Net profit per bird (Rs.)	113.32	110.10	119.02	111.79	116.23
Net profit / kg live body weight (Rs.)	55.79	54.77	57.27	55.47	56.34
Percent profit / kg live body weight (Rs.) as compared to control group	-	-1.82	2.65	-0.57	0.98

Table 4.25 Overall performance of birds at the end of six week

Parameters	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
Initial body weight (g)	44.84 ± 0.84	44.75 ± 0.19	44.80 ± 0.03	43.63 ± 0.87	45.32 ± 0.38	NS
Final body weight (g)	2031.15^{bc} ± 15.66	2010.68^{c} ± 6.12	2078.83^{a} ± 10.14	2015.78^{c} ± 12.22	2063.06^{ab} ± 13.00	43.16
Total gain in body weight (g)	1986.33^{bc} ± 16.55	1965.87^{c} ± 6.14	2034.03^{a} ± 10.12	1975.17^{c} ± 9.66	2017.73^{ab} ± 12.62	42.01
Total feed consumption (g)	3090.18^{b} ± 11.72	3092.83^{b} ± 11.57	3117.03^{ab} ± 13.84	3086.95^{b} ± 18.25	3138.23^{a} ± 15.55	34.99
Total FCR	1.556^{a} ± 0.007	1.573^{a} ± 0.005	1.533^{b} ± 0.007	1.565^{a} ± 0.006	1.555^{a} ± 0.003	0.02

Carcass traits	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
Dressed weight (g)	1618.50^{bc} ± 10.40	1586.13^{c} ± 6.19	1757.17^{a} ± 14.80	1596.67^{c} ± 12.10	1642.33^{b} ± 4.80	37.62
Dressing %	75.91 ± 1.19	76.44 ± 0.47	76.98 ± 0.48	76.54 ± 0.62	74.14 ± 0.45	NS
Edible meat (g)	1462 ± 31.28	1406.33 ± 16.36	1568 ± 31.37	1448.33 ± 26.39	1515.67 ± 43.11	NS
Edible meat %	68.53 ± 0.86	67.77 ± 0.77	68.71 ± 1.50	69.43 ± 1.24	68.40 ± 1.57	NS
Breast meat (g)	541.50 ± 5.63	548.00 ± 4.18	566.50 ± 4.26	547.00 ± 8.84	552.17 ± 7.38	NS
Breast meat %	25.40 ± 0.42	26.41 ± 0.14	24.82 ± 0.11	26.23 ± 0.64	24.93 ± 0.40	NS
Giblet weight	118.00 ± 6.56	119.67 ± 6.83	100.67 ± 4.25	102.33 ± 5.06	123.00 ± 9.54	NS
Giblet weight %	5.53 ± 0.34	5.75 ± 0.36	4.41 ± 0.19	4.90 ± 0.23	5.55 ± 0.46	NS
Liver weight %	2.19 ± 0.09	2.26 ± 0.16	1.65 ± 0.04	1.91 ± 0.09	2.25 ± 0.27	NS
Gizzard weight %	2.87 ± 0.22	3.04 ± 0.22	2.36 ± 0.11	2.39 ± 0.08	3.01 ± 0.25	NS
Heart weight %	0.46 ± 0.03	0.45 ± 0.02	0.50 ± 0.04	0.52 ± 0.05	0.48 ± 0.03	NS

Parameters	Experimental groups					CD
	T_0	T_1	T_2	T_3	T_4	
Serum Total Protein (g/dl)	3.45 ± 0.25	3.74 ± 0.17	4.53 ± 0.24	3.93 ± 0.23	4.53 ± 0.32	NS
Serum Albumin (g/dl)	1.83 ± 0.13	2.08 ± 0.22	2.61 ± 0.18	2.27 ± 0.24	2.54 ± 0.24	NS
Serum Globulin (g/dl)	1.62 ± 0.12	1.66 ± 0.07	1.93 ± 0.10	1.67 ± 0.02	1.99 ± 0.09	NS
A: G	1.13 ± 0.04	1.26 ± 0.18	1.35 ± 0.10	1.36 ± 0.15	1.27 ± 0.09	NS

Digestibility %	Experimental groups				
	T_0	T_1	T_2	T_3	T_4
Dry matter	70.61 ± 0.50	69.66 ± 0.45	72.28 ± 0.23	71.67 ± 0.55	72.28 ± 0.51
Crude protein	67.89 ± 0.23	67.89 ± 0.70	72.02 ± 0.05	69.29 ± 0.56	70.88 ± 0.41
Ether extract	81.20 ± 0.17	82.17 ± 0.60	82.03 ± 0.65	81.47 ± 0.41	82.72 ± 0.33
Crude fibre	43.21 ± 0.25	42.38 ± 0.22	43.69 ± 0.22	42.58 ± 0.33	41.87 ± 0.26
Nitrogen free extract	61.86 ± 0.54	63.58 ± 0.09	63.26 ± 0.19	64.57 ± 0.01	63.75 ± 0.18

Economics of broiler production					
Net profit per bird (Rs.)	113.32	110.10	119.02	111.79	116.23
Net profit / kg live body weight (Rs.)	55.79	54.77	57.27	55.47	56.34
Percent profit / kg live body weight (Rs.) as compared to control group	-	-1.82	2.65	-0.57	0.98

Summary and Conclusions

5. SUMMARY AND CONCLUSION

The study titled "Effect of partial replacement of soybean meal with maize gluten meal with or without protease enzyme supplementation on performance of broilers" involved 225 day-old "Vencobb 430Y" strain broiler chicks. They were randomly assigned to five treatment groups viz. T_0, T_1, T_2, T_3, and T_4, each containing 45 birds. Each treatment was further replicated into three groups, with 15 birds per replicates.

Birds from control group were fed with standard broiler diet as per BIS (2007). Birds from group T_1 and T_2 were provided with 10% protein replacement from the standard broiler diet by maize gluten meal, while birds from group T_3 and T_4 were provided with 20% protein replacement from the standard broiler diet by maize gluten meal. Furthermore, dietary treatments T_2 and T_4 were supplemented with protease enzyme at 200mg/kg and 300mg/kg diet, respectively. All the experimental diets were iso-caloric and iso-nitrogenous.

Various parameters were studied to check the effect of 10% and 20% replacement of soybean meal protein by maize gluten meal protein with or without supplementation of protease enzyme on the growth performance including weekly body weight gain, weekly feed intake, and weekly feed conversion ratio, carcass traits encompassing dressed weight, dressing percentage, edible meat percentage, breast meat weight, giblet weight, blood biochemical profile comprising of total protein, albumin, globulin and albumin : globulin ratio, nutrient digestibility, and economics of feeding broilers.

The average live body weight of birds at the end of sixth week was significantly higher in group T_2, followed by groups T_4, T_0, T_3 and T_1. It was also observed that the average weekly body weight gain of birds from the T_2 group was the highest, followed by groups T_4, T_0, T_3 and T_1. Both the average weekly live body weights and average weekly body weight gain showed statistically significant difference for various treatments. This indicated that inclusion of MGM with protease enzyme significantly improved the body weights and body weight gain of broiler birds. Also, 20% protein replacement in the broiler diet through MGM can be done as it did not show any detrimental effect on body weight.

Treatment group T_2 and T_4 had shown maximum average total feed intake as compared to the other groups. Remaining three treatments viz, T_0, T_1 and T_3 were similar pertaining to the feed intake which suggested that palatability of broiler diet was not affected by inclusion of maize gluten meal. Protease supplementation in the diet has improved feed intake which might be due to improvement in digestion of nutrients and its further utilization in the body.

The feed conversion ratio was significantly improved in group T_2 compared to the other groups. This showed that broilers can achieve better growth performance with optimal feed intake when 10% of the protein is replaced by MGM and supplemented with protease enzyme at the rate of 200mg/kg diet. This may be due to enhanced digestibility of nutrients in the diet.

Statistical analysis of various carcass traits noted non-significant difference in the carcass characteristics, including dressing percentage, edible meat percentage, breast meat percentage, giblet weight percentage, and the weights of the gizzard, heart, and liver expressed as a percentage of live weight. However, a significantly higher dressed weight of broilers was observed in group T_2 followed by T_0, T_4, T_1 and T_3 which may be due to the higher body weight of the birds from group T_2.

On blood biochemical analysis, it was evident that the levels of serum total protein, serum albumin, serum globulin and A:G ratio were at par among treatment groups however, the values were numerically higher in protease supplemented groups. Furthermore, it was seen that all the values remained within the normal range.

Nutrient digestibility studies performed during the last week of the experimental trial showed marked increase in dry matter and crude protein digestibility in birds consuming MGM diet with protease enzyme.

Economic analysis of broiler rearing demonstrated the maximum net profit from treatment group T_2 (Rs. 119.02), followed by T_4 (Rs. 116.23), T_0 (Rs. 113.32),

T_3 (Rs. 111.79) and T_1 (Rs. 110.10). Similarly, the net profit per kilogram of live body weight for experimental birds was calculated as Rs. 55.79, Rs. 54.77, Rs. 57.27, Rs. 55.47 and Rs. 56.34 for the groups T_0, T_1, T_2, T_3 and T_4, respectively. Moreover, experimental group T_2 found to increase profit/kg live body weight by 2.65% compared to control group, followed by group T_4 (0.98%). This suggested that replacement of protein up to 10% by maize gluten meal with supplementation of protease at 200 mg/kg diet was economically beneficial compared to standard broiler diet of control group.

It is evident that, incorporation of MGM to replace 10% protein from broiler diet with supplementation protease enzyme at 200mg/kg diet has improved the broiler performance and was found economical. However, up to 20% protein replacement of broiler diet through MGM can be done as it did not affect the overall growth and performance of experimental birds when compared with corn-soya based diets.

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Abstract

Appendix-G

THESIS ABSTRACT

a)	Title of the thesis (in Capital letters)	**:**	"EFFECT OF PARTIAL REPLACEMENT OF SOYBEAN MEAL WITH MAIZE GLUTEN MEAL WITH OR WITHOUT SUPPLEMENTATION OF PROTEASE ENZYME ON PERFORMANCE OF BROILERS"
b)	Full name of student	**:**	Bhujbal Prital Rangnath
c)	Name and address of MajorAdvisor	**:**	Dr. N. R. Karambele Assistant Professor, Department of Animal Nutrition, Mumbai VeterinaryCollege, Parel, Mumbai – 400 012
d)	Degree to be awarded	**:**	M. V. Sc.
e)	Year of award of degree	**:**	2025
f)	Major subject	**:**	Animal Nutrition
g)	Total number of pages in the thesis	**:**	79
h)	Number of words in the abstract	**:**	299
i)	Signature of Student	**:**	
j)	Signature, Name and address of forwarding authority (HOD / SH)	**:**	
k)	Signature of the Associate Dean	**:**	

Abstract

The 6 weeks study titled "Effect of partial replacement of soybean meal with maize gluten meal with or without protease enzyme supplementation on performance of broilers" involved 225 day-old broiler chicks. Chicks were randomly allocated to five groups viz. T_0, T_1, T_2, T_3, and T_4, each containing 45 birds, with 15 birds per replicate. Birds from control group were fed with standard broiler diet as per BIS (2007). Group T_1 and T_2 were provided with 10% protein replacement from the standard broiler diet by maize gluten meal while birds from group T_3 and T_4 were provided with 20% protein replacement by maize gluten meal. Group T_2 and T_4 were supplemented with protease enzyme at 200mg/kg and 300mg/kg diet, respectively. All the experimental diets were iso-caloric and iso-nitrogenous.

The average final live body weight (g), average gain in body weight (g), feed conversion ratio and feed intake by the experimental birds was significantly ($P<0.05$) higher in group T_2 and T_4 both receiving 10% and 20% protein replacement through MGM with protease enzyme as compared to other groups. T_2 showed consistently improved FCR and highest live body weight throughout the experimental period. Carcass parameters like dressing%, edible meat% and giblet weights except, dressed weight exhibited non-significant difference across different groups. Dressed weight was significantly higher in group T_2. Blood biochemical parameters like total protein, albumin, globulin, A:G ratio did not differ significantly for various dietary treatments. Dry matter and crude protein digestibility was improved in MGM with protease supplemented groups. Economically, group T_2 produced maximum i.e. 2.65% extra profit per kg live body weight as compared to control group. It is evident that, incorporation of MGM to replace 10% protein from broiler diet with supplementation of protease enzyme at 200mg/kg diet has improved the broiler performance with improved economics of feeding.

प्रबंध सारांश

१)	प्रबंधाचे नाव	:	मांसल कोंबड्यांच्या कार्यक्षमतेवर आंशिक प्रमाणात सोयाबीन बदलून मेझ ग्लूटेन मिलचा प्रोटीएज विकारांसहित किंवा त्याशिवाय होणारा परिणाम अभ्यासणे
२)	विद्यार्थ्याचे पूर्ण नाव	:	भुजबळ प्रितल रंगनाथ
३)	मार्गदर्शकाचे नाव आणि पत्ता	:	डॉ. एन. आर. करंबेळे सहाय्यक प्राध्यापक, पशुपोषणशास्त्र विभाग मुंबई पशुवैद्यकीय महाविद्यालय, परेल मुंबई- ४०० ०१२
४)	पदवी	:	एम.व्ही.एस.सी
५)	पदवी पुरस्काराचे वर्ष	:	२०२५
६)	मुख्यविषय	:	पशुपोषणशास्त्र
७)	प्रबंधाची एकूण पाने	:	७९
८)	सारांशाचे एकूण शब्द	:	२३९
९)	विद्यार्थ्याची स्वाक्षरी	:	
१०)	विभाग प्रमुखांचे नाव, स्वाक्षरी आणि पत्ता	:	
११)	सहयोगी अधिष्ठाता यांची सही मुंबई पशुवैद्यकीय महाविद्यालय, परळ, मुंबई-४०० ०१२.	:	

प्रबंध सारांश

सदर प्रयोगाद्वारे २२५ एकदिवसीय मांसल कोंबड्यांच्या कार्यक्षमतेवर आंशिक प्रमाणात सोयाबीन बदलून मेझ ग्लूटेन मिलचा प्रोटीएज विकारांसहित किंवा त्याशिवाय होणारा परिणाम सहा आठवड्यांकरिता अभ्यासला गेला. २२५ पिलांना प्रत्येकी ४५ याप्रमाणे टी$_{0}$, टी$_{१}$, टी$_{२}$, टी$_{३}$ आणि टी$_{४}$ अशा पाच गटांमध्ये विभागण्यात आले. नियंत्रण गटातील पक्ष्यांना BIS (२००७) च्या मानकांना अनुसरून खाद्य देण्यात आले. गट टी$_{१}$, टी$_{२}$ आणि टी$_{३}$, टी$_{४}$ मधील खाद्यातील अनुक्रमे १०% आणि २०% प्रथिने मक्याच्या ग्लूटेनद्वारे बदलण्यात आली. याशिवाय, टी$_{२}$ आणि टी$_{४}$ गटाला देण्यात आलेले खाद्य अनुक्रमे २०० मिग्रॅ/कि. आणि ३०० मिग्रॅ/कि. प्रोटीएज विकरांसह देण्यात आले. सर्व प्रायोगिक आहार समान ऊर्जा आणि समान प्रथिने असणारा होता.

प्रयोगाअंती असे लक्षात आले कि, टी$_{२}$ आणि टी$_{४}$ गटातील पक्ष्यांचे सरासरी अंतिम आठवड्यातील वजन, साप्ताहिक सरासरी वजनवाढ, खाद्य रुपांतारणाचे गुणोत्तर आणि खाद्य खाण्याचे प्रमाण इ. बाबींवर सांख्यिकीयरित्या लक्षणीय ($P<0.05$) फरक दिसून आला. संपूर्ण प्रायोगिक कालावधीमध्ये टी$_{२}$ गटातील पक्ष्यांचे वजन सर्वाधिक व सातत्याने सुधारित खाद्य रुपांतरण गुणोत्तर असल्याचे निदर्शनास आले. मांसल गुणधर्मांपैकी ड्रेसिंग वजन वगळता इतर (खाण्यायोग्य मांस, जीब्लेट वजन) कोणत्याही गुणधर्मांमध्ये फरक आढळला नाही. रक्तातील जैवरासायनिक घटकांपैकी सिरम एकूण प्रथिने, सिरम अल्ब्युमिन, सिरम ग्लोब्युलिन आणि सिरम अल्ब्युमिन : ग्लोब्युलिन गुणोत्तरामध्ये लक्षणीय फरक दिसून आला नाही. प्रोटीएज देण्यात आलेल्या प्रायोगिक गटातील पक्ष्यांमध्ये पोषक तत्वांपैकी शुष्क पदार्थ व कच्च्या प्रथिनांच्या पचनक्षमतेमध्ये कोणताही बदल आढळला नाही. आर्थिकदृष्ट्या, नियंत्रण गटाच्या तुलनेत टी$_{२}$ गटाने पक्ष्यांच्या प्रति किलो वजनामागे अधिक नफा दर्शविला. वरील प्रयोगाद्वारे असे स्पष्ट करण्यात येते कि, मांसल कोंबड्यांच्या आहारातील १०% प्रथिने मेझ ग्लूटेन मिलद्वारे बदलून त्यासमवेत २०० मिग्रॅ/कि. प्रोटीएज विकरांचा समावेश केल्यास अधिक नफा प्राप्त होतो.

Vita

VITA

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