INTRODUCTION

Poultry industry is the fastest growing sector of Indian agriculture and livestock production. The production of poultry meat has increased from 1,21,000 tones in 1971 (Narhari, 2000) to 2026,000 tons in 2009-10. Similarly, Madhya Pradesh also contributed 11,000 tons of broiler meat in 2009-10 (BAHS, 2010). At present, India is the fourth largest poultry meat producer globally (ICRA, 2011). It is giving employment to over 7 million people (Sathe, 1999). Broiler production forms a major segment of poultry industry with an annual growth rate of 12-15%. Poultry meat in recent years has become important and popular food for the non-vegetarian population. The annual per capita poultry meat consumption in India though increasing from 0.7-2.5 kg from 2001 to 2010 which remains one of the lowest, with vast gap even between recommended levels of 11 kg by National Institute of Nutrition of poultry meat per capita. This offers a tremendous opportunity for further integration and growth in industry. The domestic broiler meat demand is expected to grow at around 15-18%.

Feed costs for more than 60% to 70% of costs for a poultry farm with maize and soybean meal being the key feed ingredient. Adequate feed availability and feed prices are very crucial in sustainable operations of a poultry farm. The feed industry is evolving from being a fragmented sector to an organized one with integrated poultry players having their own feed mills though still sizeable production happens in home mixers and unorganized sector.

The feed cost can be reduced using locally available feed ingredients and agro-industrial byproducts. Soybean is a legume which is well-known for its beneficial properties. Soybean, contains 40 per cent high quality proteins, 20 per cent oil, dietary fiber and phytochemicals, as well as number of minerals, vitamins and has the best essential amino acid profile, but it is deficient in sulfur-containing amino acids (methionine, cysteine and cystine) and contains endogenous anti-nutrients including protease (trypsin) inhibitors. Because of their versatile applications soybeans have played important roles in feed industries.
Immediately post hatch, birds have low endogenous enzyme production (Noy and Sklan, 1995). Enzymatic digestion is initiated by pepsin and further aided by pancreatic proteases, peptidases, lipase and amylase (Troche, 2005). In birds, pancreatic and biliary outputs nearly complete by 7 days of age (Noy and Sklan, 1997). This is due to the fact that increased feed intake and decreased transit times, allowed for more efficient digestion of sugars and lipids. However, nitrogen digestion appeared to develop less rapidly and was only at 80% by day 7 (Noy and Sklan, 1995). Thus, low rates of nitrogen digestion results in insufficient proteolysis thereby making protein as the rate-limiting nutrient in early digestion.

The wide range of endogenous proteases synthesized and released in the gastrointestinal tract (GIT) is generally considered to be sufficient to optimize feed protein utilization (Le Heurou-Luron et al., 1993 and Nir et al., 1993). However, CP and amino acid (AA) digestibility reported for poultry indicate that valuable amounts of protein pass through the GIT without being completely digested (Parsons et al., 1997; Wang and Parsons, 1998; and Lemme et al., 2004). This undigested protein represents an opportunity for the use of supplemental exogenous proteases in broiler feeds to improve protein digestibility.

Research on the use of exogenous enzymes in broiler diets has been ongoing for decades; however, commercial use of enzymes are more recent. Keeping this in view, the present study was carried out to study the effect of protease supplementation in low protein diet on the performance of broilers with the following objectives.

1. Effect of different levels of protease supplementation on performance, nutrient utilization and carcass traits in broilers.

2. Effect of protease supplementation on economics of broiler production.
REVIEW OF LITERATURE

The present study was planned to study the effect of supplementation of protease enzyme in broiler diet with varying protein levels. The literature has been reviewed on following parameters.

2.1 Growth parameters - (Live body weight, body weight gain, feed consumption, feed efficiency ratio and performance index)

2.2 Nutrient utilization

2.3 Carcass Characteristics

2.4 Economics of feeding

2.1 Growth parameters

Yadav and Sah (2005) evaluated different levels of acid protease on performance of broiler fed reduced level of crude protein. Three hundred day-old commercial broilers were reared for 42 days on five dietary treatments comprising basal diet, diet with reduced crude protein, diet with reduced crude protein + 0.05 % protease, diet with reduced crude protein + 0.075 % protease, and diet with reduced crude protein + 0.1 % protease. Dietary protease inclusion at 0.075 % significantly improved average body weight gain in broilers.

Khan et al. (2006) conducted a 42-days trial to study the influence of exogenous enzymes supplementation to sunflower-corn based diet on digestive and performance traits in broilers with three treatments (Control, Hamecozyme and Rovabio supplemented diets) and three replicates (20 broiler chicks per replicate) per treatment. At the end of the trial, birds fed the enzymes supplemented diets ate more and grew faster (p<0.05) and had better feed conversion (p<0.05) than those fed the control diet.

Yu et al. (2007) evaluated corn soya based diet in cool and hot season by lowering protein levels in broiler feed and inclusion of commercially available enzyme preparation either mixture of protease and carbohydrases or protease alone. Both enzyme supplementations increased feed intake for all diets in cool season, but significantly decreased feed intake in the low
protein diet (LP) in hot season. Broiler chickens in the enzyme supplemented groups had better body weight gain as compared to those without supplementation, but only protease supplementation improved feed conversion. Addition of either single protease or cocktail of protease and carbohydrases to a maize soybean meal diet improved chicken growth.

Angel et al. (2011) supplemented exogenous monocomponent protease to corn-soybean meal diets of broilers from 7 to 22 d of age. A positive control diet (PC; 22.5% CP) and a low protein basal diet (20.5% CP) were formulated. Adding protease (0 mg/kg, LP0; 100 mg/kg, LP100; 200 mg/kg, LP200; 400 mg/kg, LP400; and 800 mg/kg, LP800). Birds fed the LP diets containing protease regardless of concentration grew in comparison with the birds fed PC diet except LP0. Feed conversion was impaired (P < 0.05) in birds fed the LP0 and the LP100 diets compared with those fed the PC diet, but no difference was found between birds fed the PC diet and those fed diets containing more protease (LP200, LP400, and LP800). The mono-component protease used in this trial, allowed the birds to overcome the negative effect on BWG and FCR that resulted from a 10% CP reduction in the diet.

Freitas et al. (2011) conducted two experiments to determine the effect of addition of exogenous protease to corn-soya based broiler diets. In the first experiment, the dietary treatments were a positive control, formulated with 3,050 and 3,150 kcal of ME/ kg and 22.5 and 20% CP in the starter and grower phases, respectively, and a negative control, formulated with a 4.4% reduction in ME and CP as compared with the positive control diets. A mono-component protease (75,000 protease/g) was added to the negative control diets at 0, 100, 200, 400, 800, and 1,600 ppm of feed. Broilers fed the positive control diet grew better and had a better feed-to-gain ratio (FE) than those fed the negative control diets, regardless of enzyme supplementation. Protease supplementation had no effect on BW; however, FE was improved in a quadratic manner as protease was increased. In second experiment, a factorial arrangement of 2 protein (7% difference in CP), 2 energy (3% difference in ME), and 2 protease (0 and 200 ppm) concentrations was used. Broilers fed high-protein and high-energy diets performed better (P ≤ 0.01) than those fed low-protein and low-energy diets.
Fru-Nji et al. (2011) tested the effects of novel serine protease RPA (supplemented at 15000, PROT/kg feed) on growth performance in two broiler experiments each arranged in a 2 x 2 factorial design. Each dietary treatment had 12 replicates. In experiment 1, birds were fed 12.7 MJ ME per kg iso-energetic diets in 2 phases. Each diet was fed without or with RPA (C or C + RPA, respectively) to either males or females. In experiment 2, two diets were fed in four treatments. Diet 1 (211 and 200g CP per kg feed in the starter and grower phases, respectively) was fed without or with RPA (NP or NP RPA, respectively). Diet 2 (200 and 190 g CP per kg feed in the starter and grower phases, respectively), was fed without or with RPA (LP or LP+ RPA, respectively). The FCR of Low Protein with protease was significantly better than Low Protein diet.

Kamran et al. (2011) observed the effect of lowering dietary crude protein (CP) on the performance of broilers from 1 to 35 d of age. Four experimental broiler diets were formulated. Diet A served as control with 23, 22 and 20% CP in starter, grower and finisher periods, respectively while in diets B, C and D, the CP was reduced to 22, 21 and 20% in starter; 21, 20 and 19% in grower and 19, 18 and 17% in finisher period, respectively. All diets were isocaloric within a phase (2,925, 3,075 and 3,125 kcal/kg ME for starter, grower and finisher periods, respectively). Weight gain, feed intake, feed conversion ratio (FCR), total ME intake and energy efficiency ratio were similar across treatments. Author concluded that, the dietary CP level could be reduced without harmful effects on the performance of broilers.

Mayorga et al. (2011) added mono-component protease in diets containing soybean subjected to various heat treatment periods. Body weight gain, feed intake, feed efficiency were recorded. The results indicated that feed supplementation with protease showed to be effective in improving bird performance and yield.

Widyaratne and Drew (2011) studied the effects of dietary protein level and protein digestibility on the growth performance of broilers from 1 to 35 d of age. Broiler chickens were fed 4 different ideal protein-
balanced, isocaloric diets in a 2 × 2 factorial design with 2 levels of protein [high protein HiPro; 20 and 18% or 200 and 180 g/kg] and low protein (LoPro; 18 and 16% or 180 and 160 g/kg) on d 1 to 14 and d 15 to 35, respectively. The HiDig diets were formulated using soybean meal and fishmeal, whereas the LoDig diets used wheat distillers dried grains with solubles and meat and bone meal as the primary protein sources. The results suggested that low-protein diets can support growth performance equal to high-protein diets when highly digestible ingredients are used.

Yamazaki Makoto et al. (2011) investigated the effects of dietary multi-enzyme complex supplementation on the performance and nitrogen excretion of broiler chicks. Low crude protein diets (19% CP) based on corn and soybean meal with commercial enzyme complex (cellulase, protease and pectinase) at the inclusion level of 0, 150 and 300 mg/kg diet, and a control diet (21%CP) were fed to 6-day-old male broiler chicks for 14 days. Results showed that body weight gain, feed consumption and feed efficiency were not affected by the dietary treatments. It was concluded that dietary CP content can reduce from 21% to 19% without affecting performance of chick.

Rada et al. (2013) evaluated the effect of addition of exogenous protease into low protein broiler diet on growth parameters (body weight and feed conversion ratio). A total of 990 one-day-old ROSS 308 broiler chickens were randomly divided into 9 experimental units of 110 chickens per each and located randomly to 3 different experimental treatments. The experiment was realized between the 10th and 35th day of age. The basal diet was based on wheat, corn and soybean meal. The dietary treatments were positive control diet (PC) contained normal crude protein (CP) level and two low protein diets. The level of CP in the low protein diets (LP) was reduced by 4 % compare to PC. First LP diet (LP0) was without and second LP diet (LP1) was with the supplementation of protease as 15,000 PROT PRO g feed. They concluded that, exogenous mono-component protease added into low protein broiler diet had no significant effect both on growth parameters.

Tempra et al. (2013) conducted a feeding trial to determine the effect of dietary protease supplementation on the performance of broilers in which 320 day-old broiler chicks were randomly assigned to four treatments
with eight replications of 10 birds. The dietary treatments for broilers were: Treatment 1, basal diet; Treatment 2, reduced crude protein and amino acid diet without protease supplementation; Treatment 3, treatment 2 diet with 125 ppm protease A; Treatment 4, treatment 2 diet with 200 ppm protease B. Results indicated that a 10% reduction in crude protein and amino acid content of the diet adversely affected the performance of broilers. Supplementation with protease A or B did not compensate for the reduction in nutrient content of the protease A or B did not compensate for the reduction in nutrient content of the broiler diets.

2.2 Nutrient utilization

Zanella et al. (1999) observed the effect of commercial enzyme cocktail containing xylanase, protease and amylase on performance of broilers fed corn-soya based diet. Digestibility of diets based on corn and soybean meal or soybeans treated by roasting or extrusion, with or without an enzyme supplementation were measured by "true" (Sibbald) methods, by analysis of excreta, and by analysis of ileal digesta. The amino acid (AA) digestibility of the diets was measured by analysis of the ileal contents. Enzyme supplementation improved overall CP digestibility by 2.9%, this improvement was not equal for all AA. The digestibility of Lysine, Methionine, and Arginine were not improved significantly by the enzyme supplementation; however, that of Valin was improved by 2.3% and that of Threonine was improved by 3.0%.

Jacob et al. (2000) conducted an experiment to investigate effect of dietary protein levels and supplementation of phytase and pentosanase in wheat soybean meal diet on performance and output of N and P in broilers. They concluded that addition of phytase and pentosanase in combination to reduce protein diet in male broilers significantly reduce daily N output. Retention of DM, N and P was highest in reduced protein diet supplemented with phytase and pentosanase in combination.

Ghazi et al. (2002) pretreated soybean meal with two different proteases: one was alkaline protease (isolated from bacillus species) and the other one was acid protease (isolated from Aspergillus). Incorporated soybean meal into the diets for broiler chicks revealed that acid protease treatment improved chick performance from 7 to 28 d of age and increased
apparent ileal nitrogen digestibility and apparent nitrogen retention across the whole digestive tract.

Faria Filho et al. (2005) evaluated low-protein diets based on the ideal protein concept for broiler chickens from 7 to 21 days of age reared at three environmental temperatures (low, thermo-neutral and high) and three crude protein levels in the diet (21.5, 20.0 and 18.5%). Low-protein diets showed decreased nitrogen excretion and birds reared at high environmental temperature showed lower nitrogen intake and excretion. It was concluded that the decrease in protein levels from 7 to 21 days of age contributed to lower nitrogen excretion in broiler chickens.

Yadav and Sah (2005) evaluated different levels of acid protease on performance of broiler fed reduced level of crude protein. Three hundred day-old commercial broilers were reared for 42 days on fine dietary treatments comprising basal diet, diet with reduced crude protein, diet with reduced crude protein + 0.05 % protease, diet with reduced crude protein + 0.075 % protease, and diet with reduced crude protein + 0.1 % protease. They reported significantly decreased crude protein digestibility of broiler starter and finisher diets when CP level in diet was reduced by 1%. The protease supplementation in reduced CP diet consistently and significantly improved the digestibility of CP in broiler starter and finisher diets. The crude fibre digestibility was significantly improved as 0.1% in broiler starter diet and 0.075 % and 0.1% protease supplementation in broiler finisher diets.

Khan et al. (2006) conducted a 42-days trial to study the influence of exogenous enzymes supplementation to sunflower-corn based diet on digestive and performance traits in broilers with three treatments (Control, Hamecozyme and Rovabio supplemented diets) and three replicates (20 broiler chicks per replicate) per treatment. Results showed that enzymes supplementation improved (p<0.05) apparent faecal digestibilities of dry matter, organic matter, crude protein and ether extract.

Yu et al. (2007) evaluated corn soya based diet lowering protein levels in broiler feed and inclusion of commercially available enzyme
preparation either mixture of protease and carbohydrase or protease alone. Addition of either single or cocktail of protease and carbohydrases and protease to a maize-soybean meal diet did not improve in-vivo digestibility of dry matter and protein, but significantly increased soybean meal hydrolysis rate and protein digestibility in the in-vitro assay as compared to the respective ingredients without supplementation.

Freitas et al. (2011) conducted two experiments to determine the effect of addition of exogenous protease to corn-soya based broiler diets. In the first experiment, the dietary treatments were a positive control, formulated with 3,050 and 3,150 kcal of ME/ kg and 22.5 and 20% CP in the starter and grower phases, respectively, and a negative control, formulated with a 4.4% reduction in ME and CP as compared with the positive control diets. A mono-component protease (75,000 protease/g) was added to the negative control diets at 0, 100, 200, 400, 800, and 1,600 ppm of feed. In second experiment, a factorial arrangement of 2 protein (7% difference in CP), 2 energy (3% difference in ME), and 2 protease (0 and 200 ppm) concentrations was used. Broilers fed high-protein and high-energy diets performed better ($P \leq 0.01$) than those fed low-protein and low-energy diets. Protease supplementation improved digestibilities of fat and CP ($P \leq 0.01$), regardless of dietary protein or energy concentration.

Romero and Plumstead (2012) conducted a series of studies to better understand the complex interactions of protease with different dietary ingredients and other enzymes. Two studies with 432 21-day or 288 42-day-old Ross-308 broiler males evaluated changes on the ileal energy contribution of substrates in response to xylanase and amylase without, or with protease in four broiler diets. They used a 2 x 2 x 3 factorial arrangement of treatments with two base grains (corn-soybean-meal; or wheat-soybean-meal diets); two fibrous protein ingredient levels (with, or without 10% corn-DDGS and 5% canola meal); and three enzyme levels. At 12 d or 32 d, three enzyme levels were applied: a negative control (NC); NC with xylanase from T. reesei and amylase from B. licheniformis; or NC with xylanase from T. reesei, amylase
from *B. licheniformis*, and protease from *B. subtilis* (Axtra XAP; Danisco Animal Nutrition, DuPont Industrial Biosciences). At 21 d or 42 d, birds were euthanised; ileal digesta was collected, pooled per cage, and analysed to determine the apparent digestibility of energy, starch, fat, and protein. The results revealed that, protease improved the protein digestibility and also contributed to increase the digestibility of other nutrients.

### 2.3 Carcass Characteristics

Cafe *et al.* (2002) fed nutritionally adequate diets to broilers with or without the addition of 0.1% Avizyme 1500 (xylanase, protease, and amylase). Each diet was fed to 48 pens of 65 male broilers. Five birds per pen were processed to determine dressing percentage and parts yield. Addition of Avizyme had no consistent effect on dressing percentage or yield of breast, thigh, or wing components. Abdominal fat, expressed as percentage of the carcass, was consistently increased by Avizyme supplementation, the differences being statistically significant at 42 and 49 d.

Kamran *et al.* (2004) studied the effect of decreasing dietary crude protein (CP) level on the performance of broilers in hot climatic conditions. Four isocaloric rations having CP 23 (control group), 22, 21 and 20%, with optimal amino acid balance fed significantly (P<0.01) higher weight gain on diets with CP 20 and 21%. Eviscerated carcass yield was significantly (P<0.05) higher for the group fed on diet with 20% CP. Breast meat yield, abdominal fat and composition of breast meat also remained unchanged. The overall picture of the study suggested that dietary protein level of broilers could be reduced from 23 to 20%, with beneficial effects on growth performance and carcass characteristics.

Hajati *et al.* (2009) studied the effects of a multi-enzyme (Endo-feed-W, which contained activities of enzymes including cellulase, hemicellulase, protease, alpha-amylase and alpha-galactosidase) supplementation on carcass characteristics of Cobb 500 broilers fed on corn soybean meal-wheat diets. The enzyme levels added to the diets were 0.00% (control) and 500mg kg⁻¹ DM. Enzyme supplementation significantly improved carcass and thigh percentages at 44 d of age.
Hassan et al. (2011) evaluated the effect of reducing dietary protein level without and with Avizyme (protease, xylanase and amylase) supplementation on performance and carcass characteristics of broiler. They formulated diets as high, medium and low protein (HCP, MCP and LCP). Protein levels of diet were 23, 21, 19% for starting, 21, 19, 18% for growing and 19, 18, 17% for finishing period. Results of carcass characteristics revealed highest dressing percentage value found in birds fed with HCP supplemented with avienzyme and highest abdominal fat percentage value found in birds fed with low protein diet with avinzyme.

Rada et al. (2013) evaluated the effect of addition of exogenous protease added into low protein broiler diet on carcass characteristic (carcass weight and yield). The dietary treatments were a positive control diet (PC) contained a normal crude protein (CP) level and two low protein diets. The level of CP in the low protein diets (LP) was reduced by 4 % compare to PC. First LP diet (LP0) was without and second LP diet (LP1) was with the supplementation of 15,000 PROT PRO g feed. It was concluded that the exogenous mono-component protease added into low protein broiler diet had no significant effect on carcass characteristics.

2.4 Economics of feeding

Cowieson and Adeola (2005) investigated the additive effects of xylanase, amylase, protease, and phytase in the diets of broiler chickens, using 1,152 growing broiler chicks (8 treatments with 12 replicate pens of 12 chicks). The birds were fed a corn/soybean-based negative control (NC) diet that was formulated to be nutritionally marginal in terms of metabolizable energy, Ca, and P. A nutritionally adequate positive control (PC) diet was fed for comparison. The NC diet was supplemented with phytase; a cocktail of xylanase, amylase, and protease (XAP); or a combination of phytase and XAP at 100 or 200 mg of each enzyme/kg. The use of a combination of XAP and phytase is highly effective in improving the performance of broilers, allowing for the formulation of lower-cost diets and contributing to the profitability of poultry production.
Kamran et al. (2011) studied the effect of lowering dietary crude protein (CP) on the performance of broilers from 1 to 35 d of age. Four experimental broiler diets were formulated. Diet A served as control with 23, 22 and 20% CP in starter, grower and finisher periods, respectively while in diets B, C and D, the CP was reduced to 22, 21 and 20% in starter; 21, 20 and 19% in grower and 19, 18 and 17% in finisher period, respectively. The data regarding the economic aspect of the use of the low CP diets showed that cost per kg of feed and cost per kg of live weight gain decreased as the CP content of the diet was reduced. Therefore, maximum economic returns were observed in groups fed the low CP diets and it clearly indicated that this approach is useful for economical broiler production.
MATERIAL AND METHODS

Location and Place of work

The proposed experiment was conducted in the department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.). The comprehensive programme of work was as follows:-

Experiment

The study was planned to see effect of different levels of protease supplementation on performance, nutrient, utilization and carcass traits in broilers.

Housing

The experimental chicks were reared in the battery brooder house. The battery brooders were cleaned, washed and disinfected by blow lamping and complete house was fumigated using formaldehyde and potassium permanganate four days prior to start of the experiment. Feeders and waterers were carefully cleaned with detergent. Artificial heat was provided to chicks during early period of growth using electric bulbs. In addition, room heaters were also used to maintain the room temperature as the experiment was conducted in spring season.

Duration of Experiment

Experiment was conducted for a period of five weeks. It was started on 13th February 2014 and terminated on 18th March 2014.

Experimental diet

Feed

The feed ingredients used in the experiment were maize, soybean meal and DORB. The mineral supplements used in diets were DCP, LSP, manganese sulphate, zinc sulphate, potassium iodide, sodium chloride, and vitamins (A, D₃, E, C, K and B complex). The ingredients were procured in one lot for whole experiment. They were grinded and screened properly to get uniform particle size for formulation of diets. The proximate composition of feed ingredients used in the experiment are presented in Table 1.
Table 1: Proximate composition of feed ingredients (%DM basis)

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Maize</th>
<th>DORB</th>
<th>SBM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>11.82</td>
<td>11.90</td>
<td>12.73</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>8.75</td>
<td>14.77</td>
<td>45.00</td>
</tr>
<tr>
<td>Ether extract (%)</td>
<td>3.20</td>
<td>01.48</td>
<td>1.02</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>1.90</td>
<td>20.56</td>
<td>10.01</td>
</tr>
<tr>
<td>NFE (%)</td>
<td>81.03</td>
<td>45.93</td>
<td>32.26</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>5.12</td>
<td>17.26</td>
<td>6.82</td>
</tr>
<tr>
<td>ME (Kcal/kg)</td>
<td>3340</td>
<td>2200</td>
<td>2250</td>
</tr>
</tbody>
</table>

Value analyzed in the laboratory

Computation of Ration

The feed ingredients were procured from the market and analyzed for proximate composition before formulation of diet. The experimental diets were formulated as per ICAR (1998) specifications and mineral mixture was added @ 3.0 % of the diet. All the three diets were iso-caloric containing 2800 kcal ME per kg for broilers which were be supplemented with protease at 100,200 and 300 g/ton.

The analyzed protein and ME values of feed ingredients were used for computation of rations. Composition of experimental broiler diets used in the study is given in.

Table 2: Composition of experimental broiler diets

<table>
<thead>
<tr>
<th>Feed Ingredients</th>
<th>Experimental Diets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁</td>
</tr>
<tr>
<td>Maize</td>
<td>59.50</td>
</tr>
<tr>
<td>Soya bean meal</td>
<td>37.0</td>
</tr>
<tr>
<td>DORB</td>
<td>-</td>
</tr>
<tr>
<td>Mineral Mixture</td>
<td>3.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.5</td>
</tr>
<tr>
<td>Vitamin B Complex</td>
<td>+</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Crude Protein</td>
<td>22</td>
</tr>
<tr>
<td>Energy (kcal ME/kg diet)</td>
<td>2800</td>
</tr>
</tbody>
</table>
Mineral Mixture

3.0 kg mineral mixture was added in broiler diet. The detailed composition of mineral mixture is presented in Table 3.

Table 3: Composition of mineral mixture

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicalcium phosphate</td>
<td>57.18</td>
</tr>
<tr>
<td>Lime stone powder</td>
<td>28.61</td>
</tr>
<tr>
<td>Common salt</td>
<td>12.52</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>1.34</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>0.33</td>
</tr>
<tr>
<td>Potassium iodide</td>
<td>0.03</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Dietary treatments

Different dietary treatments which were used in the study are described as below:

- **T<sub>1</sub>** - Standard diet as per ICAR (1998) feeding standard (22% CP and 2800 kcal ME/kg diet)
- **T<sub>2</sub>** - 20% CP and 2800 kcal ME/kg diet (control diet)
- **T<sub>3</sub>** - T<sub>2</sub> + Protease enzyme @ 100g/ton of feed.
- **T<sub>4</sub>** - T<sub>2</sub> + Protease enzyme @ 200g/ton of feed.
- **T<sub>5</sub>** - T<sub>2</sub> + Protease enzyme @ 300g/ton of feed
- **T<sub>6</sub>** - 18% CP and 2800 kcal ME/kg diet (control diet)
- **T<sub>7</sub>** - T<sub>6</sub> + Protease enzyme @ 100g/ton of feed.
- **T<sub>8</sub>** - T<sub>6</sub> + Protease enzyme @ 200g/ton of feed.
- **T<sub>9</sub>** - T<sub>6</sub> + Protease enzyme @ 300g/ton of feed.
Experimental birds

A total of 270 day old broiler chicks duly vaccinated against Marek’s disease were purchased from reputed Hatchery at Jabalpur. Out of which, 216 chicks were selected for experiment. During the experiment, all the chicks were vaccinated against Ranikhet disease (F1 strain) on 7th day. Then on 14th day chicks were vaccinated against IBD. On 28th day, all the chicks were immunized by giving booster dose against Ranikhet disease (Lasota strain) through intra-ocular route.

Experimental design

The design of the experiment was completely randomized design. All the day old broiler chicks were individually weighed at the start of the experiment and 216 birds of identical weight were selected. The chicks were randomly assigned to various groups so that weight of the chicks in any two groups did not differ significantly. Overall, there were nine dietary treatments. Each treatment consisted of three replicates of eight chicks each.

Feeding and Watering

The feed was offered ad-libitum in linear chick’s feeders. Aluminum plates of appropriate size and small tin boxes were used in each cage to offer water during early weeks. Due care was taken so that the chicks reach the feeder and waterer in the first week of age. Later in the experiment, large size feeders and waterers were attached to each cage in opposite direction. All-mash system of feeding was practiced during the experiment.

Fresh and clean drinking water was made available to birds all the time. Thus, in the entire study uniform condition of housing, brooding, feeding and watering was maintained for all the groups of the experiment.

Measurements and Observations

The following observations were recorded during the experimental period:

1. **Performance of broilers:**

   (a) **Body Weight:** The birds were weighed individually on weekly basis to know the body weight gain of broilers till five weeks of age. Weight gain in different groups of broilers were calculated on weekly basis considering the body weights of broilers, recorded during different intervals.
(b) **Feed intake:** Weekly feed consumption of broilers was recorded replicate wise on the basis of feed offered and left over feed recorded at the end of that week. During metabolic trial, separate record of feed consumption and left over feed was maintained to know the actual quantity of feed consumed by the bird in a particular group.

(c) **Feed efficiency ratio (FER):** To calculate FER, the body weight gain and feed consumption in each week of experiment were used and calculated using following formula:

\[ FER = \frac{\text{Body weight gain (g)}}{\text{Feed consumption (g)}} \]

(d) **Performance index (PI):** It was calculated as per the formula proposed by Bird (1955).

\[ \text{PI} = \text{Body weight gain (g)} \times \text{FER} \]

2. **Nutrient Utilization:** The utilization of nutrients in terms of energy, nitrogen and minerals from different diets was studied by conducting a metabolic trial. It was conducted on 5\(^{th}\) week of the experiment.

3. **Carcass traits:** To study the carcass traits, two broilers in each replicate were slaughtered on termination of experiment. Broilers were kept off feed for twelve hour before slaughter. During this period, they were provided clean and fresh drinking water *ad-libitum*. Before slaughter, each broiler was weighed and then by giving severe cut to the jugular vein it was killed and then allowed to bleed completely. For complete bleeding, birds were hanged in inverted position on the iron rails. After complete bleeding, weight was recorded. The weight was again recorded after manual defeathering using hot water (50-55\(^{\circ}\) c).

\[ \text{Dressed weight} = \text{Live weight} - \text{Weight loss as blood, feathers, head, shank and wing tips} \]

After recording the dressed weight, a horizontal cut was applied posterior to keel bone. Breast was pushed forward to expose the viscera which was then pulled out. Weight of carcass was recorded again. Various visceral organs like liver, heart, giblet, gizzard and pancreas were weighed. The eviscerated weight was then recorded as follows:

\[ \text{Eviscerated weight} = \text{Dressed weight} - \text{Weight of viscera} \]
Various processing losses such as blood, head, feathers, shank, fat and wing tips were recorded during the study.

4. **Gross examination of organs:** The organs (liver, heart, gizzard and pancreas) collected at the time of slaughter were used for examination of any gross abnormal changes.

5. **Mortality:** During the experimental trial number of death of broilers were recorded. The within the replicate was considered for calculating feed intake of the birds. During the experimental trial, dead birds were sent for post-mortem to the Department of Veterinary Pathology. Separate record of post-mortem report was maintained during the entire experimental period.

6. **Economics of production:** The feed cost per Kg body weight gain was calculated for each dietary treatment using average value of three replicates per treatment. The cost was calculated using existing market prices of feed ingredients.

7. **Analytical method:**

   (a) **Chemical**

   Feed ingredients as well as diets used in the study and excreta collected during the metabolic trial were analyzed for proximate composition using AOAC (2012). While, samples of feed and excreta were used for estimation of Ca and P content using method described by Talpatra *et al.* (1940).

   (b) **Statistical Analysis**

   Statistical analysis of the data was done by using analysis of variance using completely randomize design as per Snedecor and Cochran (1995). Differences among the treatments were tested for significance by Duncan’s Multiple Range Test (1955).
RESULTS

4.1 Performance of broilers

4.1.1 Effect of different level of protease enzyme in low protein (20%) diet on performance of broiler production (0-5 weeks)

The treatments means of their body weight gain indicate maximum and significantly (p<0.05) higher weight gain in broilers assigned in T1 (22% CP +without enzyme) diets and was followed by T5,T4,T3 and T2 having 20%CP+with 300gm/ton protease enzyme, 20%CP+with 200gm/ton protease enzyme, 20%CP+with 100gm/ton protease enzyme and 20%CP+without enzyme, respectively is presented in the Table 4. Among the treatment groups, significantly higher body weight gain was observed in higher level of enzyme added T5 diet. Treatment group T2 and T3 didn't differ significantly however, body weight gain of T2 was higher than that of T3. Similarly the body weight gain of T5 were significantly higher than the groupT4. The feed intake was also highest in broilers assigned to T1 diet followed by T5, T4,T3 and T2. Significantly (p<0.05) lower feed intake was registered in a groups assigned to T2 and diet. The FER was highest in broilers assigned to T1 followed by T5,T4,T3,T2. Significantly (p<0.05) lower FER was register in among treatment groups T2. The PI was maximum and significantly higher in broilers allotted T1 diet. It was minimum in the groups assigned to T2 diet. Among the treatment groups, significantly higher FER and PI was observed in higher level of enzyme added T5 diet.

Table 04: Effect of different level of protease enzyme in low protein (20%) diet on performance of the broilers (0-5 weeks)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight gain(g)</th>
<th>Feed intake (g)</th>
<th>FER</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1454±7.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2052±4.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1030.20±11.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T2</td>
<td>1121.31±10.41&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1815±4.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.61±0.03&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>684.96±11.81&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T3</td>
<td>1175.33±16.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1810.33±9.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.59±0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>657.26±22.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T4</td>
<td>1188±13.54&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1841.66±2.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65±0.09&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>766.06±19.13&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T5</td>
<td>1230±12.97&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1865±6.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66±0.06&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>811±17.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*means bearing different superscript differ significantly (p<0.05)
4.1.2 Effect of different level of protease enzyme in low protein (18%) diet on Performance of broiler production(0-5 weeks)

The treatments means of their body weight gain indicate maximum and significantly (p<0.05) higher weight gain in broilers assigned in T₁ (22% CP +without enzyme) diets and was followed by T₉, T₈, T₇, T₆ having 18%CP+with 300gm/ton protease enzyme, 18%CP+with 200gm/ton protease enzyme, 18%CP+with 100gm/ton protease enzyme 18%CP+without enzyme, respectively is presented in the Table 5. Among the treatment groups significantly higher body weight gain was observed in higher level of enzyme added in T₉ diet. Treatment group T₆ and T₇ didn't differ significantly however, body weight gain of T₇ was higher than that of T₆, similarly the body weight gain of T₉ were non -significantly higher than the group T₈. The feed intake was also highest in broilers assigned in T₁ diet followed by T₉, T₈, T₇ and T₆. Significantly (p<0.05) lower feed intake was registered in a groups assigned T₆ diet. The FER was highest in broilers assigned in T₁ followed by T₉,T₈, T₇ and T₆. Significantly (p<0.05) lower FER was registered in among treatment groups T₆. The PI was maximum and significantly higher in broilers allotted T₁ diet. It was minimum in the groups assigned T₆ diet. Among the treatment groups significantly feed intake, FER and PI was observed in higher level of enzyme added in T₉ diet.

Table 05: Effect of different level of protease enzyme in low protein (18%) diet on performance of the broilers (0-5 weeks)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Weight gain(g)</th>
<th>Feed intake (g)</th>
<th>FER</th>
<th>PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>1454±7.57</td>
<td>2052±4.09</td>
<td>0.70±0.03</td>
<td>1030.20±11.81</td>
</tr>
<tr>
<td>T₆</td>
<td>918±8.32</td>
<td>1820±29.05</td>
<td>0.49±0.01</td>
<td>453.26±14.18</td>
</tr>
<tr>
<td>T₇</td>
<td>970.66±6.56</td>
<td>1835.66±18.62</td>
<td>0.53±0.01</td>
<td>528.7±0.45</td>
</tr>
<tr>
<td>T₈</td>
<td>1010±20.03</td>
<td>1870±15.27</td>
<td>0.55±0.01</td>
<td>560.96±24.26</td>
</tr>
<tr>
<td>T₉</td>
<td>1046.66±34.80</td>
<td>1880±69.32</td>
<td>0.56±0.01</td>
<td>591.73±24.14</td>
</tr>
</tbody>
</table>

*means bearing different superscript differ significantly (p<0.05)
4.2 Nutrients utilization

4.2.1 Effect of different level of protease enzyme in low protein (20%) diet on nutrients utilization in broiler production (0-5 weeks)

The treatment means indicated that maximum and significantly (p<0.05) higher dry matter retention was observed in broilers assigned T5 diet which was statistically similar with T4 diet is furnished in the Table 6. It was minimum and significantly (p<0.05) lower in groups assigned T2 and T3 diets. The maximum and significantly (p<0.05) higher crude protein and NFE retention was observed in broilers assigned T5 diet which was statistically similar with those of T4, T3 and T1 diet. Crude fiber retention was maximum and significantly (p<0.05) higher in groups fed T5 and T4 diet. It was minimum and significantly (p<0.05) lower in groups assigned T2 diet. Retention of other nutrients ether extract, calcium and phosphorus did not differ significantly (P>0.05) in broiler diet as is presented in Table 6.

4.2.2 Effect of different levels of protease enzyme in low protein (18%) diet on nutrients utilization in broiler (0-5 weeks).

The treatment means indicated that maximum and significantly (p<0.05) higher dry matter, crude protein, NFE and calcium retention was observed in broilers assigned T1 diet is presented in Table 7. It was minimum and significantly (p<0.05) lower in groups assigned T6 diet. It was observed that addition of the protease enzyme on 18 % of protein in compare with control T1 (22%CP+without enzyme), no significant effect was observed but when compare with T6 diet (18%CP+without enzyme), retention of dry matter, crude protein, NFE and crude fiber was observe in T9 and T8 diet (18%CP+with 300gm/ton protease enzyme, 18%CP+with 200gm/ton protease enzyme).
Table 06: Effect of different level of protease enzyme in low protein (20%) diet on nutrients utilization broilers (0-5 weeks)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DM</th>
<th>CP</th>
<th>EE</th>
<th>CF</th>
<th>NFE</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>72.06±0.29&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>64.03±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.11±0.44</td>
<td>41.95±0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.75±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.09±0.03</td>
<td>53.75±0.34</td>
</tr>
<tr>
<td>T2</td>
<td>70.29±0.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>60.17±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.46±0.41</td>
<td>40.70±0.28&lt;sup&gt;c&lt;/sup&gt;</td>
<td>79.13±0.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.08±0.07</td>
<td>53.41±0.35</td>
</tr>
<tr>
<td>T3</td>
<td>71.20±0.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.68±0.64&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>63.24±0.34</td>
<td>41.54±0.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>80.70±0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.61±0.03</td>
<td>53±0.35</td>
</tr>
<tr>
<td>T4</td>
<td>73.63±0.95&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>64.03±1.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62.94±0.49</td>
<td>43.64±1.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.36±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.75±0.03</td>
<td>53.02±0.34</td>
</tr>
<tr>
<td>T5</td>
<td>74.79±1.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.25±1.76&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.29±0.23</td>
<td>43.85±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.15±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.76±0.23</td>
<td>52.84±0.58</td>
</tr>
</tbody>
</table>

*means bearing different superscript differ significantly (p<0.05)

Table 07: Effect of different level of protease enzyme in low protein (18%) diet on nutrients utilization broilers (0-5 weeks)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>DM</th>
<th>CP</th>
<th>EE</th>
<th>CF</th>
<th>NFE</th>
<th>Ca</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>72.06±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.03±0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.11±0.44</td>
<td>41.95±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.75±1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.09±0.03</td>
<td>53.75±0.34</td>
</tr>
<tr>
<td>T6</td>
<td>67.22±0.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.07±0.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>62.75±0.29</td>
<td>39.88±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>76.19±0.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59.84±0.30</td>
<td>51.18±0.32</td>
</tr>
<tr>
<td>T7</td>
<td>67.80±0.88&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.50±0.91&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>62.98±0.28</td>
<td>40.89±1.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.64±0.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.90±0.33</td>
<td>51.91±0.33</td>
</tr>
<tr>
<td>T8</td>
<td>69.77±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.95±1.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.99±0.75</td>
<td>40.51±0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.05±0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.20±0.30</td>
<td>52.65±0.02</td>
</tr>
<tr>
<td>T9</td>
<td>69.76±0.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.99±1.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.69±0.25</td>
<td>40.59±0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.49±0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.52±0.19</td>
<td>52.80±0.35</td>
</tr>
</tbody>
</table>

*means bearing different superscript differ significantly (p<0.05)
4.3 CARCASS TRAITS OF BROILERS

4.3.1 Effect of different level of protease enzyme in low protein (20\%) diet on carcass yields (% live weight) of broilers (0-5 weeks)

Treatment means of the carcass yields showed that use of protease enzyme at higher doses did not differ significantly (P>0.05). There was no difference between enzyme and without enzyme groups. Non significantly maximum yield (dressed, eviscerated and drawn weights) was noted in broilers assigned T_5 diet while, it was minimum in those assigned T_1 diet is presented in Table 8. When the control diet was compared with low protein diet, the carcass yield was observed to be higher in enzyme added group T_5 (20\%CP+with 300gm/ton protease enzyme).

Table 08: Effect of different level of protease enzyme in low protein (20\%) diet on carcass yields (% live weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dressed weight (%)</th>
<th>Eviscerated weight (%)</th>
<th>Drawn weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_1</td>
<td>80.80±0.51</td>
<td>62.63±2.19</td>
<td>66.84±2.21</td>
</tr>
<tr>
<td>T_2</td>
<td>81.50±0.72</td>
<td>63.05±1.00</td>
<td>67.73±0.64</td>
</tr>
<tr>
<td>T_3</td>
<td>81.34±0.17</td>
<td>64.62±1.97</td>
<td>69.55±1.75</td>
</tr>
<tr>
<td>T_4</td>
<td>81.67±2.43</td>
<td>64.95±1.16</td>
<td>69.35±1.13</td>
</tr>
<tr>
<td>T_5</td>
<td>82.06±1.89</td>
<td>67.39±2.06</td>
<td>72.027±1.89</td>
</tr>
</tbody>
</table>

4.3.2 Effect of different level of protease enzyme in low protein (18 \%) diet on carcass yields (% live weight) of broilers(0-5 weeks).

Treatment means of the carcass yields showed that use of protease enzyme at higher doses did not differ significantly (P>0.05) influenced the carcass yield from broilers. Maximum yield non significant (Dressed, eviscerated and drawn weights) was noted in broilers assigned T_8 diet while, it was minimum in those assigned T_1 diet is presented in Table 9.
Table 09: Effect of different level of protease enzyme in low protein (18%) diet on carcass yields (% live weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dressed weight (%)</th>
<th>Eviscerated weight (%)</th>
<th>Drawn weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>80.80±0.51</td>
<td>62.63±2.19</td>
<td>66.84±2.21</td>
</tr>
<tr>
<td>T₆</td>
<td>81.20±0.81</td>
<td>64.37±1.76</td>
<td>68.91±1.67</td>
</tr>
<tr>
<td>T₇</td>
<td>81.21±1.19</td>
<td>64.08±0.91</td>
<td>68.68±0.71</td>
</tr>
<tr>
<td>T₈</td>
<td>81.90±0.69</td>
<td>66.02±1.47</td>
<td>70.40±1.07</td>
</tr>
<tr>
<td>T₉</td>
<td>81.33±0.80</td>
<td>64.11±1.44</td>
<td>70.28±1.99</td>
</tr>
</tbody>
</table>

4.4 Organ weight

4.4.1 Effect of different level of protease enzyme in low protein (20 %) diet on organ weight (% Dress weight) of broilers (0-5 weeks)

Treatment means of the carcass yields showed that use of protease enzyme at higher doses did not differ significantly (P>0.05). However, higher weight of heart was recorded in T₁ diet is presented in Table 10. Lower weight of heart was recorded in T₅ diet, higher weight of gizzard liver pancreas and giblet was observed in T₅ diets. The lowest weight of liver and pancreas was observed in T₂ diet.

Table 10: Effect of different level of protease enzyme in low protein (20%) diet on organ weight (% Dress weight ) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Heart</th>
<th>Gizzard</th>
<th>Liver</th>
<th>Spleen</th>
<th>Pancreas</th>
<th>Giblet</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>0.723±0.04</td>
<td>2.103±0.22</td>
<td>2.523±0.04</td>
<td>0.087±0.01</td>
<td>0.287±0.04</td>
<td>5.357±0.25</td>
</tr>
<tr>
<td>T₂</td>
<td>0.593±0.01</td>
<td>2.680±0.20</td>
<td>2.443±0.24</td>
<td>0.110±0.00</td>
<td>0.240±0.03</td>
<td>5.783±0.46</td>
</tr>
<tr>
<td>T₃</td>
<td>0.670±0.05</td>
<td>2.397±0.22</td>
<td>2.877±0.40</td>
<td>0.123±0.01</td>
<td>0.247±0.03</td>
<td>5.940±0.46</td>
</tr>
<tr>
<td>T₄</td>
<td>0.587±0.05</td>
<td>2.467±0.19</td>
<td>2.470±0.22</td>
<td>0.127±0.009</td>
<td>0.257±0.01</td>
<td>5.530±0.17</td>
</tr>
<tr>
<td>T₅</td>
<td>0.563±0.02</td>
<td>2.263±0.19</td>
<td>2.963±0.32</td>
<td>0.133±0.02</td>
<td>0.263±0.01</td>
<td>5.803±0.34</td>
</tr>
</tbody>
</table>
4.4.2 Effect of different level of protease enzyme in low protein (18%) diet on organ weight (% Dress weight) of broilers (0-5 weeks)

Treatment means of the carcass yields showed that use of protease enzyme at higher doses did not differ significantly (P>0.05). Higher weight of heart was recorded in T₁ diet is furnished in Table11. Lower weight of heart was recorded in T₉ diet. Higher weight of gizzard, liver, pancreas and giblet was observed in T₁ diets lowest weight of gizzard, liver, pancreas and giblet was observed in T₉ diets.

Table11: Effect of different level of protease enzyme in low protein (18%) diet on organ weight (% Dress weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Heart</th>
<th>Gizzard</th>
<th>Liver</th>
<th>Spleen</th>
<th>Pancreas</th>
<th>Giblet</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>0.723±0.04</td>
<td>2.103 ± 0.22</td>
<td>2.523±0.04</td>
<td>0.087 ±0.01</td>
<td>0.287 ± 0.04</td>
<td>5.357±0.25</td>
</tr>
<tr>
<td>T₆</td>
<td>0.630±0.04</td>
<td>2.450±0.04</td>
<td>2.550±0.06</td>
<td>0.150±0.02</td>
<td>0.273±0.02</td>
<td>5.537±0.10</td>
</tr>
<tr>
<td>T₇</td>
<td>0.650±0.07</td>
<td>2.760±0.29</td>
<td>2.237±0.42</td>
<td>0.167±0.003</td>
<td>0.280±0.01</td>
<td>5.760±0.35</td>
</tr>
<tr>
<td>T₈</td>
<td>0.613±0.09</td>
<td>2.437±0.29</td>
<td>2.413±0.15</td>
<td>0.173±0.003</td>
<td>0.293±0.003</td>
<td>5.473±0.264</td>
</tr>
<tr>
<td>T₉</td>
<td>0.537±0.02</td>
<td>3.53±0.30</td>
<td>2.693±0.06</td>
<td>0.130±0.02</td>
<td>0.300±0.03</td>
<td>5.037±0.69</td>
</tr>
</tbody>
</table>

4.5 Processing losses

4.5.1 Effect of different level of protease enzyme in low protein (20%) diet on organ weight (% Dress weight) of broilers (0-5 weeks)

Treatment means of the processing losses indicated that blood, shank, feather and wing tips losses were maximum non-significantly (P>0.05) higher in T₃ diet is presented in Table12. While, losses were minimum in higher level of enzyme added group was T₅ diet. The head weight was higher in T₂ diet.
Table 12: Effect of different level of protease enzyme in low protein (20%) diet on processing losses (% Live weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Blood</th>
<th>Feather</th>
<th>Head</th>
<th>Shank &amp; wing tips</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>3.747±0.40</td>
<td>7.763±1.22</td>
<td>2.907±0.20</td>
<td>5.487±0.22</td>
</tr>
<tr>
<td>T₂</td>
<td>3.593±0.35</td>
<td>6.370±0.18</td>
<td>3.097±0.13</td>
<td>5.330±0.36</td>
</tr>
<tr>
<td>T₃</td>
<td>4.523±0.23</td>
<td>6.840±0.68</td>
<td>2.737±0.17</td>
<td>5.540±0.27</td>
</tr>
<tr>
<td>T₄</td>
<td>4.030±0.44</td>
<td>6.670±0.62</td>
<td>2.753±0.08</td>
<td>5.427±0.22</td>
</tr>
<tr>
<td>T₅</td>
<td>3.667±0.42</td>
<td>6.523±0.26</td>
<td>2.717±0.02</td>
<td>5.020±0.33</td>
</tr>
</tbody>
</table>

4.5.2 Effect of different level of protease enzyme in low protein (18 %) diet on organ weight (% Dress weight) of broilers (0-5 weeks)

Treatment means of the processing losses indicated that blood loss was maximum but non-significantly (P>0.05) higher in T₇ diet having 18%CP+with 100gm/ton protease enzyme added is presented in Table 13. Minimum blood loss was observe in T₉ diet containing 18%CP+with 300gm/ton protease.

Table 13: Effect of different level of protease enzyme in low protein (18%) diet on processing losses (% Live weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Blood</th>
<th>Feather</th>
<th>Head</th>
<th>Shank &amp; wing tips</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>3.747±0.40</td>
<td>6.670±0.62</td>
<td>2.907±0.20</td>
<td>5.427±0.22</td>
</tr>
<tr>
<td>T₆</td>
<td>3.333±0.32</td>
<td>6.683±0.25</td>
<td>2.887±0.41</td>
<td>5.177±0.34</td>
</tr>
<tr>
<td>T₇</td>
<td>4.067±0.17</td>
<td>6.217±0.75</td>
<td>2.843±0.18</td>
<td>5.633±0.27</td>
</tr>
<tr>
<td>T₈</td>
<td>3.550±0.09</td>
<td>7.017±0.43</td>
<td>2.957±0.07</td>
<td>5.247±0.11</td>
</tr>
<tr>
<td>T₉</td>
<td>3.147±0.11</td>
<td>7.133±0.23</td>
<td>3.160±0.22</td>
<td>6.013±0.10</td>
</tr>
</tbody>
</table>
4.6 Economics of broiler production

4.6.1 Effect of different level of protease enzyme in low protein (20\%) diet on economics of broiler production

The cost of feed per kg weight gain was maximum in broilers assigned T_2 diet followed by those allotted T_3 diet. In both these groups, differences were maximum and significant (P<0.05) is presented in Table 13.

It was minimum in broilers offered T_1 which was statistically similar with those of T_4 and T_5 diets. While, total cost of feed for total weight gain was maximum in groups assigned diet T_1. It was significantly (P>0.05) lower in broilers assigned T_4 diet followed by T_3,T_5 and T_2. Between these groups difference were statistically non- significant.

Receipt obtained from sale of broilers was maximum and significantly higher (P<0.05) in groups assigned T_1 diet followed by T_5 ,T_4,T_3 and T_2. Among the treatment groups maximum receipt obtained from sale of broilers was T_5 diets containing 20\%CP+with 300gm/ton protease (Table 14).

The net return over feed cost per kg weight gain was maximum and significantly higher (P<0.05) in T1 diets followed by T_5 and T_4. Between these groups difference were statically non- significant. Minimum return was noticed in broilers offered T_2 diet (Table 14).

Total cost of feed for total weight gain was maximum and significantly higher (P<0.05) in groups assigned T_1 diet is presented in Table 14. It was followed by T_5,T_4,T_3 and T_2. As the enzyme level is low total cost of feed for total weight gain is less.
Table 14: Effect of different level of protease enzyme in low protein (20 %) diet on economics (%Live weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cost of feed/kg (Rs)</th>
<th>Cost of feed/kg b.wt gain (Rs)</th>
<th>Total cost of feed for wt. gain (Rs)</th>
<th>Gain (Rs)</th>
<th>Net return over feed cost /kg b.wt gain (Rs)</th>
<th>Net return over feed cost total b.wt gain (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₁</td>
<td>29.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.11±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.79±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.88±0.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.87±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.09±0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₂</td>
<td>26.89&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.36±0.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.21±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.30±1.10&lt;sup&gt;d&lt;/sup&gt;</td>
<td>22.63±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.08±1.33&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₃</td>
<td>26.98&lt;sup&gt;d&lt;/sup&gt;</td>
<td>44.17±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.53±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.25±0.70&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>23.82±0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.71±0.64&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₄</td>
<td>27.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.37±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.33±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79.46±0.92&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>26.62±0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.79±4.09&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>T₅</td>
<td>27.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.26±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.56±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.69±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.76±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.12±0.88&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means bearing different superscript differ significantly (p<0.05)
4.6.2 Effect of different level of protease enzyme in low protein (18%) diet on economics of broiler production

The cost of feed per kg weight gain was maximum in broilers assigned T$_6$ diet followed by those allotted T$_7$, T$_8$ and T$_9$ diet. In these groups difference were non-significant. It was minimum in broilers offered T$_1$ (Table 15). Between the treatments cost of feed per kg weight gain was maximum in broilers in T6 containing 18%CP+with out protease enzyme.

Receipt obtained from sale of broilers was maximum and significantly higher (P<0.05) in groups assigned T1 diet followed by T$_9$, T$_8$, T$_7$ and T$_6$ is presented in Table 15. In the enzyme added groups maximum receipt obtained from sale of broilers was in the 18%CP+with 300gm/ton protease(T$_9$), while the lowest was seen in the (T$_6$).

Total cost of feed for total weight gain was maximum and significantly higher (P<0.05) in groups assigned T$_1$ diet. When enzyme added in the groups the total cost of feed for total weight gain was influenced maximum and non-significantly higher (P<0.05) in groups assigned T9 diet (18%CP+with 300gm/ton protease enzyme) (Table 15), while the lowest total cost of feed for total weight gain was seen in T$_6$ diet.

The net return over feed cost per kg weight gain was maximum and significantly higher (P<0.05) in T$_1$ diet and it was minimum and (P<0.05) lower in broiler allotted T6 diet while, the net return over feed cost on total weight gain was maximum and significantly (P<0.05) higher in broilers offered T$_1$ diet and was minimum in groups assigned T$_6$ diet (Table 15).
Table 15: Effect of different level of protease enzyme in low protein (18 %) diet on economics (%Live weight) of broilers

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Cost of feed/kg (Rs)</th>
<th>Cost of feed/kg b.wt gain (Rs)</th>
<th>Total cost of feed for wt. gain (Rs)</th>
<th>Gain (Rs)</th>
<th>Net return over feed cost /kg b.wt gain (Rs)</th>
<th>Net return over feed cost total b.wt gain (Rs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>29.13±0.0^a</td>
<td>41.11±0.26^c</td>
<td>59.79± 0.11^a</td>
<td>98.88±0.52^a</td>
<td>26.87±0.25^a</td>
<td>39.09±0.57^a</td>
</tr>
<tr>
<td>T6</td>
<td>25.40±0.0^e</td>
<td>51.26±1.26^a</td>
<td>47.04±0.73^bc</td>
<td>62.42±0.56^bc</td>
<td>16.73±1.26^c</td>
<td>15.38±1.28^c</td>
</tr>
<tr>
<td>T7</td>
<td>25.49±0.0^d</td>
<td>46.80±0.75^b</td>
<td>45.41± 0.45^c</td>
<td>65.98±0.59^c</td>
<td>21.19±0.75^b</td>
<td>20.57±0.86^b</td>
</tr>
<tr>
<td>T8</td>
<td>25.58±0.0^c</td>
<td>46.13±1.07^b</td>
<td>46.22±0.09^bc</td>
<td>68.67±1.36^bc</td>
<td>21.86± 1.07^b</td>
<td>22.11±1.52^b</td>
</tr>
<tr>
<td>T9</td>
<td>25.67±0.0^b</td>
<td>45.45±1.15^b</td>
<td>47.56±1.78^b</td>
<td>71.17±2.36^b</td>
<td>22.54±0.95^b</td>
<td>23.61±1.31^b</td>
</tr>
</tbody>
</table>

Means bearing different superscript differ significantly (p<0.05)
DISCUSSION

The present experiment was conducted with the objective to study the effect of supplementation of protease enzyme in broiler diet with varying protein levels.

The parameters such as growth performance, feed consumption, feed efficiency ratio, carcass characteristics, nutrient utilization, and economics of broiler production were studied. The data obtained was statistically analyzed for interpretation of the results.

PERFORMANCE OF BROILERS

Incorporation of different levels of protease enzyme by decreasing protein up to 20 and 18 %. revealed that the body weight gain were significantly more in group receiving 22% protein than that of the diets containing 20 and 18% protein without enzyme and with enzyme with inclusion levels 100,200 and 300 gm per ton of feed. It was at par with group receiving control diet suplemented with 22% of protein without enzyme. The body weight gains were quite comparable for diet receiving 20% protein diet and 18% deficient diet without protease supplementation and with100,200 and 300 gm per ton of feed protease supplementation, respectively. However, the significantly higher body weight gain were observed in group receiving with 200 and 300 gm per ton of feed protease supplementation, indicated that the range of protease activity in this study may not have been great enough to produce a response or to enhance growth on protein deficient diet at higher level, since beginning period, rather simply reducing CP level at 20 and 18 percent with and without protease supplementation proved to be non beneficial in enhancing body weight gain of broilers (Table 4 and Table 5).

The findings corroborates with Yamazaki et al. (2011) as they concluded that dietary CP level could be reduced from 21 to 19% without affecting performance of broilers and also reported that commercial enzyme supplementation did not affect the body weight gain in broilers. Tempra et al. (2013) justified that supplementation of protease enzyme did not compensate for the reduction in protein content of the broiler diet. Kamran et al. (2004)
reporting significant increase in body weight gain fed on low protein diet with CP 20 to 21%. Ramesh et al. (2012) conducted a biological trial to study the effect of protease supplementation exogenously in low protein diet. Although there was numerical improvement in body weight and feed efficiency in protease supplemented group, it was statistically not significant, indicating supplementation of protease in nutritionally marginal low protein diet was not effective in improving the brooder performance of the turkey poults. Kamran et al. (2011) reduced protein level up to 17% in broiler diet without harmful effects when supplemented with limiting amino acid. Angel et al. (2011) found non-significant effects on birds fed low CP diet supplemented with protease enzyme on growth performance compared to standard CP diets. Frietas et al. (2011) also found non-significant effect of commercial mono-component protease supplementation on growth performance. Widyaratne and Drew (2011) concluded that low protein diets can support growth performance of broilers equal to high protein diet when highly digestible ingredients are used. Bertechini et al. (2009) reported increased true AA digestibility for soybean meal and Carvalho et al. (2009) reported increased true AA digestibility for corn, in the presence or absence of a mono-component protease. Walk et al. (2011) reported no effect on broiler performance with the addition of a protease.

The feed consumption was significantly more in control groups, 22% (CP) than treatment groups receiving 20 and 18% reduced protein diet respectively, may be to satisfy the nutrient requirement of birds, however it was comparable with 20% control diet receiving without protease enzyme and with protease enzyme indicated that supplementation of protease enhances feed consumption in normal course (Table 4). The protease enzyme supplementation in 18% protein reduced diet could not elevate the consumption of feed, thereby resulting reduced body weight gain also and (Table 5).

These results are in agreement with Yadav and Sah (2005) who indicated that the reduction of crude protein in diet increased the feed consumption of broilers while protease enzyme supplementation of the reduced crude protein diets generally decreased the feed consumption of
broilers. The increase in feed consumption of birds with reduction of crude protein in diet could be due to the tendency of the birds to eat more in order to compensate for lower protein diets. However decrease in feed consumption of birds with protease supplementation of reduced CP diets could be due to the exogenous protease, which significantly increased the digestibility of crude protein, as also evident in the present study or also amino acid availability by the enzyme inclusion (Yu et al., 2007). Significant improvement in feed consumption in control diet due to supplementation of protease are supported by Khan et al. (2006) who found that enzyme supplementation increased feed consumption compared to control diet.

The significantly better FER and PI was noticed in control group containing 22 % CP and non significant better FER was notice in diet receiving 20% and 18% less protein diet without protease enzyme and with protease enzyme. Among the treatment groups in 20% and 18% better FER and PI was noticed in group feed 300 gm per ton of protease enzyme, indicated better conversion of feed into bodyweight even on the reduced protein level diet, however effect could not be seen on higher level of protein reduction. But simply supplementation of protease in normal standard diet could enhance the performance of broiler in terms of FER and PI. Addition of protease enzyme in protein deficient diet could not proved to be beneficial in conversion of feed into meat (Table 4 and Table 5).

The results are in accordance with Kamran et al. (2004) who reported significantly improved FCR by decreasing CP level. The improvement in FCR in control diet with protease supplementation is supported by Freitas et al. (2011) reporting better FCR on protease in control diet, improvement in body weight gain and FCR with addition of protease are expected to occur through increase amino acid availability that can promote growth, supported by Yadav and Sah (2005), however they also reported depressed FCR with reduction in crude protein level. Rada et al. (2013) reported non significant effect of exogenous mono component protease added into low protein diet on body weight and FCR. Tempra et al. (2013) reported that protease supplementation could not improve FCR in low protein diet, suggesting the importance of evaluating the availability and levels of
substrate for enzyme in feed and matching them with specific types of enzyme activities to achieve the optimal response. Fru-Nji et al. (2011) tested that the FCR of low Protein supplemented with protease was significantly better than low protein diet without protease enzyme.

**Nutrient utilization**

The significantly higher dry matter utilization was seen in 20% protein reduced group with 300gm/ton protease supplementation (Table 6). The significantly higher dry matter utilization was seen control diet when it is compare with 18% protein reduced level supplemented with and without protease enzyme (Table 7). The results are in agreement with Olukosi et al. (2007) who found that DM digestibility was higher ($P < 0.05$) in positive control and when enzyme alone was supplemented in the diet. Khan et al. (2006) found that digestibility of DM was increased ($p < 0.05$) with supplementation of enzymes. Zanella et al. (1999), reported that supplementation of broiler diet with exogenous enzyme improved starch digestibility and consequently DM digestibility. They explained that the solubilization and disruption of grains endosperm cell walls by enzyme supplementation was primarily responsible for the improvement in digestibility. Jacob et al. (2000) found that retention of DM was highest in groups fed low protein diet supplemented with enzyme. However, Yu et al. (2007) found that addition of either single or cocktail of protease and carbohydrases and protease to a maize-soybean meal diet did not improve digestibility of dry matter. Barekatain et al. (2013) reported significant improvement in the CP digestibility coefficient of the birds was observed receiving 300 g DDGS/kg with addition of protease.

Crude protein retention was significantly higher in group fed 20% protein reduced diet with 300gm/ton enzyme supplementation when compared with $T_2$ diet. The CP utilization in control group was significantly higher than that of 18% low protein diet either protease supplemented or non supplemented group, indicated that effect of protease could not be seen in normal diet in improving CP utilization rather it worked better on CP reduced diet and exceptionally well on 20% protein reduced diet (Table 6). As it compare with 18% CP supplemented group with control diet there is significant utilization in control group (Table 7). This implies greater utilization
of protein when fed at lower level. These results are consistent with the findings of Angel et al. (2011) who found improvements in apparent CP digestibility of the protease-supplemented diets when compared with the positive control (PC) or low protein (LP0) diets. These effects were similar for all concentrations of protease supplementation. The lowest apparent CP digestibility was observed in birds fed the PC and LP0 diets. When compared with the LP0 diet, addition of the protease at any concentration increased apparent CP digestibility. Yadav and Sah (2005) found that the protease supplementation in reduced CP diet consistently and significantly improved the digestibility of CP in broiler starter and finisher diets.

On the other hand Freitas et al. (2011) found that addition of protease enzyme improves CP digestibility when added to the high-protein diets as compared with the low-protein diets. Whereas Yu et al. (2007) did not find improvement in digestibility of CP after addition of either single or cocktail of protease and carbohydrases and protease to a maize-soybean meal diet. Fru-Nji et al. (2011) results showed protease significantly increased ileal protein and energy digestibility. Yamazaki et al. (2011) investigated the effects of dietary multienzyme complex supplementation on the performance and nitrogen excretion of broiler chicks. It was concluded that, nitrogen excretion was lower for chicks fed low-protein diets compared to the control diet, however, no significant effect of enzyme supplementation was observed.

The CF and NFE retention was significantly better in group receiving 20% reduced protein levels with supplementation with 300 gm/ ton of protease enzyme. The supplementation of protease also proved to be better in enhancing crude fiber utilization with 18 percent protein reduction. Further, simply reducing protein level in diet without enzyme supplementation that much not improved CF utilization (Table 6 and Table 7 ). These results are in agreement with Yadav and Sah (2005) who found that the crude fiber digestibility was significantly improved as 0.1% in broiler starter diet and 0.075 % and 0.1%protease supplementation in broiler finisher diets. Romero and Plumstead (2012) found that protease enzyme contributed to increase digestibility of starch and all other nutrients.
The ether extract calcium and phosphorus was non-significantly effected in the control diet and low protein diets (Table 6 and Table 7). Botha (2011) reported that many times enzyme supplementation did not increase the nutrient the nutrient digestibility. Madrid et al. (2010) concluded that the multienzyme complex of protease and carbohydrazes enzymes might be effective for improving nutrient digestibility in broilers fed with a wheat soybean meal based diet under commercial farm conditions.

Effects of protease on poultry diet do not appear to be completely limited to protein digestion, but also affect the digestibility of other nutrients. Increased digestion of corn starch with the use of protease, attributed to the disruption of protein matrix in starch granules. The protein hydrolysis catalyzed by the exogenous protease may be responsible for the improvement in apparent CP digestibility. The fiber degradation may be one of the mechanisms by which protease increases the digestion of nutrients in chickens (Romero and Plumstead, 2012)

CARCASS TRAITS OF BROILERS (live weight)

It was observe that addition of different levels of enzyme did not effect on dressed weight (%) eviscerated weight (%) and drawn weight (%), there is no significantly difference was observed in control diet and enzyme supplementation diets (Table 8 and Table 9). Results of present study are in agreement with Kamran et al. (2004) who found that carcass yield was significantly (P<0.05) higher for the group fed low protein diet with 20% CP compared to control diet with 23% CP. However, Cafe et al. (2002) did not find consistent effect on dressing percentage due to addition of enzyme. Whereas, Jha et al. (2010)) found that enzyme supplementation has no significantly effect on dressed, eveserated, giblet and drawn weight. Berwal et al. (2009) found that no significantly effect on drawn weight by enzyme supplementation. Kilic et al. (2006) in his study enzyme addition had no influence on broiler carcass characteristics except for abdominal fat content which was decreased due to enzyme addition.
ORGAN WEIGHT (% DRESS WEIGHT) OF BROILERS

Gizzard percentage in different groups was non significant among the groups. This percentage did not increased with reduction in protein levels either with enzyme or without enzyme over the control (Table 10 and Table 11). These results are in agreement with Hajati et al. (2009) found that addition of enzyme has no significant effect on gizzard percentage and enzyme supplementation decreases the relative size of digestive organs. However, Hassan et al. (2011) who found that birds fed low protein diet showed significant increase in percentage of gizzard and addition of enzyme also increased gizzard percentage.

Heart percentage of groups was non significant (P>0.05) among the groups. Protein reduction at 20% and 18% with and without protease enzyme supplementation could achieve significant weight as compare to control, however enzyme supplementation in treatments could not yield better heart weight. The observations are consistent with Hassan et al. (2011).

Liver percentage of groups showed no significant difference among the groups. It is revealed that 20% and 18 % protein reduction proved to be beneficial either in protease or non protease group for getting higher liver weight but there is no significant difference as compare to control diet(Table 10 and Table 11). The liver weights are expected due to increased lipogenic activity in the liver of low protein fed chickens (Kamran et al., 2011). The pancreas and spleen weight was statistically non significant weight gain were observe in the low protein supplemented with protease enzyme or without protease enzyme also compare with control group.

These results are in accordance with Hassan et al. (2011) who found that enzyme supplementation improves liver weight. However, El-Katcha et al. (2014) observed that enzyme supplementation had no significant effect on liver weight. Rada et al. (2013) showed that the exogenous mono-component protease added into low protein broiler diet had no significant effect on carcass characteristic. As there is no significant effect is observe in liver heart gizzard so there is non significant effect is observe in the giblet weight.
**Processing losses**

The processing losses indicated that blood, shank, feather and wing tips losses were maximum but not significantly (P>0.05) higher in low protein 20% with 100gm/ton of protease enzyme of diet. While losses were minimum in higher level of enzyme added group in low protein 20% with 300gm/ton of protease enzyme of diet. Head weight was higher in low protein with 20% diet. The feather, shank and wing tip weight were non significantly higher in the control diet. There was no statistically difference among the groups. When the protein level was lower to 18% no significant difference was observed in the control group and the protease enzyme supplemented and without enzyme supplemented groups, and also the addition of different levels of enzyme showed no significant difference among groups (Table 12 and Table 13).

**Economics of broiler production**

Effect of different level of protease enzyme in low protein (20%) diet on economics of broiler production the cost of feed per kg was maximum in broilers assigned with control diet as compare to low protein diet 20 and 18% supplemented with enzyme or without enzyme. The cost of feed per kg weight gain was maximum and significantly higher (p<0.05) with broilers low protein 20% diet without enzyme. It was minimum in broilers offered control diet which was statistically similar with those of low protein 20% 200 and 300 gm per ton of enzyme supplemented diets. While, total cost of feed per kg of body weight gain was maximum in control group diet and low in 20 and 18% low protein groups. The total cost of feed for body weight gain was significantly maximum in control diet as compare to low protein diet. In low protein diet minimum cost is required in the enzyme supplemented group. The net return over feed cost per kg weight gain was maximum and significantly higher (P<0.05) in control diets followed by the enzyme added groups. Among these groups difference were statically non-significant. Minimum return was noticed in broilers offered low protein without enzyme diet. Net return over feed cost total weight gain was maximum and significantly higher (P<0.05) in groups assigned 22% of diet as compare to
low protein diet. Among enzyme supplemented group with low protein diet total cost of feed for weight gain was maximum non significant in enzyme supplemented group as the weight gain is maximum in enzyme group added with 300gm per ton of protease enzyme in 20 and 18 percent low protein diets (Table 14 and Table 15).

These results are in agreement with Kamran et al. (2004) who revealed that decreasing CP levels from 23 to 20% resulted in reduced feed cost per kg of live weight gain, which clearly indicated that this approach was useful especially in severe summer conditions to increase economic returns. Cowieson and Adeola (2005) found that use of enzyme complex is highly effective in improving the performance of broilers, allowing for the formulation of lower-cost diets and contributing to the profitability of poultry production. Kamran et al. (2011) found that maximum economic returns were observed in groups fed the low CP diets and it clearly indicated that this approach is useful for economical broiler production. Fru-Nji et al. (2011) observe that with low protein supplemented with protease was significantly better than low protein diet without protease supplementation. Results showed that enzyme addition improved protein and energy digestibility which lead to significant improvements in broiler performance also helped to address some of the current challenges faced by the livestock industry as feed cost and demands for sustainable farming Doskovic et al. (2013) reported that Enzyme supplementation in broiler diets had no adverse effect on cost of production.
SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

Summary

The present study was planned to see the effect of different levels of protease enzyme supplementation in low protein diets on performance, nutrient utilization and carcass traits and economic of broilers production. The Experiment was conducted on broilers chicks for a period of five weeks. It was started on 13\textsuperscript{th} February 2014 and terminated on 18\textsuperscript{th} March 2014. in the department of Animal Nutrition, College of Veterinary Science and Animal Husbandry, N.D.V.S.U., Jabalpur (M.P.). The present experiment was conducted to determine the effect of protease supplementation on performance, nutrient utilization and carcass traits of broilers. Out of 270 day old procured broiler chicks, a total of 216 chicks were randomly assigned to nine dietary treatments and each dietary treatment consisted of three replicates of eight chicks in each. The basal diet (Diet 1) was formulated as per ICAR (1998) specifications and contained 22% CP and 2800 kcal ME per kg diet. Diet 2 was same as basal diet except that in this diet CP content was reduced to 20%. Whereas Diet 6 was also same as basal diet except that in this diet CP content was further reduced to 18%. Diet 3, 4 and 5 were same as diet 2 except that in these diets protease was supplemented @ 100,200 and 300 g/ton. Similarly, Diet 7, 8 and 9 were same as diet 6 except that in these diets protease was supplemented @ 100,200 and 300 g/ton.

The observations were recorded for daily feed offered, weekly feed consumption, weekly live weight and from these observations average weekly feed consumption, average weekly gain in weight, average weekly feed conversion ratio was calculated. Nutrient utilization was studied at the end of experiment by conducting metabolic trial for three consecutive days. The carcass evaluation study was carried out at the end of trial by selecting two birds from each replicate to assess the carcass traits such as dressing weight, eviscerated weight, drawn weight in percentage, processing losses like feather, blood, head, shank and wingtips, pancreas, spleen, liver, heart and gizzard weight at the end of experiment and economics of broiler production was calculated.
Incorporation with different levels of protease enzyme in the low protein diets produced significant effect on the performance and the nutrient utilization. The control diet showed significantly higher (p<0.05) weight gain, feed intake, FER and PI. among the treatment groups containing low protein 20 and 18 percent show higher significant weight gain, feed intake, FER and PI in the group supplement with 300gm/ton of protease enzyme. Effect of different levels of protease enzyme in low protein (20%) diet on nutrients utilization there was significantly(p<0.05) increase the utilization of dry matter, crude protein, crude fiber and NFE there is no significant effect was notice in the ether extract calcium and phosphorus in the enzyme supplemented group with 300gm/ton of protease enzyme. Effect of different levels of protease enzyme in low protein (18%) diet on nutrients utilization revealed reduced the dry matter and other nutrient utilization.

Effect of different level of protease enzyme in low protein 20 and 18 percent diets on carcass yields (% live weight) organ weight (% Dress weight) processing losses(% live weight) of broilers, showed that use of protease enzyme at higher doses did not differ significantly (P>0.05) and there was no difference between enzyme and without enzyme supplemented groups.

The net return over feed cost per kg weight gain was maximum and significantly higher (P<0.05) in control diets but there was no such statistical difference between 20 percent crude protein enzyme supplemented with 300 and 200gm/ton of protease. There was statistically difference in net return over feed cost per kg weight gain was maximum and significantly higher (P<0.05) in control diets as compared to T6,T7,T8 and T9 groups.
Conclusions

Based on the present study on broilers it can be concluded that the high protein diet (22% CP) was found to be better than low protein diets (20% and 18% CP). In low protein diets supplementation of protease enzyme @ 300 gram per ton showed better response on performance and nutrient (DM, CP, CF and NFE) utilization, however it was uneconomical. Further, the effect of protease enzyme on carcass yield was not observed.
Suggestions for further work

1. The studies should also be carried out by adding combination of enzyme protease and xylanase in broiler diet.

2. Experiments should be carried out by adding protease enzyme by decreasing 0.5% protein level in broiler diet.
REFERENCES


