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ORIGINAL ARTICLE

Effect of Protease Supplementation on Growth Performances, Carcass and Meat Quality Characteristics of Broiler Chicken Fed with Low Protein Diets

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Abstract

The effect of feeding low dietary crude protein (CP) with different levels of supplemental protease enzyme on commercial broilers was investigated. Three-hundred broiler chicks were divided into five treatments with six replicates in a completely randomized design. The experimental diets were positive control (recommended CP levels, T1), negative control (level of CP reduced by 5%, T2), negative control + 300 g t^{-1} protease (T3), negative control + 400 g t^{-1} protease (T4) and negative control + 500 g t^{-1} protease (T5). Growth, blood serum, carcass quality and meat quality parameters were measured. Data were analysed using one-way Analysis of Variance. The highest live weight (2.86 ± 0.07 kg), weight gain (2.66 ± 0.05 kg) and the lowest feed conversion ratio (1.84 ± 0.06) were observed in birds fed with T5. There was no influence of treatments (p>0.05) on NH_3 emission from litter, dressing percentage, meat quality and blood serum parameters. The feed cost per producing 1 kg of live weight and sellable carcass weight was significantly low (p<0.05) in T5. Thus, it can be concluded that low protein diets supplemented with protease enzyme at 500 g t^{-1} support better growth performances in broiler chicken with a lower cost of production.

Keywords*: Broilers, Low protein diets, Performances, Protease enzyme*

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1. Introduction

The demand of the world market for poultry products is rapidly rising with the growing population. The broiler sector has a vital role to make people food secure, in both economically and nutritionally. It has been estimated by the Food and Agriculture Organization that as of 2024, broiler meat production will reach 134 million metric tons (Anon 2018). With the increasing population, it was predicted that meat consumption per person per year will increase by 26% from 2006 to 2030 and this increase is mainly for chicken meat (OECD-FOA 2010). Therefore, the poultry industry has to grow continuously to meet the increasing demand. Hence, to supply the increasing demand, the broiler industry needs to be further commercialized, aiming at higher profits to the owner.

The poultry industry had changed dramatically in both nutritional and genetic components for achieving production targets (Gunal et al. 2006). In 1925, a broiler chicken took about 120 days to reach 1.5 kg of body weight, while in 2005, it was only 30 days due to intensive selection & breeding, and nutrition (Bessei 2006). Though genetic components are rapidly changed, it is not adequate to achieve higher production. In addition, improved nutrition and proper management are also essential.

Proper feeding of broilers is one of the main practices that producers have to consider, where feed cost contributes 60 - 70% of total production cost (Dosković et al. 2013). For cost-saving on feed, knowledge on digestive tract characteristics of poultry, nutrient requirements, digestion and nutrient utilization at certain growth and development stages is essential (Dosković et al. 2013). Currently, farmers are using high-cost feed ingredients and supplements to supply enough nutrients for broilers to achieve higher performances. However, birds do not absorb all the nutrients available in feed rations. The undigested nutrients, especially proteins and amino acids, are utilized by the microbes for their metabolism in the hindgut resulting in an acidic condition (Moughan et al. 2014). Many studies have been conducted on broiler nutrition based on exogenous enzymes that could use in broiler feeds to attain maximum utilization efficiency.

Generally, the aims of adding enzymes to the broiler rations are to increase the digestibility, remove the anti-nutritional factors, improve the availability of nutrients and reduce the environmental issues (Hedstrom 2002). Many studies suggested

that various types of endogenous protease are synthesized and released by the gastrointestinal tract, and they are sufficient to utilize feed protein at the optimum level. However, Dosković et al. (2013) suggest that the birds in their early stages might produce limited amounts of endogenous enzymes. These enzymes are necessary to digest higher amounts of vegetable proteins (Bedford 2009). It affects the nutrient digestibility of broilers in young ages. Therefore, protease enzyme can be used as supplementation to increase the digestibility of protein ingredients in the ration while it decreases the amount of dietary protein in the feed ration. This also reduces the protein waste and excretion of nitrogen into the environment (Ghazi et al. 2002).

Further, most of the broiler farmers use soybean meal as the protein source but, it contains several anti-nutrient factors. Though heat treatments apply to reduce the anti-nutrient factors, they are not totally inactivated (Maidala et al. 2013). Further, prolonged heating will result in the reduction of nutritional value. Hence, the use of exogenous enzymes to inactivate the proteinaceous anti-nutritional factors is a better method. Addition of exogenous enzymes results in better flock performances, quality of litter and improved bird health which in return, has a positive influence on total production costs (Cowieson and Ravindran 2008). Ultimately, supplementation of exogenous proteases on broiler rations improves the production efficiency by increasing the low-quality ingredient digestibility and reducing the losses of nutrients through excreta. It retains nutrient levels in rations resulting economic benefits to the farmer (Ghazi et al. 2002). Therefore, this research aimed to investigate the possibility of decreasing dietary protein levels in feed by adding protease enzyme to the broiler feed rations without interfering growth performances and carcass quality.

2. Materials and Methods

Experiment Location

Fieldwork and laboratory analysis were conducted at the livestock farm and laboratory of New Hope Lanka Ltd. *Ekala*, *Ja-Ela*, Sri Lanka. Blood serum parameters and meat colour were analysed at the Animal Science laboratory in the Faculty of Agriculture, Rajarata University of Sri Lanka, *Puliyankulama*, *Anuradhapura*, Sri Lanka.

Experimental Design

Three hundred "Cobb 500", day-old broiler chicks were purchased from a commercial hatchery (CIC farm, Sri Lanka) and randomly assigned into five treatments designated as

T1, T2, T3, T4 and T5 in a Complete Randomized Design (CRD). Each treatment was replicated six times and there were 10 birds in each replicate.

Treatment Rations

Three concentrations of protease were added to the basal feed mixture and treatments were as follows;

Treatment 1: Recommended level of crude protein (CP) and amino acids (AA) concentration (Positive control)

Treatment 2: Level of CP and AA reduced by 5% (Negative control)

Treatment 3: Negative control + 300 g t^{-1} of protease

Treatment 4: Negative control + 400 g t^{-1} of protease

Treatment 5: Negative control + 500 g t^{-1} of protease

Feed Mixing

Feed rations were mixed according to the National Research Council (NRC) recommendations (NRC 1994), and the composition of each is presented in supplementary table 1, 2 and 3. The floor was cleaned to avoid contamination of any foreign materials. Macro and micro-ingredients were weighed and mixed using a shovel, while mixing, vegetable oil. Finally, treatment additives were mixed. For the booster, starter and finisher period, 150 kg, 750 kg, 1000 kg of feeds were prepared, respectively. Prepared rations were allocated to each pen and stored in labelled bags until feeding.

Feeding Management

Rations were offered in 3 feeding phases; broiler booster (day 8 to day 14), broiler starter (day 15 to day 28) and broiler finisher (day 29 to day 42). The rations and clean drinking water were provided *ad libitum* throughout the experimental period. General feeding regime of day 1 to day 7 was practised following VRI recommendations (Department of Animal Production and Health 2014). The treatment rations were provided from day 8 to 42.

Broiler Management Practices

Day-old chicks were introduced to preheated brooder pens and brooded up to seven days. Electrical bulbs were used as heat sources and paddy husk was used as litter material. Chicks were introduced on the paper layer. Just after the introduction of chicks to pens, glucose and vitamins were supplied with drinking water, and vitamin supplement was provided continuously up to day seven.

NH_3 Emission from Litter

Amount of ammonia emitted from the litter was measured for each replicate weekly, using ammonia estimating machine (ADKS-1-NH3, China).

Slaughtering of Birds

After 42 days of the growing period, two birds were randomly selected from each pen and weighed. Birds were slaughtered and bled. Feathers were removed with the skin. The digestive tract, respiratory tract, heart, and liver were removed and gall bladder was peeled away. Gizzard was cleaned and the inner layer was removed. Breast, thighs, and drumsticks with bone were cut and weighed. The weight of the internal organs; liver, gizzard, and heart were measured and expressed as a percentage of carcass weight.

Chemical Analysis of Feed Samples

Feed samples (100 g) were collected randomly after the mixing of feeds and ground to a fine powder. Then samples were stored in bags until the analysis. Experimental rations were analysed to determine moisture, crude protein (CP), crude fibre (CF), crude fat (EE) and ash, following the standard methods described by AOAC (2003).

Serum Lipid Profile

Blood samples were collected to plain sterilized tubes from one randomly selected fasting bird from each replicate at day 42. Immediately, the serum was separated at 1500 rpm for 20 min using centrifugation (C0060, USA). Then, samples were stored in -20^0C until further analysis. The serum samples were tested for total cholesterol, high-density lipoproteins (HDL), triglycerides (TAG) and low-density lipoproteins (LDL) using a commercial kit (02160 MAIZY, France) and a spectrophotometer (Uvd 2960, USA).

Meat Quality Parameters

Meat samples were taken from the breast area and stored in -20^0C. Proximate analysis was done according to the AOAC methods (AOAC 2003).

For pH measurement, 1 g of meat sample from each replicate was taken, thawed for 30 min and blended with 9 mL of distilled water. Samples were filtered (Whatman- No. 4) and pH of filtrates was determined using a pH meter (E-08328 ALELLA, Barcelona).

Meat samples were cut into cubes (2.0 ± 0.10 g) to measure the water holding capacity (WHC). Those were carefully placed between 2 pieces of filter papers (Whatman- No. 4)

and left under a 10 kg weight for 5 min separately. After recording the final weight of each sample, WHC was calculated using equation 1;

Equation 1

$$WHC\ (\%) = 100 - \left[\frac{(Wi - Wf) \times 100}{Wi}\right]$$

where W_i and W_f were the initial and final weights of the sample, respectively.

One sample for each replicate was used to evaluate the colour values at the dorsal surface of the intact skinless breast muscles using a colourimeter (CR 10 plus, Konica Minolta, Japan). The values of lightness (L*), redness (a*), and yellowness (b*) were obtained at 3 sites on the same sample.

Data Collection and Calculations

Amount of feed given and remained per pen were measured and feed intake was calculated daily throughout the study period (Eq. 2). Body weight was measured weekly and weight gain and feed conversion ratio (FCR) were calculated (Eq. 3). Live weight and carcass weights were recorded and dressing percentage was calculated (Eq. 4). The weight of internal organs (liver, gizzard, and heart) were taken and expressed as a percentage of the carcass weight.

Equation 2

$$Feed\ intake\ (g) = Given\ feed\ (g) - Remain\ feed\ (g)$$

Equation 3

$$Feed\ conversion\ ratio = \frac{Feed\ intake\ (kg)}{Live\ weight\ gain\ (kg)}$$

Equation 4

$$Dressing\ percentage\ (\%) = \frac{Carcass\ weight\ (kg)}{Live\ weight\ (kg)} \times 100$$

The feed cost for different treatments was recorded throughout the study period. Feed intake per bird during the study period was calculated. The data were used to obtain the cost of feed per kg of live weight (Eq. 5) and carcass weight (Eq. 6)of the bird.

Equation 5

$$Cost\ of\ feed\ per\ kg\ of\ live\ weight\ (Rs/kg) = \frac{Cost\ of\ feed\ per\ bird}{Live\ weight\ of\ a\ bird}$$

Equation 6

$$Cost\ of\ feed\ per\ kg\ of\ carcass\ weight\ (Rs/kg) = \frac{Cost\ of\ feed\ per\ bird}{Carcass\ weight\ of\ a\ bird}$$

Data Analysis

Weight gain, feed intake, FCR, dressing percentage of birds, serum lipid profile, proximate composition of the meat samples and feed cost were analysed using the Analysis of Variance (ANOVA) procedure of Statistical Software for Data Analysis (SAS), Ver. 9.0 (SAS 2002). Mean separation was done by Turkey's Standardized Range Test (TSRT). Statistical significance was declared at $p < 0.05$.

3. Results and Discussion

Feed Intake

Effect of treatments on feed intake, weight gain and FCR of broilers fed with different rations supplemented with protease enzyme are presented in Table 1, 2 & 3, respectively.

There were significant differences (p<0.05) in feed intake of broilers fed with different treatment rations during booster period (from day 8 to 14), starter period (from day 15 - 28) and whole study period (from day 8 - day 42) (Table 1). However, there was no significant difference (p>0.05) in feed intake of birds during the finisher period (from day 29 - 42). During booster period, feed intake of birds fed with T1 was significantly higher *(*p<0.05) compared to birds fed with treatment 2, 3 and 5. However, during the starter period and from day 36 to day 42 birds fed with T3 recorded significantly (p<0.05) higher feed intake. Similar observations were recorded during the total study period.

The results are also in confirmation with the results of Angel et al. (2011). They showed that when *Ross 708* broilers fed with mono-component protease enzyme supplementation (75,000 protease units per g) to a corn-soy meal containing positive control ration (22.5%) and low CP (20.5%) ration, there is a positive influence on feed consumption from day 7 to day 22. Further, Law et al. (2018) stated that there were no significant CP × protease interactions for feed intake except feed intake during day 29 to 35 for birds fed with low protein rations. Moreover, they found feed intake was not affected by the enzyme inclusion during 2nd and 3rd weeks of age, except during the 1st week. However, Ajayi (2015) showed that feed intake of birds significantly decreased with inclusion of different levels of protease to the basal ration formulated with 12.5%, 14.4% and 20% CP levels with similar metabolisable energy level.

Table 1: Average feed intake of broilers fed different rations supplemented with protease enzyme

Period	Feed intake (g/bird) in different treatments*					P-value	SE
	1	2	3	4	5		
Day 8 – 14	437^{a}	429^{b}	430^{b}	434^{ab}	427^{b}	0.03	2.42
Day 15 – 21	840^{d}	888^{c}	976^{a}	976^{a}	933^{b}	0.00	0.67
Day 22 – 28	1104^{e}	1204^{a}	1177^{b}	1138^{c}	1132^{d}	0.00	0.52
Day 29 – 35	1203	1204	1304	1220	1194	0.68	59.56
Day 36 – 42	1094^{d}	1203^{bc}	1228^{a}	1207^{b}	1189^{c}	0.00	5.16
Day 15 – 28	1944^{e}	2091^{c}	2153^{a}	2114^{b}	2064^{d}	0.00	0.93
Day 29 – 42	2297	2407	2532	2427	2381	0.13	60.44
Day 8 – 42	4677^{c}	4926^{b}	5113^{a}	4974^{ab}	4871^{b}	0.00	61.13

$^{a, b, c}$ means within the same row with different superscripts are significantly different (p<0.05).

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} proteases.

Weight Gain

Table 2: Average weight gain of broilers fed different rations supplemented with protease enzyme

Period	Weight gain (g/bird) in different treatments*					P-value	SE
	1	2	3	4	5		
Day 8 - 14	295^{ab}	271^{c}	280^{bc}	281^{abc}	303^{a}	0.04	7.43
Day 15 - 21	484	452	499	503	479	0.43	20.36
Day 22 - 28	477	531	500	461	514	0.25	23.39
Day 29 - 35	601	669	623	628	643	0.86	45.11
Day 36 - 42	597^{b}	460^{c}	549^{bc}	517^{bc}	726^{a}	0.00	43.38
Day 15 - 28	960	982	999	963	992	0.50	18.62
Day 29 - 42	1198^{b}	1129^{b}	1172^{b}	1145^{b}	1367^{a}	0.04	57.68
Day 8 - 42	2451^{b}	2380^{b}	2449^{b}	2388^{b}	2663^{a}	0.00	53.58

$^{a, b, c}$ means within the same row with different superscripts are significantly different ($p<0.05$).

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease.

The body weight gain was significantly (p<0.05) different among the treatments from day 8 - 14, day 36 - 42, day 29 - 42 (finisher period) and day 8 - 42 (whole study

period) (Table 2). The birds fed with 500 g t^{-1} of protease added to 5% reduced CP ration (T5) had the highest body weight gain in day 36 - 42, day 29 - 42 and day 8 - 42.

The present study is also in agreement with the study of Fosnaught and Odetellah (2005) who experimented on broilers by adding Versazyme protease enzyme at 0.1% to corn-soybean meal based rations. Their results revealed that body weight gain of birds at 21, 35 and 42 days of age was improved by the addition of Versazyme protease enzyme. Further, Mohammadigheisar and Kim (2018) showed that supplementing low CP diets with protease alleviated the negative effects of lowering dietary CP on body weight gain during day 1 - 2. Zakaria et al. (2010) also reported higher weight gains in *Lohmann* broilers at 42 days of age fed with rations supplemented with enzyme mixture (protease, alpha-amylase, pectinase, phytase and cellulose). Law et al. (2018) also observed improved weight gains in broilers fed with protease supplementation.

Feed Conversion Ratio

The FCR was significantly (p<0.05) different among the treatments from day 36 - 42, day 15 - 28 and day 8 - 42 periods (Table 3).

The results are also in confirmation with results of Café et al. (2002). As per the result of the research, in commercial broilers fed with rations (nutritionally complete broiler ration based on the corn-soybean meal) supplementing Avizyme at 0.1% at 35 and 49 days of age showed a non-significant effect on FCR. However, they observed FCR of broilers had a significant effect at 16 days and 42 days of age.

Table 3: Average feed conversion ratio of broilers fed different rations supplemented with protease enzyme

Period	FCR in different treatments*					P-value	SE
	1	2	3	4	5		
Day 8 – 14	1.51	1.60	1.55	1.55	1.42	0.07	0.05
Day 15 – 21	1.77	1.98	1.97	1.96	1.98	0.38	0.09
Day 22 – 28	2.34	2.3	2.38	2.5	2.24	0.53	0.11
Day 29 – 35	2.11	1.87	2.13	1.96	1.9	0.74	0.18
Day 36 – 42	1.85^{b}	2.66^{a}	2.32^{ab}	2.67^{a}	1.65^{b}	0.01	0.24
Day 15 – 28	2.03^{b}	2.14^{ab}	2.15^{a}	2.19^{a}	2.08^{ab}	0.04	0.04
Day 29 – 42	1.95	2.16	2.2	2.17	1.75	0.09	0.13
Day 8 - 42	1.92^{bc}	2.08^{ab}	2.1^{a}	2.1^{a}	1.84^{c}	0.00	0.06

$^{a, b, c}$ means within the same row with different superscripts are significantly different (p<0.05).

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease.

As shown in Table 2, during day 8 – day 14, weight gain was similar in control, T3, T4 and T5. It has been suggested that the underdeveloped intestines of the birds at starter period are unable to produce sufficient amounts of digestive secretions. Hence, supplementation of the rations with enzymes makes them utilize the nutrients efficiently and perform better in terms of growth performances (Dosković et al. 2013).

Further, as shown in Table 2 and 3, the results indicate that the feed efficiency of the broilers was depressed with a reduction of the crude protein content of their ration. Moreover, feed utilization was enhanced with protease supplementation in the ration. Better feed conversion and a higher weight gain were obtained with T5, suggesting that the higher enzyme dosage allows birds to utilize the nutrients efficiently without causing any dietary imbalances.

Carcass Characteristics

The live weights were significantly (p<0.05) different among the treatments at day 42 and the highest live weight was recorded in birds supplemented with T5 (Table 4). However, carcass weight and dressing percentage were not significantly (p>0.05) different among the treatments (Table 4).

These results are in agreement with Freitas et al. (2011). Also, Yadav and Sah (2005) studied the effect of supplementation of acid protease to corn-soybean meal based diets at 0%, 0.05%, 0.075% and 0.1% to control diet (18.5% CP) and reduced CP diet (17.5% CP). However, they showed that there was no effect of supplementation of protease enzyme on dressing percentage of broilers.

Table 4: Carcass characteristics of broilers fed different rations supplemented with protease enzyme

Parameter	Treatment*					P-value	SE
	1	2	3	4	5		
Live weight (kg)	2.64^{b}	2.60^{b}	2.65^{b}	2.52^{b}	2.86^{a}	0.02	0.07
Carcass weight (kg)	1.81	1.92	1.65	1.62	1.90	0.09	0.09
Dressing percentage (%)	68.41	73.64	62.34	64.14	66.43	0.10	3.00
Carcass cuts (% carcass weight)							
Brest	45.4^{a}	38.68^{b}	39.84^{b}	37.60^{b}	36.51^{b}	0.00	1.70
Drumstick	15.94	14.99	15.87	16.98	16.87	0.39	0.79
Wings	8.26	6.17	8.84	8.93	7.47	0.22	0.92
Thighs	29.2^{bc}	26.92^{c}	37.05^{a}	32.45^{abc}	34.92^{ab}	0.02	2.28
Internal organs (% carcass weight)							
Liver	3.29	3.32	3.28	3.39	3.24	0.98	0.18
Heart	0.89	1.15	0.92	1.29	0.84	0.21	0.18
Gizzard	2.07	1.89	2.40	2.07	1.68	0.16	0.22

$^{a, b, c}$ means within the same row with different superscripts are significantly different (p<0.05).

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease

The results were contradictory to the findings of Espino et al. (2000) who observed a slight increase in the dressing percentage of broilers fed rations containing a mixture of enzymes.

Further, Ajayi (2015) reported improved dressing percentages with protease inclusion compared to the birds fed with control ration without protease. These differences may occur due to the variation of broiler diets, enzymes and their level of inclusions.

There was no significant (p>0.05) effect of supplementation of protease enzyme on giblet percentage of commercial broilers (Table 4). However, the highest and the lowest liver and heart percentages were recorded in birds fed with T4 and T5 rations, respectively. Further, the highest and the lowest gizzard percentage were recorded in birds fed with 300 g t^{-1} and 500 g t^{-1} of protease added to 5% reduce CP rations (T3 and T5).

Zakaria et al. (2010) reported that multi-enzyme (protease, alpha-amylase, pectinase, phytase and cellulase) supplementation at 250, 500, 750 g t^{-1} of feed did not affect giblet weights in *Lohmann* broilers at 42 days of age, which confirms the results of the present study. Further, Ndazigaruye (2019) showed that the protease enzyme increased the relative liver weight of broilers at 21 days of age, but disappears the effect at 35 days of age.

Meat Quality Parameter

Meat quality parameters of the present study were not significantly (p>0.05) different among the treatments (Table 5).

The present results are in agreement with Yadav and Sah (2005). Accordingly, male broilers fed with supplementing acid protease to corn-soybean meal based diets at 0%, 0.05%, 0.075% and 0.1% to control diet (18.5% CP) and reduced CP diet (17.5% CP) from day 0 to 42 did not show a difference in crude protein, crude fat and crude ash percentages. Further, in the study of Yang et al. (2010), *Arbor Acres* male broilers were fed by supplementing multi-enzyme additive containing amylase, protease, and xylanase in rations for 36 days. There was no difference in dry matter and crude protein in meat as compared with birds fed with lower enzyme diet or the control diet.

Blood Serum Parameters

Blood serum parameters were not significantly (p>0.05) different among the treatments (Table 6). The results in this study are in agreement with the findings of Zakaria et al. (2010). As the results, the

Lohmann broilers supplemented with multi-enzyme (protease, alma amylase, pectinase, phytase and cellulase) at 250, 500, 750 g t^{-1} of feed showed no significant effect on blood serum parameters. Further, Ndazigaruye (2019) reported that neither dietary CP nor protease enzyme affects serum triglycerides, total cholesterol, HDL and LDL.

Table 5: Meat quality parameter of broilers fed different rations supplemented with protease enzymes

Quality Parameter	Treatment*					P-value	SE
	1	2	3	4	5		
Dry matter %	24.69	25.18	24.59	25.76	23.97	0.09	0.45
Crude protein %	9.49	11.08	10.42	11.64	9.66	0.09	0.62
Ash %	4.25	4.25	4.19	4.09	4.28	0.89	0.15
Ether extract %	3.24	3.21	3.35	3.43	3.48	0.30	0.11
Colour (L*)	51.08	51.17	53.68	49.93	49.96	0.52	1.69
pH	5.74	5.92	5.77	5.82	5.84	0.05	0.04
WHC %	67.52	56.9	65.5	65.59	65.55	0.44	4.24

*The treatments were, T1 = positive control (recommended CP level), T2 = negative control (level of CP reduced by 5%), T3 = negative control + 300 g t^{-1} protease, T4 = negative control + 400 g t^{-1} protease and T5 = negative control + 500 g t^{-1} protease.

Table 6: Blood serum parameters of broilers fed different rations supplemented with protease enzyme

Parameter (mg/dL)	Treatment*					P-value	SE
	1	2	3	4	5		
Triglyceride	78.30	75.80	74.79	70.35	67.15	0.25	3.76
Total cholesterol	155.82	147.04	147.62	142.72	145.20	0.10	3.36
HDL	71.39	72.18	66.78	63.57	62.79	0.33	3.95
LDL	68.77	59.70	65.66	62.78	67.65	0.77	5.56

*The treatments were, T1 = positive control (recommended CP level), T2 = negative control (level of CP reduced by 5%), T3 = negative control + 300 g t^{-1} protease, T4 = negative control + 400 g t^{-1} protease and T5 = negative control + 500 g $^{-1}$ protease.

NH_3 Emission from Litter

Ammonia emission from the litter was not significantly (p>0.05) different among the treatments (Table 7).

Table 7: NH_3 emission of the litter of broilers fed different rations supplemented with protease enzyme

Parameter	Treatment*					P-value	SE
	1	2	3	4	5		
NH_3 in litter ppm	6.52	6.43	6.51	6.38	6.36	0.61	0.08

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease.

Cost Analysis

Feed cost per kg of live weight and feed cost per kg of the saleable carcass were significantly (p<0.05) different among the treatments (Table 8). The highest feed cost per kg of live weight and saleable carcass weight were recorded in T4. The lowest feed cost per kg of live weight and saleable carcass weight were recorded in T5. The production cost per kg of live weight at 42 days of age and production cost per kg of carcass weight due to supplementation of protease enzyme were similar compared to non-supplemented rations (positive and negative controls) with 5% protease enzyme supplemented ration. Similarly, Yadav and Sah (2005) analysed the effect of protease supplementation on production costs and the authors received the highest income from the rations supplemented with 0.075% protease. Therefore, protease supplementation profoundly decreased the production cost as compared to the basal diet.

Table 8: Cost analysis of broilers fed different rations supplemented with protease enzyme

Parameter	Treatment*					P-value	SE
	1	2	3	4	5		
Feed cost per kg of live weight (LKR)	169.34^{bc}	180.40^{ab}	184.47^{ab}	188.37^{a}	162.06^{c}	0.01	5.73
Feed cost per kg of saleable carcass (LKR)	249.89^{b}	250.48^{b}	297.97^{a}	295.70^{a}	244.03^{b}	0.02	14.26

$^{a, b, c}$ means within the same row with different superscripts are significantly different (p<0.05).
*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease

4. Conclusions

Feeding 500 g t^{-1} of protease with 5% crude protein reduced ration is a better solution to improve protein digestibility without interfering the growth performances of broilers with lower cost and maximum economic benefits.

Conflicts of Interest: The authors have no conflicts of interest regarding this publication.

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Supplementary Table 1: Composition of chick booster feed mixed with different protease concentration

Ingredient %	Treatments*				
	1	**2**	**3**	**4**	**5**
Broken rice	56.22	59.17	59.14	59.13	59.12
DDGS	5.5	5.5	5.5	5.5	5.5
Vegetable fat	1.4	1.1	1.1	1.1	1.1
Soybean meal	25.5	23.2	23.2	23.2	23.2
Corn gluten meal	2	2	2	2	2
Fish meal	2.5	2	2	2	2
Meat & bone meal	4	4	4	4	4
DCP	0.5	0.5	0.5	0.5	0.5
Limestone -powder	0.82	0.95	0.95	0.95	0.95
Salt	0.2	0.2	0.2	0.2	0.2
Sodium bicarbonate	0.2	0.2	0.2	0.2	0.2
Choline chloride 60%	0.1	0.1	0.1	0.1	0.1
L-lysine 98.5%	0.225	0.245	0.245	0.245	0.245
DL-Methione 98.5%	0.265	0.265	0.265	0.265	0.265
L-Threonine 99%	0.115	0.115	0.115	0.115	0.115
Toxin binder	0.1	0.1	0.1	0.1	0.1
Phytase 5000	0.01	0.01	0.01	0.01	0.01
NutriMin CPM116	0.2	0.2	0.2	0.2	0.2
Vitamin MPV118	0.05	0.05	0.05	0.05	0.05
Salinomycin	0.04	0.04	0.04	0.04	0.04
Probiotics-100	0.02	0.02	0.02	0.02	0.02
Protease	-	-	0.03	0.04	0.05
Tributyrin-45%	0.035	0.035	0.035	0.035	0.035

*The treatments were, T_{1} = positive control (recommended CP level), T_{2} = negative control (level of CP reduced by 5%), T_{3} = negative control + 300 g t^{-1} protease, T_{4} = negative control + 400 g t^{-1} protease and T_{5} = negative control + 500 g t^{-1} protease.

Supplementary Table 2: Composition of starter feed mixed with different protease concentration

Ingredient %	Treatments*				
	1	**2**	**3**	**4**	**5**
Broken rice	55.035	58.2	58.17	58.16	58.15
Wheat shorts	2	2	2	2	2
DDGS	7	7	7	7	7
Vegetable fat	2.4	2	2	2	2
Soybean meal	23.75	21	21	21	21
Corn gluten meal	2	2	2	2	2
Meat & bone meal	5	5	5	5	5
DCP	0.45	0.45	0.45	0.45	0.45
Limestone -powder	0.8	0.8	0.8	0.8	0.8
Salt	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.22	0.22	0.22	0.22	0.22
Choline chloride 60%	0.08	0.08	0.08	0.08	0.08
L-lysine 98.5%	0.2	0.2	0.2	0.2	0.2
DL-Methione 98.5%	0.25	0.235	0.235	0.235	0.235
L-Threonine 99%	0.125	0.125	0.125	0.125	0.125
Toxin Binder	0.1	0.1	0.1	0.1	0.1
Phytase 5000	0.01	0.01	0.01	0.01	0.01
NutriMin CPM116	0.2	0.2	0.2	0.2	0.2
Vitamin MPV118	0.04	0.04	0.04	0.04	0.04
Protease	-	-	0.03	0.04	0.05
Maduramycin 1%	0.04	0.04	0.04	0.04	0.04
Probiotics-100	0.015	0.015	0.015	0.015	0.015
Tributyrin-45%	0.035	0.035	0.035	0.035	0.035

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease.

Supplementary Table 3: Composition of finisher feed mixed with different protease concentration

Ingredient %	Treatments*				
	1	**2**	**3**	**4**	**5**
Broken rice	53.6	55.4	55.4	55.4	55.4
Rice polish	5	5	5	5	5
DDGS	10	10.2	10.2	10.2	10.2
Vegetable fat	4.5	4.5	4.5	4.5	4.5
Soybean meal	16.9	15.2	151.7	151.6	151.5
Corn gluten meal 60%	2.5	2.01	2.01	2.01	2.01
Meat & bone meal	5	4.8	4.8	4.8	4.8
DCP	0.3	0.4	0.4	0.4	0.4
Limestone -powder	0.5	0.8	0.8	0.8	0.8
Salt	0.25	0.25	0.25	0.25	0.25
Sodium bicarbonate	0.25	0.25	0.25	0.25	0.25
Choline chloride 60%	0.05	0.05	0.05	0.05	0.05
L-lysine 98.5%	0.34	0.34	0.34	0.34	0.34
DL-Methione 98.5%	0.26	0.25	0.25	0.25	0.25
L-Threonine 99%	0.115	0.115	0.115	0.115	0.115
Antioxidant	0.02	0.02	0.02	0.02	0.02
Toxin Binder	0.1	0.1	0.1	0.1	0.1
Phytase 5000	0.01	0.01	0.01	0.01	0.01
Protease	-	-	0.03	0.04	0.05
NutriMin CPM116	0.2	0.2	0.2	0.2	0.2
Vitamin MPV118	0.035	0.035	0.035	0.035	0.035
Maduramycin 1%	0.05	0.05	0.05	0.05	0.05
Probiotics-100	0.02	0.02	0.02	0.02	0.02

*The treatments were, T_1 = positive control (recommended CP level), T_2 = negative control (level of CP reduced by 5%), T_3 = negative control + 300 g t^{-1} protease, T_4 = negative control + 400 g t^{-1} protease and T_5 = negative control + 500 g t^{-1} protease.