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Research Article

Effect of supplementation of different levels of protease enzyme on growth performance and protein digestibility of broiler

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Abstract

The objective of this study was to examine the impact of supplementation of protease enzyme on feed conversion ratio (FCR), weight gain and digestibility of crude protein in broilers. In a 42-day randomized trial, 240 un-sexed (ROSS 308) day-old broiler chicks were purchased from a local hatchery and randomly assigned to four experimental groups: T1 (Control), T2 (250g/ton of feed), T3 (500g/ton), and T4 (protease 750 g/ton). Each group had 3 replicates with 20 chicks. At the end of the 42-day experimental feeding, FCR, weight gain, and feed consumption were evaluated using weekly data, and 2 birds from each replicate were slaughtered to evaluate carcass weight and dressing percentage. Total Collection Method was used to estimate the digestibility of Crude Protein, Crude Fiber, Ether Extract and Dry Matter with the help of kjeldahl, Labconco, Soxhlet, and Hot Air Oven, respectively. JMP was utilized to statistically evaluate the cumulative data. T4 had the highest feed intake (3836.6 g/bird), followed by T2 (3808.3g/bird), T1 (3780g/bird), and T3 (3741.6g/bird). Feed intake was non-significant (P>0.05) in all groups. T3 had the highest body weight (2209g), followed by T4 (2201g), T2 (2090g), and T1 (2053g). Significantly greater (P<0.05) body weight was seen in T3 as compared to T2 and T1. Our results showed that T3 had the best FCR (1.69), highest dressing percentage (65.53%) and carcass weight (1450g) whereas, group T1 contained the lowest (1277g) carcass weight. The mortality rates for T1, T2, T4, and T3 were 6.6%, 4.5%, 3.33 %, and 2.0%, respectively. Crude protein digestibility was highest in T3 (76.43%), followed by T4 (73.43%), T2 (71.41%), and T1 (65.34%). However, digestibility of crude fat, crude fiber, and dry matter were non-significant (P>0.05) among all the groups. All other groups had lower net profits after broiler sales than that of T3. It was concluded that the Protease dose @500g/ton of feed improved broilers' FCR, Body Weight, Mortality, and Economical performance of the broilers.

Keywords: Body Weight; Economics; FCR; Mortality; Poultry

Introduction

The poultry business is a significant and rapidly evolving sector globally, with over 50 billion poultry birds being raised each year for the production of both eggs and meat. This sector attracts about $700 billion in investments and provides employment for over 1.5 million people. Poultry accounts for 31% of the overall meat production in the country. Additionally, the poultry sector contributes 1.4% into the Gross Domestic Product (GDP), 7.1% into the agricultural sector, and 12.2% into the value of total livestock [1]. In order to meet the protein requirements of the rapidly expanding human population, there is intense competition to produce raw feed ingredients and improve the nutrient digestibility in poultry birds. Poultry is considered a crucial source of nutrition. Broiler birds, specifically bred for meat production, is in high demands worldwide. In 2018, broiler meat had the highest consumption rate internationally [2]. The cost of feed makes up more than 70% of broiler production expenses. Protein is the most expensive nutrient in the feed. Poultry birds lack the necessary enzymes to fully break down protein compounds, therefore, supplementing exogenous enzymes can have a positive impact on improving performance and digestibility [3, 4]. The cost of feed ingredients used in poultry rations has significantly increased in recent years, and protein feedstuffs have a significant impact on the overall cost of feed production. The rising costs of feed ingredients, limited availability, and society's need for less environmental contamination from animal farming have highlighted the significance of utilization of enzymes in feed. Research on the utilization of exogenous enzymes in broiler diets has been ongoing for several decades, starting with studies on the insufficient production of digestive enzymes in young broilers [5]. Since, monogastric animals lack endogenous secretions of enzymes, hence, it is necessary to include exogenous enzymes in their diets to enhance nutrient utilization from complex feed matrices [6]. Protein in corn-soy diets isn't broken down easily, having 80-85% digestibility, compared to starch (90%). Additionally, a significant amount of protein passes through the gastrointestinal tract without being fully digested [7]. The presence of undigested protein serves as an opportunity to enhance protein digestibility and growth performance in broiler diets by supplementing exogenous protease enzymes. A significant amount of research has been conducted in the field of chicken nutrition to investigate the application of exogenous enzymes and enhance nutrient utilization [8]. Plants possess certain chemicals that are indigestible to animals, typically due to the animal's inability to create the required enzyme for their breakdown. Nutritionists can assist the animals by detecting these indigestible substances and providing the appropriate enzymes for proper digestion. The enzymes are derived from microorganisms that are meticulously chosen for the specific purpose and cultivated under regulated circumstances [9]. Protease enzymes can enhance the digestibility of protein in the feeds. This, in turn, decreases the growth of dangerous bacteria involved in the gut damage [6]. Supplementing exogenous protease in a low-protein diet can lead to enhanced weight gain and increased feed conversion ratio in birds, compared to those fed a basal diet [10]. Several studies have concluded an increment in protein and amino acid digestion when protease enzyme was supplemented in the diets of broilers [11]. While it is clear that exogenous protease is linked to improved digestion of crude protein, it should be noted that the intestinal system is complex and the impact of proteases extends beyond the simple breakdown of dietary crude protein. This extended function may enhance the digestion of various other nutrients of the feed. Additionally, changes in microbial population may result in the availability of easily accessible proteins in different sections of the intestinal lumen [12, 13]. In a study it was also found that the addition of a protease enzyme to low crude protein diets did not have a significant influence on amino acid digestibility and carcass weight but it increased feed conversion ratio [14]. An efficient supplementary protease has the potential to not only decrease the feed expenses by effectively utilizing the nutrients, but it also decreases the overall nitrogen contents in the manure [15]. Thus, based on the aforementioned findings, the objective of this research was to study the effect of supplementation of various doses of protease enzyme on FCR, weight gain and digestibility of crude protein in broilers.

Materials and Methods

A 42-day random trial was conducted using 240 unsexed (ROSS 308) day-old broiler chicks obtained from a local hatchery. The chicks were vaccinated according to (Table 1).

Table 1. Vaccination schedule

Days	Vaccines	Route
3rd	Newcastle disease + Infectious Bronchitis.	Eye Drop
9th	Infectious Bursal Disease.	Eye Drop
22nd	Newcastle disease.	Drinking Water

And placed in an environmentally controlled shed at the Poultry Experimental Station, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam. They were then randomly divided into four treatment groups: T1 (Control), T2 (with a protease dose of 250g/ton), T3 (with a protease dose of 500g/ton), and T4 (with a protease dose of 750g/ton). Each treatment group contained 3 replicates, with 20 chicks in each replicate. Performance data such as Feed Intake, Live Body weight, FCR, Carcass weight, and Mortality Percentage were recorded weekly. To estimate the digestibility of Crude Protein, Crude Fiber, Ether Extract, and Dry Matter, the Total Collection Technique was used between day 36-39. The data from each group was calculated in Microsoft Excel and then uploaded to JMP software, developed by SAS Institute Inc., located in Cary, NC, for statistical analysis. The study was conducted utilizing the Complete Randomized Design (CRD) design through Analysis of Variance. Additionally, LSD (Least Significant Difference) was used to compare the mean values and determine if there was a statistically significant difference at a probability level of 0.05. The experimental design is presented in (Table 2).

Table 2. Experimental design

Groups	Treatment	Protease Dose	Route of Administration	No. of birds
T1	Control	-	-	60
T2	Protease Ghazi Brothers Pvt Ltd	250 g/ Ton of feed	In feed supplement	60
T3	Protease Ghazi Brothers Pvt Ltd	500 g/ Ton of feed	In feed supplement	60
T4	Protease Ghazi Brothers Pvt Ltd	750 g/ Ton of feed	In feed supplement	60

Real estate and administration

The control housing system was implemented with a floor area allocation of 1 square foot per bird. The shed was thoroughly cleaned using water and disinfectants. At the end, the house was fumigated and sealed for a duration of 24 hours. The process of brooding preparation was finished before to the arrival of the birds. After arrival of day-old chicks, temperature and humidity were provided in accordance with the prescribed standards for broilers. The initial brooding temperature was 95°F, which was then decreased and ultimately maintained at 75°F for the rest of the duration of the research period. Rice husk had been utilized as a bedding material at a depth of 3-5 inches. The litter was sun-dried for 24 hours before using as bedding material and then placed on the floor with the addition of limestone to minimize the risk of illnesses in the shed. To mitigate the production of hazardous gases in the chicken house, the litter had been aerated twice daily.

Experimental diet

Experimental feed was provided to all the birds on *ad libitum* basis and all the feeds had been formulated according to the Ross broiler specifications (Table 3).

Table 3. Ingredients and feed formulation of diet (ROSS, 2014)

Ingredients	Starter Feed (%)	Grower Feed (%)	Finisher Feed (%)
Rice	13.0	17.0	36.0
Maize	50.0	50.0	40.0
Fish Meal	7.0	9.0	12.0
Soybean Meal	24.0	19.0	11.0
Canola Meal	5.0	4.0	0.0
Lime stone	1	1	1
Calculated Nutritive values			
Crude Protein	22	21	20
Metabolizing Energy	3000	3100	3200
Dig Lys	1	0.95	0.90
Dig Meth	0.5	0.48	0.45
Dig M+C	0.65	0.65	0.65

Parameters of study

Live body weight

At the end of the experiment 42 days, three chickens from every treatment were randomly selected and weighed with electric weighing scale.

Feed consumption

Fresh feed was given twice in a day and feed consumption had been recorded by using following formula:

$$\text{Feed consumption (g/b)} = \frac{\text{Total feed given - Total feed rejected}}{\text{Total chicks}}$$

Feed efficiency

Feed Conversion Ratio was obtained by using this formula:

$$\text{FCR} = \frac{\text{Total feed consumed}}{\text{Total weight gain}}$$

Carcass characteristics

At the end of the trial, two chickens were randomly selected from each replicate. Birds were slaughtered and defeathered to calculate the carcass weight and carcass yield. Carcass yield was gained by using following formula:

$$\text{Dressing (\%)} = \frac{\text{Total Carcass weight}}{\text{Total body weight}} \times 100$$

After that, the relative weight of heart, liver, gizzard, and spleen weighed with electric weighing scale and recorded according to the following formula:

$$\text{Relative weight (\%)} = \frac{\text{Weight of organs in (g)}}{\text{Total body weight (g)}} \times 100$$

Digestibility analysis

Three birds were selected from each group and shifted into individual pens during the age of 36-39 days of the experiment. After the adaptation period of three days, fecal samples were collected by Total Collection Technique two times in a day morning and evening and were stored at -5°C for analysis. Feces were dried under hot air oven at 105^0C for overnight. For proximate analysis, samples were grinded in the grinder and evaluated according to the AOAC protocols. Apparent nutrients retention was calculated by following formula:

(Nutrients in feed - Nutrients in feces/Total Nutrients in feed x 100) expressed as a percentage

Dry Matter

To determine dry matter, 3g sample was put in a pre-weighed empty petri dish, it was kept in hot air oven for 3 hours at 105^0 C, it was then cooled in a desiccator and then weighed. Following formula was used to obtain moisture percentage:

$$\text{DM (\%)} = \frac{\text{W1-W2}}{100} \times 100$$

Ether extract

Ether extract was obtained by the Soxhlet apparatus technique, for that 1g wrapped sample and fat solvent diethyl ether for a time period of 5-6 hours at $55\text{-}60^0$ C was put in specially made Soxhlet's apparatus. The sample was put in Soxhlet flask and filled ¾ with diethyl ether. The water condenser was run for 2-3 hours. After that, the sample was removed and diethyl ether was allowed to evaporate, which consequently left the fat in the dish. The weight of fat was measured.

Crude fiber

Crude fiber was obtained on labconco by dissolving 1g of sample in sodium hydroxide 10% for 30 minutes at 55^0 C and then in 10% Sulphuric acid at same temperature. After that, the sample was filtered and dried in a pre-weighed crucible by using hot air oven. Finally, the sample was shifted into the muffle furnace at 850^0 C for 20 minutes then weighed.

Protein digestibility

To estimate protein digestibility, Kjeldahl apparatus was used (AOAC, 2000). For that, 1gram Feed/Feces sample was initially weighed and put into the digestion flask along with 5 gram of digestion mixture containing (copper sulfate, ferrous sulfate and potassium sulfate). Then 25ml Sulfuric Acid (Lab-grade) was poured into the digestion flask and then it was put on 1000W heater for 3-3.5 hours for digestion. It was then removed from the heater when light green color appeared. Then the sample was cooled at normal room temperature for 30 minutes. Sample was diluted with distilled water. 10ml diluted sample and 10ml NaOH 40% was put in Kjeldahl apparatus for distillation and the released ammonia was captured in 10ml boric acid 2% in the beaker from another end. Finally, that boric acid was titrated with 0.1 normal H_2SO_4 to capture the ammonia. The indication of completion of the reaction was the change of the yellowish color of the sample into light pink color. The reading of utilization of 0.1 normal H_2SO_4 was noted and put into the following formula:

Constant = 10.937, Reading = Volume used x Constant

Mortality percentage

Mortality percentage was recorded on weekly basis by the following formula:

$$\text{Mortality (\%)} = \frac{\text{Total No. of dead birds}}{\text{Total birds}} \times 100$$

Economics

Economics of experimental birds was calculated by the "Gross Margin Analysis"

$$\text{Gross Profit Margin} = \frac{\text{Net Sales} - \text{COGS}}{\text{Net Sales}}$$

Results

The Figure-I represents the data of total amount of feed consumed by the broiler chicks. The feed contained varying levels of protease enzyme as a supplement. There was not found any kind of statistically significant difference (P>0.05) in feed intake among all the groups. However, the birds that were given a dose of 750g/ton of feed had the highest feed intake (3836.6 g/bird), followed by birds given 250g/ton of feed (3808.3 g/bird). Birds given control feed had intake of 3780 g/bird, and the birds given 500g/ton of feed had feed intake 3741.6 g/bird. The figure-II reflects the results of live body weight (g/bird) of broiler chickens when treated with various amounts of protease enzyme in the feed. The data revealed a statistically significant difference (P<0.05) in the results among these groups. Birds that were given feed supplemented with 500g/ton experienced a notably greater increase in body weight, with a gain of 2209 grams. This was followed by birds given feed supplemented with 750g/ton, who gained 2201 grams. Birds that were given feed supplemented with 250g/ton gained 2090 grams. Whereas, those birds given the control feed gained 2053 grams. The figure-III explores the results of the FCR of broiler chickens when supplied with varying amounts of protease enzyme. The results of our study showed a significant difference (P<0.05) in the FCR in the birds that were supplemented with 500g/ton of protease in the feed (FCR=1.69) as compared to the birds that were given the control diet (FCR=1.84), and the birds that were supplemented with 250g/ton of feed (FCR=1.81). Analysis of variance revealed a non-significant difference (P>0.05) among the birds that were given a dose of 750g/ton of feed. The figure-IV indicates the carcass weight (in grams per batch) of four groups that were given varying quantities of protease enzyme in their feeds. It was found that the hens supplemented with a dose of 500g/ton of feed had a significantly higher carcass weight (1450g) as compared to the hens supplemented with 250g/ton of feed (1325.33g) and the hens of the control group (1277.67g). However, there was no significant difference in carcass weight among the hens supplemented with 750g/ton of feed (1342g) and the control group (P>0.05). The dressing percentage of four distinct groups which were supplemented with varying quantities of protease enzyme in the feed, is illustrated in Figure-V. The statistical analysis of the data of dressing percentage explained that there was no significant difference (P>0.05) among these groups. The dressing percentages for the control group, the group with a dose of 250g/ton protease in the feed, the group supplemented with a dose of protease of 500g/ton in the feed, and the group supplemented with a dose of 750g/ton protease in the feed were 62.16%, 63.37%, 65.53%, and 60.89%, respectively. The group of birds that received a supplementation dose of 500g/ton protease in the feed had the highest dressing percentage, which was 65.53%. On the other hand, the group of birds that received a supplementation dose of 750g/ton of protease in the feed had the lowest dressing percentage, which was 60.89%.

Figure-VI elaborates the proportionate weight of edible organs in four distinct groups that were given varying doses of protease enzyme in their rations. The statistical data showed a non-significant difference (P>0.05) among these groups. The control group had the highest liver weight (3.21), followed by the groups of birds supplemented with doses of 250g/ton, 750g/ton, and 500g/ton of feed. Birds in the group supplemented with a dose of protease 250g/ton of feed exhibited a comparatively larger heart weight, while birds in the group supplemented with a dose of protease 750g/ton of feed had a lower heart weight. The greatest weight of the gizzard was observed 2.31g in the group T3, while the minimum weight of gizzard was found 2.20g in the T2 group. Furthermore, the control group had the highest spleen weight (2.52). Whereas, the group of birds fed with a dose of 750g/ton of feed had the lowest spleen weight. Nevertheless, the analysis of variance revealed that there were no significant differences (P>0.05) among any of the groups in terms of the weight of all edible organs. The figure-VII expresses the findings on the mortality rate of broiler chickens that were given varying amounts of protease enzyme in their feeds. It was found that the birds in the control group had the highest mortality rate at 6.6%. The Birds supplemented with 250g/ton of feed had a mortality rate of 4.5%, while those supplemented with 750g/ton of feed had a mortality rate of 3.33%. The lowest mortality rate of 2% was observed in birds supplemented with 500g/ton of feed. The analysis of variance revealed a significant (P<0.05) difference between the T1 and T2 groups, but the T3 and T4 groups did not exhibit a significant difference (P>0.05) between each other. The nutrient digestibility percentages of broiler chicks treated with varying levels of protease enzyme are displayed in figure-VIII. The data indicates a statistically significant (P<0.05) variation in the groups regarding to the digestibility of crude protein. Broilers that were given a supplement of 500g/ton protease of feed had a significantly higher crude protein digestibility value of 76.43%. This was followed by the broilers given a protease supplement of 750g/ton of feed with a digestibility value of 73.43%. The broilers that were given a supplement of 250g/ton of protease in the feed had a digestibility value of 71.41%, and the control group had a value of 65.34%. However, no significant difference had been observed for the digestibility values (P>0.05) of dry matter, crude fat, and crude fiber while supplementing various doses of protease enzyme. Furthermore, the birds that received a supplementation of 750g/ton protease of feed had the greatest reported crude fat digestibility (71.47%), while the control group had the lowest digestibility (60.75%). In addition, birds that received a protease supplementation of 500g/ton of feed exhibited a higher level of crude fiber digestibility (37.9%), while the control group had a lowest digestibility level (29.74%) of crude fiber. Nevertheless, the control group had a high dry matter digestibility percentage of 91.15%, whereas the group supplemented with a dose of 750g/ton of feed demonstrated a lower percentage of 89.79%. Economics is displayed in (Table 4).

The total feed cost per chicken in the control group and the groups with additional protease had been found as Rs. 230.3, 231.2, 226.5, and 232.5 rupees, respectively. The aggregate revenue generated by the avian population in groups A, B, C, and D, subsequent to the marketing process, amounted to Rs. 266.5, 271.7, 286, and 286 rupees, respectively. In addition, the post-market net profit was highest in T3 group at 59.5 rupees per bird, followed by T4 group at 53.5 rupees per bird, T2 group at 40.5 rupees per bird, and the control group at 36.2 rupees per bird.

Table 4. Economics of broiler

S. No.	Particulars	Groups			
		T1	T2	T3	T4
1.	Cost of day old chick (Rs/ bird)	43	43	43	43
2.	Total Feed Consumed (kg/bird)	3.78	3.80	3.68	3.83
3.	Cost of feed (Rs/ bird)	166.32	167.2	164.56	168.52
6.	Litter cost (Rs/ bird)	6	6	6	6
7.	Vaccination cost (Rs/ bird)	5	5	5	5
8.	Miscellaneous expenditure	10	10	10	10
9.	Total expenditure (Rs/ bird)	230.3	231.2	226.5	232.5
10.	Final weight of bird (kg)	2.05	2.09	2.20	2.20
11.	Broiler sale rate (Rs/kg)	130	130	130	130
12.	Total income (Rs/kg)	266.5	271.7	286	286
13.	Net profit (Rs/bird)	36.2	40.5	59.5	53.5

Discussion

The addition of protease enzymes at a dosage of 500g per ton of feed resulted in significant improvements in various performance parameters, such as weight gain, feed conversion ratio (FCR), feed intake, carcass weight, and dressing percentage. It was observed that both lower and higher doses of protease enzymes had less pronounced effects. Additionally, the inclusion of protease enzymes efficiently improved digestibility of crude protein, crude fiber, ether extract, and dry matter. At a dose of 500g/ton of feed, feed intake was lower compared to other doses. This decrease in intake may be due to the more efficient utilization of nutrients present in the feed. The reduction in feed consumption due to protease supplementation, improved the FCR, and weight gain. These findings are according to the studies conducted by **[16-18]**. The study's current findings indicate that broiler birds supplemented with 500g/ton protease of feed exhibited increased live body weight as compared to the broiler birds supplemented with 750g/ton protease of feed, 250g/ton protease of feed, and the control group. The increased body weight of birds is potentially associated with improved feed conversion ratio and protein digestibility. According to **[18]**, chickens that were given a diet low in CP and supplemented with a protease enzyme experienced enhanced weight gain. The study conducted by **[19]** found that the supplementation of protease enzyme from 1 to 42 days of age resulted in a significant increase in average daily gain. The increased carcass weight was achieved when a dose of 500g/ton of feed was administered. This may be attributed to the improved feed conversion ratio and protein digestibility in the birds. The addition of protease enzyme to the diet of broiler chickens resulted in increment in the weight of their carcass, while also improving the efficient utilization of the nutrients, particularly the crude protein **[14, 17]**. Furthermore, the addition of external protease had a substantial impact on both the weight and the yield of the carcass, as observed by **[20]**. The present investigation indicated that the protein digestibility was notably greater in the group supplemented with protease at a medium dose rate of 500g/ton, as compared to the control group and the other groups. Protease has a crucial role in maintaining the balance of microorganisms in the intestines of poultry birds. This leads to better utilization of the nutrients, particularly crude protein and metabolizable energy of the diet. As a result, the birds have enhanced feed intake and exhibit better performance. Based on our findings, it was found that adding protease enzyme to the diet of broilers improved their performance by enhancing feed utilization and promoting the growth of beneficial gut microorganisms, as demonstrated by **[21]**. Additionally, they contribute significantly to the process of digestion and the absorption of nutrients by increasing the activity of digestive enzymes and facilitating the breakdown of nutrients that are difficult to digest **[22]**.

All parameters were assessed by using the American Association of Official Analytical Chemists. **[23]**.

Conclusion

It was concluded that the production and the economic performance of broilers had been improved at a protease dose of 500g/ton of feed as compared to the lower and higher doses.

Authors' contributions

Conducted experiments and wrote the article: AZ Kaimkhani, Designed the experiment: GA Mughal & M Urooj. Proofread the article: AZ Kaimkhani, GA Mughal & ZA Khan, Analyzed the data: AZ Kaimkhani & MN Manzoor, Checked the references: AZ Kaimkhani, GA Mughal, MN Manzoor, ZA Khan & M Urooj.

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References

1. Pakistan GO (2016-17). *Economic Survey of Pakistan*, Ministry of Finance, Planning and Development, Islamabad.
2. Statista (2019). *Production of Meat Worldwide* from 2016 to 2018 (in Million Metric Tons).
3. Khattak FM, Pasha TN, Hayat Z & Mahmud A (2006). Enzymes in poultry nutrition. *J Poul Sci* 16: 1-7.
4. Ajayi HI (2015). Effect of protease supplementation on performance and carcass weights of broiler chickens fed low protein diets. *Nig J Agric Food Environ* 11: 29-32.
5. Carvalho CC, Souza SF, Tinôco IF & Vieira MA (2009). Segurança, saúde e ergonomia de trabalhadores em galpões de frangos de corte equipados com diferentes sistemas de abastecimento de ração. *Eng Agríc* 31: 438-447.
6. Fru-Nji F, Kluenter AM, Fischer M & Pontoppidan K (2011). A feed serine protease improves broiler performance and energy digestibility. *J Poul Sci* 48: 239-246.
7. Lemme A, Ravindran VB & Bryden V (2004). Ileal digestibility of amino acids in feed ingredients for broilers. *World Poul Sci J* 60: 423-437.
8. Campbell GL & Bedford MR (1992). Enzyme application for mono gastric feeds a review. *Can J Anim Sci* 72: 449-466.
9. Wallis I (1996). Enzymes in poultry Nutrition. Technical Note, SAC. West Mains road, Edinburgh.
10. Ghazi S, Rooke JA & Galbraith H (2013). Improvement of the nutritive value of soybean meal by protease and a-galactosidase treatment in broiler cockerels and broiler chicks. *Br Poul Sci*

44: 410-418. Antalya, Turkey. *World Poul. Sci. Assoc.*, 16: 160-168.

11. Romero JB, Jong Jde & Langhout DJ (2014). Effect of a xylanase enzyme supplementation to wheat-based diets in broiler chicks about dietary factors. Proc. Sec. Europ. Symp. on Feed Enzy Noordwijkerhout, *Netherlands* 14: 95-101.
12. Morita ZR, Qiao SY & Lu WQ (1998). Effects of Enzyme Supplementation on Performance Nutrient Digestibility Gastrointestinal Morphology and Volatile Fatty Acid Profiles in the Hindgut of Broilers Fed Wheat-based Diets. *Poul Sci* 84: 875-881.
13. Scott T, Yu R & Chung TK (2013). Effects of Multiple Enzyme Mixtures on Gr Performance of Broilers Fed Corn-Soybean Meal Diets. *J App Poul Res* 13: 178-182.
14. Rada J.D, Schelle C & Kwakernaak C (2016). Wheat characteristics are related to its feeding value and the response of enzymes. Proceedings 10th European Symposium on Poultry Nutrition Antalya Turkey. *World Poul Sci Assoc* 16: 160-168.
15. Vieira SL, Freitas DM, Pena JEM, Barros & Xavier PS (2009). Performance and amino acid utilization by broilers supplemented with a novel exogenous protease. *Poul Sci* 88: 37.
16. Cowieson AJ & Ravindran V (2008). Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers growth performance and digestibility of energy minerals and amino acids. *J Food Agri Sci* 35: 547-558.
17. Rada V, Lichovníkova M, Foltyn M (2014). The effect of serine protease on broiler growth and carcass quality. *J Acta Fyt Zootech* 17:87–89.
18. Angel CR, Saylor W, Vieira SL & Ward N (2011). Effects of a mono-component protease on performance and protein utilization in 7 to 22-day-old broiler chickens. *Poul Sci* 90: 2281-2286.
19. Luan AI, Pawlik JR & Marquardt RR (2015). Improvements in nutrient retention and changes in excreta viscosities in chicks fed rye-containing diets supplemented with fungal enzymes sodium taurocholate and penicillin. *Can J Anim Sci* 68: 483-491.
20. Kamran Z, Sarwar M, Nisa M, Nadeem MA & Mahmood MA (2013). Effect of Low Protein Diets Having Constant Energy-to-Protein Ratio on Performance and Carcass Characteristics of Broiler Chickens from One to Thirty-Five Days of Age. *J Poul Sci* 87: 468-474.
21. Ritz CW, Hulet RM, Self BB & Denbow DM (1995). Effects of protein level and enzyme supplementation upon growth and rate of digesta passage of male turkeys. *Poul Sci* 74: 1323-1328.
22. Gracia MI, Aranibar MJ, Lazaro R, Medel P & Mateos GG (2003). Alpha-amylase supplementation of broiler diets based on corn. *Poul Sci* 82: 436-442.
23. AOAC (1990). Official Method of Analysis, 15th edition Association of Official Analytical Chemists, Washington D.C.