Effect of supplemented ProAct (CT) Protease enzyme on performance and the amount of protein excreted in feces of Broiler Chickens

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Abstract

Soybean and corn meal provide most of broiler chickens' protein and energy in diets. Although soybean and corn meals are highly digestible, it is yet possible to improve their digestibility of energy and protein content. This study aimed at assessing the effect of protease enzyme on performance and the amount of protein excreted in feces of broiler chickens fed on a corn-soybean based diet. 300 Ross 308 broiler chickens were randomly selected and categorized in five treatments and four repeats. All the breeding conditions such as room temperature, ventilation, etc. were the same for population. At the end of days 21 and 42, the chickens were examined for amount of feed consumption, experimental rations, and the amount of protein present in the material excreted from ileum. The results showed that the highest and lowest amounts of feed consumption were respectively related to treatments 3, and 5 and 1. The weight of treatment 4 fed on a low-level-protein meal with 200 g of external enzyme was lower than that of treatment fed on a higher-level-protein meal, but the difference was not significant. It can be concluded that through addition of enzyme to the diets containing soybean and corn meal, digestibility of energy and raw protein increases and the accessibility of this part of energy and protein for poultry is economically very important.

Key words: Protease; Chicken; Corn; soybean; broiler.

Introduction

In today's world, the problem of food and population is among the most important problems for human beings. The increasing world population from one side and food shortage from the other side is the crises threatening the world in near future. The demand for protein products is increasing rapidly and poultry-dependent production allocates the highest rate of increase. It is while porcine products are in the next rank (Raney et al, 2009). Through the past decades, the goal of breeding broiler chickens was to reach the highest genetic performance in the shortest time. To increase the productive traits, the poultry should be fed on a diet providing all their growth requirements. During the recent years, to have the highest performance of broiler chickens, the attention has been focused on using slowly growing strains, reduced stocking density, prolonged breeding period, and increased and improved poultry welfare (Doskovic et al, 2012). One of the poultry's requirements for reaching the highest genetic performance is provision of a proper protein level in diets (Bregendahl and Zimmermand, 2000).

Soybean and corn meals provide much of the protein and energy required by broiler chickens in diets. Although soybean and corn meals are highly digestible, it is yet possible to improve their digestibility of energy and protein content, because a majority of corn and soybean cell walls comprises non-starch polysaccharides (Chesson, 2001). Because of these non-starch polysaccharides, the metabolizable energy of soybean meal is considerably low in poultry (Pierson et al, 1980). In addition, about 10% of soybean meal protein is within the dry material located inside the cell wall (Chesson, 2001). If the non-starch polysaccharides were hydrolyzed by carbohydrates, these protein and energy sources could be accessible for poultry (Mandels, 1985). In fact, through the addition of carbohydrates to diets containing soybean and corn meals, energy and raw protein digestibility increases (Oloffs et al, 1999; Saki et al, 2005). Therefore, it is economically very important to make this part of energy and protein accessible for poultry. Digestion efficiency in poultry is mainly dependent on microorganisms present naturally in their gastrointestinal tract (Cowieson and Ravindran, 2008).

The undigested material could be converted into very viscous feces, leading in problems caused by moist and juicy wastes. The most important function of enzymes in birds' gastrointestinal tract is to degrade cell walls of some nutritional items in the diet, yielding a homogenous mixture of nutrients. As a result, the digestibility of nutrients (especially carbohydrates) increases (Saki et al, 2005). The problems caused by viscous materials in intestine include decreased intestinal capability to mix the contents, inhibition of cellular access to enzymes assisting the cells to degrade nutrients, lower contact of nutrients with intestine wall, decreased absorption of nutrients into the blood, excretion of undigested food and absorbed water from the body in the form of very viscous and juicy excreta, prolonged gastrointestinal transit time as a result of reduced food consumption, weaker growth and increased nutritional experimental rations. Addition of enzymes to diets results in removal of anti-nutritional factors, completion of bird's natural enzymes, increased digestibility of nutritional items present (increased digestion of non-starch polysaccharides indigestible for birds), lowered feeding costs, improved substratum quality, improved nutritional experimental rations, assistance to environmental health through decreased excretion of organic material, improved nutrient uptake, sustaining the performance of low-quality diets, lowering formulation costs, widening the spectrum of nutritional items (raw material) usage, overcoming the anti-nutritional factors in raw material, and reduced nutrient and water excretion (Saki et al, 2005; Sorbara, 2009).

Different types of interactions can occur between enzymatic supplements. For example, for carbohydrate digestion and absorption, enzymes with different activities are necessary with the ability to act on different carbohydrate substrates comprising the diet (Zanella et al, 1999; Olukosi et al, 2007; Jiang et al, 2008). Using multiple enzymes improves the bird's performance because of degradation in complex cell wall matrix by multi-carbohydrases. This leads in increased digestibility by exposing the nutrients inside the cellulose wall to digestive enzymes (Bedford, 2000). Protease enzyme plays different roles in the environment. This enzyme participates in many physiological processes such as homeostasis, apoptosis, signal transduction, reproduction and immunity. The protease also influences blood coagulation and wound healing process. However, hydrolyzing proteins to amino acids by peptidases can be introduced as a specific role. There are some intestinal proteases forming a protease system able to act on different protein sources (Kalmendal and Tauson, 2012). This study aimed at evaluating the effect of protease enzyme on performance and the amount of protein excreted in feces of broiler chickens fed on a corn-soybean based diet and reducing the costs through betted absorption of nutrients present in the diet (especially proteins), along with reducing environmental pollution caused by nitrogen release in the feces of chickens.

Materials & Methods

This investigation was conducted in a broiler chicken farm located in Miandoab, Western Azarbayjan, Iran. 300 Ross 308 broiler chickens were randomly selected and categorized in five treatments and four repeats. So that 15 chickens were held in each pan. All the breeding conditions such as room temperature, ventilation, etc. were the same for population. Treatment 1 was the positive control group and no enzyme was added to their diet and the chickens received a diet of 23% protein and 2950 Kcal energy up to day 21 and a diet of 21% protein and 3050 Kcal energy up to slaughter at day 45. Treatment 2 was the negative control group with no enzyme and fed on a diet of 20% protein and 2950 Kcal energy up to day 21 and a diet of 18% protein and 3050 Kcal energy up to day 45. Treatment 3 included the diet on Treatment 2 plus to 100 g protease per ton of meal with minimal protein. Treatment 4 had the diet on Treatment 2 plus to 200 g protease per ton of meal with minimal protein. Treatment 5 comprised the diet on Treatment 2 plus to 300 g protease per ton of meal with minimal protein. Hygienic and managerial conditions were the same for all treatments. At the end of days 21 and 42, the chickens were examined for live weight, amount of feed consumption, experimental rations, and the amount of protein present in their ileum excreta. To collect the ileum content, the chickens were slaughtered naturally two hours after mandatory feeding. Table 1 shows the transform coefficients nitrogen in protein (Deutz et al, 1995). The volume of consumed acid was according to the formula below. The protein coefficient is calculated by Kjeldahl method (Kjeldahl, 1883), showing the amount of protein.

The volume of consumed acid, acid normality 100^{14}

Sample weight 1000g

Nitrogen percent × protein coefficient = Protein percent (1)

Table1. Transform coefficients nitrogen in protein (Deutz et al, 1995).

Type of food	Nitrogen in protein (%)	Conversion ratio
Mixed	16	25.6
Meat	16	25.6
Soya	51.17	71.5
Corn	16	25.6
Rice	81.16	95.5

Results

Table 2 compares the average live weight between different treatments in three weeks and there is a significant difference between treatments 2 and three and other treatments. Between the treatments fed on higher-protein diet and also the treatment fed on low-protein diet but according to the manufacturer's suggestions, 300 g and a little more than 300 g, the protease was used, the grams of live weight in three weeks were higher than that of treatments 2 and 3 and there was no significant difference between them and the highest weight was corresponding to the treatment fed on higher protein..

Table2. Comparison of different treatments average live weight (gram) of at ages 3 and 6 weekly.

Live wt.	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5
3 weeks	0.060 ± 680^{a}	610 ± 0.075^{b}	618 ± 0.065^{b}	650 ± 0.071^{a}	660 ± 0.073^{a}
6 weeks	2050 ± 0.079^{a}	1900 ± 0.041^{b}	1910 ± 0.052^{b}	2030 ± 0.48^{a}	2030 ± 0.068^{a}

a, b and c represents the difference between the average live weight in the table 0.05 ($P < 0.05$).

In the week 6, the highest weight relates to the treatment fed on the highest protein percent and the lowest live weight relates to the treatment fed on the lowest protein percent with no enzyme. There was no significant difference between treatments 2 and 3. Among treatments 1, 4, and 5 with the highest live weight, there were not any significant differences. Among treatments 4 and five fed on a lower protein percent, and respectively with 200 g and 300 g of enzyme, no significant difference was detectable.

According to the contents of Table 3, a comparison between the grams of amount of feed consumption in six weeks for different treatments showed that the highest amount was related to treatment 2, fed on the minimum protein percent and this treatment showed significant difference from the others. It is while no significant difference was observed between other treatments. The maximum amount of feed consumption was for treatments 3 and five and the minimum amount was for treatment 1 (fed on a high-protein diet). It was also considered that there was no significant difference between treatment 1 (fed on a high-protein diet), treatment 4 (fed on a diet containing low protein percent plus to 200 g of protease), and treatment 5 (fed on a diet containing low protein percent plus to 300 g of protease). No significant difference was detected between treatment 2 (4138±0.028) and treatment 3 (4080±0.035), but they were significantly different from other treatments. Treatment 3 had the maximum amount of feed consumption (4080±0.035), which was significantly different from other treatments, except treatment 2. Among treatments 1, 4, and 5 with insignificant difference, the maximum amount of feed consumption was related to treatment 5 (4020±0.012), fed on a low protein diet supplemented with 300 g of protease.

Table3: Comparison of the mean difference between treatments feed consumption ages 3 and 6 weekly.

Live wt (gram)	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
3 weeks	1014 ± 0.065^{a}	610 ± 0.066^{b}	1020 ± 0.069^{a}	1018 ± 0.072^{a}	1020 ± 0.067^{a}
6 weeks	3865 ± 0.018^{a}	1900 ± 0.028^{c}	4080 ± 0.035^{b}	3920 ± 0.021^{a}	4020 ± 0.012^{ab}

a, b and c represents the difference between the average mean difference data in the table 0.05 ($P < 0.05$).

Table 4 summarizes the results of comparison of experimental rations percent between different treatments during three weeks. Accordingly, treatment 1 (1.49±0.05) showed the lowest experimental rations and treatment 2 (fed on a low-protein diet) showed the highest experimental rations (1.69±0.051). There were also no significant differences between treatments 1, 4, and 5. Treatments 2 and 3 had a significant difference from other treatments, but were not significantly different from each other, respectively with (1.69±0.51) and (1.65±0.048). In evaluating percent of experimental rations during six weeks, the minimum value corresponded to treatment 1 (1.90±0.059), which was not significantly different from treatments 4 and 5, but had a significant different in comparison with treatments 2 and 3. The maximum experimental rations were observed for treatment 2 (fed on a low-protein diet) (2.17±0.053), which showed no significant difference proportional to treatment 3 (2.13±0.056), but significantly different from the other treatments.

Table 4: Comparing the average conversion ratio between treatments the ages of 3 and 6 weekly.

Live weight	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
3 weeks	1.49 ± 0.051^{a}	1.69 ± 0.051^{b}	1.56 ± 0.048^{b}	1.56 ± 0.042^{a}	1.54 ± 0.057^{a}
6 weeks	1.90 ± 0.059^{a}	2.17 ± 0.053^{c}	2.13 ± 0.036^{c}	1.93 ± 0.062^{a}	1.98 ± 0.063^{ab}

a, b and c represents the difference between the average conversion ratio data in the table 0.05 ($P < 0.05$).

As summarized in Table 5 for the cadaver weight in different treatments at the age of 42 days, the maximum weight was seen for treatment 4 (fed on a low-protein diet supplemented with 200 g of protease) (1481±0.362), which did not significantly differ from treatment 5 (1451±0.2815) and treatment 1 (1435±0.318), but significantly differed from treatments 2 and 3. Treatment 2 (1235±0.312) showed the minimum cadaver weight, which was not significantly different from treatment 3 (1260±0.312), but showed a significant difference in comparison to other treatments. Treatment 2 had no significant difference from treatment 3, but showed a significant difference in comparison to other treatments.

Table 5: Comparing the average cadaver weight **Live weight** (gram) of different treatments in 42 days.

Live wt.	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
3 weeks	-	-	-	-	-
6 weeks	1435 ± 0.318^{ab}	1235 ± 0.312^{c}	1260 ± 0.312^{c}	1481 ± 0.322^{a}	1451 ± 0.285^{a}

a, b and c represents the difference between the average cadaver weight data in the table 0.05 ($P < 0.05$).

Table 6: The mean percentage of protein present in their ileum excreta different treatments ages 3 and 6 week.

Live wt.	**Treatment 1**	**Treatment 2**	**Treatment 3**	**Treatment 4**	**Treatment 5**
42 days	24 ± 0.029^{c}	23 ± 0.025^{c}	20 ± 0.018^{ab}	17 ± 0.022^{a}	15 ± 0.012^{a}

a, b and c represents the difference between the mean percentage amount of protein data in the table ($P < 0.05$).

According to Table 6, no significant difference was observable between treatment 4 (with 200 g of enzyme), treatment 5 (with 300 g of enzyme), and treatment 3 (with minimum amount of enzyme, 100 g). Treatments 1 and 2 were significantly different in comparison to these three treatments and there was also no significant difference between treatments 1 and 2.

Discussion

Nowadays, noting the remarkable content of broiler chicken breeding all over the world, most executive problems have focused on reducing the costs and increasing the performance. Along with the developments achieved, some problems had also diverted man's attention to themselves in this industry. One of these problems is environmental pollutions caused by phosphorus and nitrogen excretion by poultry. Noticing that the major phosphorus sources used in this industry are different grains and about the two thirds of total phosphorus available in these grains is in the form of phytate complex, unusable in monogastric animals because of lack of endogenous phytase and excreted in feces (Nelson, 1967), the excreted materials through wasting different nutrients, as well as increasing the production costs, endanger the environment by increasing phosphorus and nitrogen concentration in soil and water (Correll, 1998). The results summarized in Table 1 showed that the treatments fed on diets containing protein from the first day up to day 22 (23% protein) and from day 22 to the end (21% protein) had the highest live weight. Opposite to treatment 1 was treatment 2 the chickens in this treatment had the lowest live weight in the third and sixth weeks, showing the effective role of protein source in chickens' growth and development. Although the average live weight in three-week samples in treatment 5 with 300g of enzyme was a little more than that of treatment 4 with 200g of enzyme, there was no significant difference between them and this was better reflected in long-term application up to week six; so that the weight of both treatments 4 and 5 increased equally up to sixth week. It can also be concluded that in comparison with Treatment 1 fed on a higher-protein diet, the weight of treatment 4 fed on a low-protein diet with 200g of exogenous protease was lower. But this difference was not significant, showing that through correct and proper use of Pro Act protease, the lower-protein diets could be fed and the protease makes this possible by better protein hydrolysis followed by higher amino acid absorption by the birds and the bird gains the maximum absorption from minimum protein administered. The weight difference was also lower in one-, two-, and three-week treatments than that of six-week, which can be related to limited digestive enzyme production at lower ages.

According to Table 3, in Treatment 1, the average amount of feed consumption at the third and sixth weeks was lower than other treatments. This minor difference could be related to the changes in other items of diet such as higher oil content in the diet of day 22 to the end. Since most of food uptake is based on energy, but Treatments 1 and 2 were not significantly different in food consumption, showing that the protein-diluted diets irregularly increased use in diets. Noticing that the energy is to some extent equal in diets, this can cause metabolic complications such as ascites, renal failure, and abdominal accumulation of fat.

Based on the contents of Table 4, the average experimental rations were almost similar in Treatments 1, 4, and 5 with no significant difference, suggesting that the manually added protease could greatly compensate for protein shortage in the diet, resulting in better digestion and absorption of protein factors and as a result, better performance. The higher the flock age, the more was the difference in experimental rations.

It can be inferred from the results summarized in Table 5 that carcass weight was higher in Treatment 4. Treatment 4, with 200g of protease, the direct effect of protease on better and higher digestion and absorption of proteins available in the diet was observable, which improves the conditions of carcasses delivered to the market and the reason possibly lies in improved performance and physiological status of intestine and other organs such as liver and kidneys. The protease can act synergistically with pancreatic proteases and intestinal pepsin. Enzyme addition also lessens nitrogen excretion and the substratum ammonia gas minimizes and metatarsus scorch and hocks and also the amount of respiratory and dirty waste reach a minimum. Based on results displayed in Table 6 and noting that the amounts of weight and experimental rations were better in treatment 1, but the percent of omitted protein via digestive system were the highest in treatments 1 (23±0.025) and 2 (24±0.029) respectively.

These findings indicate that protease causes a minimization in the amount of nitrogen excreted in guano and this is achieved by better peptide digestion and amino acid absorption, leading in better performance and decreased unfavorable effects of nitrogen excretion in the surroundings. This is in accordance with our findings (Pierson et al, 1980). Our findings are compatible with the results reported by Angel et al. in 2011. They evaluated the protease effect and eventually, found no noticeable difference between the groups fed on high-protein diets and the diets supplemented with enzyme (Angel et al, 2011). In an investigation in 2004, enzymes were exploited to increase growth rate and improve nutritional experimental rations, in which one of the functions of enzymes was to remove anti-nutritional effects of NSP. In addition of increased nutrient usage, enzymes have other advantages including total reduction in the amount of excreted organic material because of better absorption, reduced feces moisture caused by lower viscosity, improved animal health originated from prevention of comorbidities or microflora reproduction, etc.; and these findings are compatible with the findings of the present study (Choct et al, 2004). Our findings are also consistent with the studies by Oxenboill et al., 2011 on protease effect on improved environment. During this study, protease was added to a diet with normal protein levels. The results showed reduction in fecal nitrogen excretion and also remarkable decrease in ammonia excretion (Oxenboill et al., 2011).

Eidi Vand and collaborators studied the effects of pectinase, cellulase and hemicellulase supplementation on performance and nutrient digestibility in broiler chickens fed on corn-soybean meal based diets. Chicks were given experimental diets containing 0.33, 2 and 2 U/g of diet cellulose, hemicellulase and pectinase. Combinational of these enzymes is effective in improving digestibility. Supplementation with these enzyme preparations, can improve uniformity and decrease mortality in broiler chickens fed on maize-based diets. This is in compliance with our findings (Eidi Vand et al, 2010).

In another study 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, was evaluated. Data indicate that it is possible to improve the nutritional value of a maize/soy-based diet for broiler starters through the utilization of exogenous enzymes. Energy and amino acid values of maize-based diets for broilers can be increased by supplementation with an enzyme cocktail of xylanase, amylase and protease, offering potential economic benefits to producers. Their findings were consistent with the results of our project (Cowieson and Ravindran, 2008).

Tabook and co-workers studied the effect of date fibre supplemented with an exogenous enzyme on the performance and meat quality of broiler chickens in 2006. Two experiments were conducted to evaluate the use of date fibre as a partial replacement of maize as a source of energy for growing broiler chicken. This study indicated that date fibre can be included at levels of 5% in broiler diets without affecting performance. The results are similar with the present study (Tabook et al., 2006).

The enzyme exploited in the present study might act coordinately and effectively via hydrolysis of cell wall non-starch polysaccharides present in soybean and corn meal. Based on improved digestibility of raw protein and dry material in the diet, diminution in raw protein level content seems rational. Using Pro Act enzyme properly and at appropriate amounts also set the stage for application of lower-protein diets through better hydrolysis of proteins, followed by higher amino acid absorption by the chickens. These oral enzymes decrease feeding costs and also nitrogen excretion. As mentioned, most of corn and soybean cell wall are made up of non-starch polysaccharides. If these non-starch polysaccharides are degraded by enzymes, this source of protein and energy can be accessible for the chickens.

Conclusion It can be concluded that, by addition of enzymes to diets containing soybean and corn meals, digestibility of energy and protein increases and the accessibility of this part of energy and protein for poultry are economically very important.

References

1) Angel CR, Vieir SL, Iwaniuk M, Ward NE, 2011. Effects of a Mono Component Protease on True Ideal Amino Acid Digestibility of Selected Ingredients for turkey Poults, Department of Animals and Avian Science, University of Marryland, Animal Feed Science, 61: 224.

2) Bedford MR, 2000. Exogenous Enzymes in Monogastric Nutrition-Their Current value and Future Benefits. Animal Feed Science and Technology, 86: 11-13.

3)Bregendahl K, Zimmermand SJL, 2000. Effect of Low-Protein Dieted on Growth Performance and Body Composition of Broiler Chicks. Poultry Science, 81: 1156-1167.

4) Chesson A, 2001. Non-Starch Polysaccharide Degrading Enzymes in Poultry Diets: Influence of Ingredients on the Selection of Activities. World’s Poultry Science Journal, 57: 251-263.

5) Choct M, Kocher A, Waters DLE, Pettersson D, Ross G, 2004. A Comparison of Three Xylanases on the Nutritive Value of 2 Wheats for Broiler Chickens. British Journal of Nutrition, 92: 53-61.

6) Correll DL, 1998. The Role of Phosphorus in the Eutrofication of Receiving Waters. Journal of Environmental Quality, 27: 261-266.

7) Cowieson AJ and 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. British Poultry Science, 49: 37-44.

8) Deutz NE, Ten Have GA, Soeters PB, Moughan PJ, 1995. Increased intestinal amino-acid retention from the addition of carbohydrates to a meal. Clinical Nutrition, 14: 354-64.
9) Doskovic V, Bogosavljevic Z, Pavlovski B, Milosevic Z, Skrbic S, Radonjac V, 2012. The Effect of Protease on Productive and Slaughter Traits in Broiler Chickens. Biotechnology in Animal Husbandry, 28: 817-826.
10) Eidi Vand S, Mahdavi A, Alami F, Hosseini A, Mostafa Tehrani A, Ghasemloo V, 2010. Effect of pectinase, cellulase and hemicellulase on performance and digestibility of diets in broilers. Veterinary Journal (Pajouhesh & Sazandegi), 92: 13-22.
11) Jiang Z, Zhou Y, Lu F, Han Z, Wang T, 2008. Effects of Different Levels of Supplementary Alpha Amylase on Digestive Enzyme Aactivities and Pancreatic Amylase mRNA Expression of Young Broilers. Asian-Australian Journal of Animal Science, 21: 97-102.
12) Kalmendal R, Tauson R, 2012. Effects of Xylanase and Protease, Individually or in Combination and an Ionophore Coccidistat on Performance, nutrient utilization and intestinal morphology in broiler chickens fed a wheat –Soybean Meal- Based Diet. Department of animal Nutrition and management Swedish University of Agriculture Science. 24: 130-136.
13) Kjeldahl J, 1883. Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern" (New method for the determination of nitrogen in organic substances), Zeitschrift für analytische Chemie, 22: 366-383.
14) Mandels M, 1985. Application of Cellulases. Biochemical Society Transactions, 13: 414-415.
15) Nelson TS, 1967. The Utilization of Phytate by Poultry-A review. Poultry Science, 46: 862-871.
16) Oloffs K, Samli E, Jeroch H, 1999. Effect of Supplementation of Non-Starch-Polysaccharide (NSP)Hydrolyzing Enzymes and Wheat Variety on Pre cecal and Faecal Digestibility as Well as Metabolizable Energy of Lying Hens. Proceedings 12th European Symposium on Poultry Nutrition. WPSA Dutch Branch, Veldhoven, The Netherlands.
17) Olukosi OA, Cowieson AJ, Adeola O, 2007. Agerelated Influence of a Cocktail of Xylanase, Amylase, Protease or Phytase Individually or in combination in broilers. Poultry Science, 86: 77-86.
18) Oxenboll KM, Pontoppidan K, Fru-Nji F, 2011. Use of Protease in Poultry Feed Offers Promising Environmental Benefits. International Journal of Poultry Science, 10: 824-848.
19) Pierson EM, Potter LM, Brown RD, 1980. Amino acid Digestibility of Dehulled Soybean Meal by Adult turkeys. Poultry Science, 59: 355-360.
20) Raney TS, Gerosa K, Shoet J, Skoet H, Steinfeld A, Mcleod C, Opio cluff M, 2009. The State of Food and Agriculture. Livestock in balance. FAO.
21) Saki, A.A., Mazugi, M.T., & Kamyab A, 2005. Effect of Mannanase on Broiler Performance, Ileal and In-vitro Protein Digestibility, Uric Acid and Litter Moisture in Broiler Feeding. International Journal of Poultry Science, 4: 21-26.
22) Sorbara JOB, Murakami AE, Nakage ES, Piracés F, Potença A, Holanda Guerra RL, 2009. EnzymaticPrograms for Broilers. Brazilian Archives of Biology and Technology, 52: 233-240.
23) Tabook NM, Kadim IT, Mahgoub O, Al-Marzooqi W, 2006. The effect of date fibre supplemented with an exogenous enzyme on the performance and meat quality of broiler chickens. British Poultry Science, 47: 73-82.
24) Zanella I, Sakomura NK, Silversides FG, Fiqueirdo A, Pack M, 1999. Effect of Enzyme Supplementation of Broiler Diets Based on Corn and Soybeans. Poultry Science, 78: 561-568.