

Supplementing diets for broilers that are low in crude protein and amino acids with protease

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(Submitted 18 February 2020, Accepted 4 July 2020, Published online 20 August 2020)

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Abstract

The study evaluated supplementation of broiler diets with a protease enzyme. Six hundred Ross 308 female broiler chicks were acquired at one day of age and randomly distributed to three treatments with five replicates of 40 chicks each. The experimental treatments consisted of i) a positive control diet with normal levels of crude protein, digestible lysine and digestible sulphur containing amino acids (PC); ii) a negative diet with reduced levels of crude protein, digestible lysine and digestible sulphur containing amino acids (NC); and iii) the NC diet augmented with Ronozyme ProAct protease at 200 mg/kg (NC+ENZY). Compared to the other diets, feeding the PC produced the highest final bodyweight (BW), bodyweight gain (BWG), and feed intake (FI) and best feed conversion ratio (FCR) over 0 - 42 days. The PC diet also produced the highest hot and cold carcass yields, ileal protein digestibility and improved the serum biochemistry parameters. Relative to the NC, the NC+ENZY diet increased BW, BWG and FI, improved FCR, increased ileal protein digestibility, hot and cold carcass yields, and the crude protein content of meat. However, it decreased serum triglyceride and very low density lipoprotein (VLDL) cholesterol levels and the crude fat content of meat of broilers. As a result, protease supplementation at the level of 200 mg/kg may not remove the detrimental effects that are caused by a 6% reduction in crude protein and digestible amino acids on performance, hot and cold carcass yields and ileal digestibility of crude protein of broilers.

Keywords: broiler, growth performance, low crude protein diet, meat, nutrient digestibility, protease, serum biochemistry parameters

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Introduction

Broiler breeding plays an important role in meeting the animal protein requirements of the increasing human population in the world and in Turkey. In recent years, the goal of the broiler industry has been to obtain faster growth and high-quality meat production in the minimal time (Dosković *et al.*, 2013). For this purpose, broilers should be fed on diets that meet all their nutrient requirements and have high digestibility and biological values (Dosković *et al.*, 2013). One of the most important nutritional requirements for optimum performance of broilers is to ensure adequate dietary crude protein levels. However, some studies have evaluated practices that allow a reduction of dietary crude protein level, since this would not only decrease nitrogen excretion into the environment (Rada *et al.*, 2016; Mohammadigheisar & Kim, 2018), but reduce diet costs (Dosković *et al.*, 2013; Mohammadigheisar & Kim, 2018). Feed costs account for about 60% - 70% of broiler production (Dosković *et al.*, 2013).

Protein is the second most important and expensive component of poultry diets. Protein in modern broiler production is supplied predominantly from soybean meal (Ndazigaruye *et al.*, 2019). Soybean meal is a protein source feedstuff with highly digestible protein and a balanced amino acid profile. It meets about 80% of the protein and amino acid requirements of all poultry types at all life stages (Ndazigaruye *et al.*, 2019). But the digestibility of protein of soybean meal must be improved because its nutritional value is generally reduced by the presence of anti-nutritional factors such as trypsin inhibitors, lectins and non-starch polysaccharides (Dessimoni *et al.*, 2019). Moreover, synthesized amounts of protease in the gastrointestinal system could improve protein hydrolysis in the presence of anti-nutritional factors and could be sufficient to optimize protein utilization by increasing the efficiency of amino acid consumption in poultry (Dosković *et al.*,

2013; Mohammadigheisar & Kim, 2018). Besides, some soy protein is passed through the gastrointestinal tract without being completely digested (Freitas *et al.*, 2011; Rada *et al.*, 2016) resulting in environmental pollution (Law *et al.*, 2018). The supplementation of synthetic amino acids and exogenous protease enzyme played a positive role in reducing the dietary crude protein level in a lower cost of production without excessive excretion of nitrogen (Mohammadigheisar & Kim, 2018).

Chickens that are fed the standard levels of dietary protein can synthesize non-essential amino acids from excess essential amino acids. However, this means that fewer essential amino acids are available for non-essential amino acid synthesis when diets with low amino acid are used (Ndazigaruya *et al.*, 2019). In recent years, the use of synthetic amino acids in low crude protein (LP) diets has been studied. But despite supplementation with synthetic amino acids, at excessive levels LP diets still have negative effects on growth performance, nitrogen digestibility, and production costs (Law *et al.*, 2018). For these reasons, research has concentrated on studies in which the protease enzyme is supplemented to LP diets. Supplementation with single exogenous protease increased the apparent amino acid and protein digestibility effectively in broiler diets, especially LP diets (Law *et al.*, 2018). Also, protease supplementation decreased the negative effects of lectins and trypsin inhibitors by increasing amino acid utilization in poultry (Du Plessis & Van Rensburg, 2014). But the results of studies conducted with protease supplementation are inconsistent because of the diversity of feed ingredients, the types and definitions of protease, and the differences in methodology (Rada *et al.*, 2016; Mohammadigheisar & Kim, 2018). In recent years, several studies have investigated the effects on performance and nutrient digestibility of protease supplementation to LP diets for broilers (Favero *et al.*, 2009; Fidelis *et al.*, 2010; Angel *et al.*, 2011; Freitas *et al.*, 2011; Rada *et al.*, 2014; Mahmood *et al.*, 2017; Law *et al.*, 2018; Mohammadigheisar & Kim 2019). However, little research has focused on the effects on growth performance of protease supplementation to diets that are reduced in crude protein and amino acids (Manangi *et al.*, 2009; Rosa *et al.*, 2009; Fru-Nji *et al.*, 2011; Ndazigaruya *et al.*, 2019).

Therefore, the study investigated the effects of protease supplementation at the level of 6% to diets with reduced crude protein, digestible lysine and digestible total sulphur amino acids on growth performance, nutrient digestibility, nutrient contents of meat and certain serum biochemistry parameters of broilers.

Material and Methods

The experimental protocols for the management and care of animals were approved by the Local Ethics Committee for Animal Experimentation at the University of Tokat Gaziosmanpasa in Tokat, Turkey (Resolution No. 2009-HADYEK-007). Six hundred one-day-old Ross 308 female broilers that were supplied from a commercial hatchery (Anadolu Ross, Ankara, Turkey) were used in a 42-day trial. The broiler chicks were weighed, wing-banded and distributed randomly to three treatments with five replicates of 40 chicks each. From hatching until six weeks old, the chicks were kept on floor pens bedded with fresh wood shavings as litter. Temperature was kept at 32 °C for the first week, 28 °C for the second week and thereafter at 21 °C. A fluorescent lighting schedule of 23 hours light and 1 hour darkness with an average light intensity of 20 lux was used. The diets, in the form of mash, and drinking water were provided ad libitum throughout the experiment.

The ingredients were ground through a 1-mm screen in preparation for chemical analysis. Prior to formulating the diet, these ingredients were analysed for crude protein (CP), ether extract, starch, and total sugar contents (AOAC, 2007). Metabolizable energy (ME) was calculated based on the analysed values of feedstuffs (WPSA, 1989). The contents of digestible lysine and digestible total sulphur amino acids (methionine and cystine) were analysed in Degussa in Turkey (Istanbul, Turkey). All diets were formulated according to phase feeding practices as chickens advanced in age and weight (Ross 308, 2009). The starter period lasted from 0 to 10 days, the grower period from 11 to 28 days and the finisher period from 29 to 42 days.

The experimental diets included PC: a positive control diet with normal crude protein, digestible lysine and digestible sulphur containing amino acids (methionine+cystine); NC: a negative diet, which had 6 % less crude protein, digestible lysine and digestible sulphur containing amino acids; and NC+ENZY: NC supplemented with protease at 200 mg/kg. The ingredients and nutrient composition of the positive and negative control diets are presented in Table 1.

Table 1 Ingredients and chemical composition (dry matter basis, g/kg) of the positive and negative control diets for rearing broiler chickens

Ingredients	Days					
	0 - 10		11 - 28		29 - 42	
	Positive control diet	Negative control diet	Positive control diet	Negative control diet	Positive control diet	Negative control diet
Corn	553.75	602.50	549.23	579.10	607.40	643.20
Soybean meal	350.0	310.0	340.0	324.5	292.7	262.6
Fish meal	20.3	24.7	16.5	3.4		
Vegetable oil	35.0	25.4	57.6	54.8	60.3	54.4
Dicalcium phosphate	19.4	16.5	17.2	18.9	19.4	19.4
Limestone	9.7	9.0	9.0	8.8	8.7	9.0
Salt	2.7	2.7	2.9	3.2	3.3	3.3
Vitamin-trace mineral premix ¹	2.5	2.5	2.5	2.5	2.5	2.5
DL-Methionine	3.3	3.1	2.8	2.7	2.8	2.6
L-Lysine	1.4	1.4	0.25	0.6	1.1	1.4
L-Threonine	1.2	1.6	0.5	1.1	0.7	1.1
Cholin chloride	0.75		1.52		1.10	
Sodium Bicarbonate		0.6		0.4		0.5
Chemical composition (calculated)						
Dry matter	884.7	882.3	885.5	885.6	885.6	883.9
Crude protein	230.4	216.3	220.7	207.1	207.1	178.5
Metabolizable energy (Kcal/kg)	3025	3025	3152	3152	3201	3203
Calcium	10.0	10.0	9.0	9.0	9.0	9.0
Available phosphorus	5.0	9.0	4.5	4.5	4.5	4.5
Digestible methionine+cysteine	9.5	8.9	8.7	8.2	8.0	7.5
Digestible lysine	12.8	12.0	11.5	10.8	10.2	9.6
Digestible threonine	8.6	8.6	7.7	7.7	6.8	6.8
Digestible tryptophan	2.5	2.4	2.4	2.3	2.1	1.9
Digestible arginine	14.4	13.2	13.7	12.9	11.8	10.9
Digestible leucine	17.1	16.1	16.3	15.5	14.4	13.7
Digestible isoleucine	9.1	8.2	8.6	8.0	7.3	6.8
Digestible valine	9.8	9.0	9.2	8.6	7.9	7.4
Crude fat	63.7	55.1	84.8	81.3	86.9	81.8
Crude fibre	22.9	22.3	22.3	22.4	21.7	21.4
Crude ash	34.4	32.0	32.2	29.6	27.2	25.6
Sodium	1.6	1.6	1.6	1.6	1.6	1.6

¹ Vitamin-trace mineral premix/kg diet: vitamin A: 12 000 IU, vitamin D₃: 1 500 IU, vitamin E: 50 mg, vitamin K₃: 5 mg, vitamin B₁: 3 mg, vitamin B₂: 6 mg, vitamin B₆: 5 mg, vitamin B₁₂: 0.03 mg, niacin: 25 mg, calcium-d-pantothenate: 12 mg, folic acid: 1 mg, D-biotin: 0.05 mg, apo-carotenoic acid ester: 2.5 mg, choline chloride: 400 mg, manganese: 80 mg, iron: 60 mg, zinc: 60 mg, copper: 5 mg, cobalt: 0.2 mg, iodine: 1 mg, selenium: 0.15 mg

The enzyme (Ronozyme® Proact, DSM Nutritional Products Ltd, Istanbul, Turkey) that was used in this study was a purified mono component serine protease produced by submerged fermentation of *Bacillus licheniformis*, which contained transcribed genes from *Nocardiopsis prasina*. The enzyme activity for this protease is measured in protease units, with 1 unit being defined as the amount of enzyme that releases 1 µmol of p-nitroaniline from 1 µM substrate per minute at pH 9.0 and 37 °C. The product, which consisted of

of 75,000 protease units/g of enzyme, was added according to the manufacturer's recommendation at 200 ppm of protease (15,000 protease units/kg) (Freitas *et al.*, 2011).

Throughout the experiment, broilers were handled according to the principles for the care of animals in experimentation (Ross 308, 2009). During the 42-day period, their growth performance was evaluated by recording BWG, FI, and FCR. Bodyweight was recorded at the beginning of the experiment then weekly. Feed supplied and left over were recorded weekly. The FI data were adjusted for mortalities. Feed conversion ratio was calculated weekly as a ratio of FI to BWG. Mortality was recorded as it occurred. The effects of treatments on initial and final BWs and BWGs, FIs, and FCR of broilers from hatching to 42 days are shown in Table 3.

At the end of the experiment, 15 broiler chickens from each group whose BWs were similar to the group average were selected, legbanded and weighed after eight-hour fasting. A sodium pentobarbital injection (100 mg/kg) was applied as anaesthesia before slaughter. A total of 45 chickens were slaughtered by severing the jugular vein to determine the pre-slaughter BW, the relative weight of certain digestive organs, and hot and cold carcass yields. When the broilers were completely immobilized, carcasses were scalded at 55 °C to 60 °C for 45 seconds and defeathered in a rotary plucker. The carcasses were immediately plucked, processed (removal of head and feet), eviscerated, weighed and then chilled overnight in a refrigerator (+4 °C). The measurements included hot and cold carcass yields. The weights of the proventriculus, spleen, gizzard, heart, abdominal fat, bursa fabricius, liver, small intestine, and pancreas were measured individually to the nearest ± 0.001 g and values were recorded. These weights were calculated as a percentage of the pre-slaughter BW of the chickens. The carcasses were vacuum packed and stored in a freezer at -80 °C until required for analysis of dry matter, crude protein, crude fat and crude ash contents. The carcasses were removed from the freezer, thawed and minced with a chopper. The dry matter, crude protein, crude fat and crude ash contents of meat were determined (AOAC, 2007).

At the end of the experiment (42 days), blood samples from the 45 chickens were collected in test tubes without anticoagulant and centrifuged at 3000 rpm for 15 min. at +4 °C. After centrifugation, the serum was removed and stored in Eppendorf tubes at -80 °C until analysis. Serum samples were analysed with an audit autoanalyser using its test kits for total cholesterol, VLDL cholesterol, triglyceride, total protein, uric acid and NH₃ at the biochemistry laboratory of Medicine Faculty of Tokat Gaziosmanpasa University.

Fifty broilers were raised in metabolic cages from 35 days to 42 days old as an adjustment period to determine the ileal digestibility of crude protein. On day 35, 0.3% chromium oxide was added to all diets as an indigestible marker to determine the ileal digestibility of crude protein. On the 42nd day, the chickens were slaughtered and after washing the ileum with bidistile water, one sample pool was formed from the 10 ileum contents of each treatment. As a result, 15 ileum sample pools for three treatment groups were formed. The contents of the ileum (from the Meckels diverticulum to the ileosecal connection) were collected and weighed, lyophilized, milled and sieved through a 0.5 mm diameter sieve and stored at -80 °C until analysis. All diets and ileum samples were analysed in triplicate for crude protein (AOAC, 2007) at Agricultural Faculty, Department of Animal Science Laboratory, Tokat Gaziosmanpasa University. Chromium was analysed with inductively coupled plasma - optical emission spectrometry (ICAP 7000 Series, Waltham, MA USA) (Williams *et al.*, 1962). In vitro protein digestibility was calculated as:

$$\text{Percent digestibility} = 100 \left(1 - \frac{N_i \cdot C_d}{N_d \cdot C_i} \right)$$

Where: N_i is the concentration of protein in the contents of the ileum,

N_d is the concentration of protein in the diet,

C_d is the concentration of chromium in the diet and

C_i is the concentration of chromium in the contents of the ileum (Mohammadigheisar & Kim, 2018).

A linear model (Release 6.1, SPSS Inc., Chicago, Illinois, USA) for one-way analysis of variance was applied to data from the experiment. Significant differences between treatment means were separated using Duncan's multiple range test (Duncan, 1955). Results were presented as least square means and standard error of means. All statements of significance were based on $P < 0.05$.

Results and Discussion

The PC diet increased final BW ($P < 0.001$), BWG ($P < 0.001$) and FI ($P < 0.05$) and improved the FCR ($P < 0.01$) of broilers compared with the other diets. Moreover, the NC+ENZY diet enhanced final BW ($P < 0.001$), BWG ($P < 0.001$), and FI ($P < 0.05$), and improved FCR ($P < 0.01$) of broilers compared with the NC diet. The effects of the diets on the final BW and BWG, FI and FCR of broilers at 0 - 6 weeks are given in Table 2.

The current results agree with the findings of Manangi *et al.* (2009), Rosa *et al.* (2009) and Fru-Nji *et al.* (2011), who reported that protease supplementation to a diet with reduced crude protein and digestible amino acid contents at the level of 5% or 6% significantly improved the growth performance of broilers compared with the diet without protease supplementation. Similarly, the results for growth performance agree with the findings of Mohammadigheisar and Kim (2018) feeding chickens the LP diet had negative effects on BWG and FCR compared with the PC diet and the LP diet supplemented with protease. In addition, Law *et al.* (2018) noted that reducing dietary crude CP linearly reduced BWG and reduced the FCR of broilers, while protease supplementation improved their BWG and FCR. The improvements in BWG and FCR of broilers fed the NC+ENZY diet in the current trial might have been derived from the improvement in digestibility for most essential amino acids, which was caused by the supplementation of protease to the diet (Mohammadigheisar & Kim, 2018). Similarly Angel *et al.* (2011) reported that protease supplementation at a concentration of 200 ppm to the LP diet improved the BWG and FCR of broilers compared with the PC diet. The NC diet in the current trial decreased FI significantly compared with the other diets ($P < 0.05$). This may be because one or more amino acids are regulating FI rather than CP concentrations per se (Sklan & Plavnik, 2002; Law *et al.*, 2018)

Table 2 Effects of experimental diets on the final bodyweight, bodyweight gain, food intake, and food conversion ratio of broilers from 0 to 6 weeks

Diet	Initial bodyweight, g	Final bodyweight, g	Gain, g	Feed intake, g	Feed Conversion ratio
Positive control	41.85	2279.28 ^a	2237.43 ^a	3821.46 ^a	1.710 ^a
Negative control	41.85	1951.78 ^c	1909.93 ^c	3672.35 ^c	1.927 ^c
Negative control with protease ¹	41.84	2040.37 ^b	1998.53 ^b	3777.30 ^b	1.891 ^b
SE	0.02	43.43	43.44	26.73	0.03
<i>P</i> -value	0.99	<0.01	<0.01	0.04	<0.01

^{a,b,c} Within a column, means with a common superscript did not differ at $P = 0.05$

¹ protease supplemented at 200 mg/kg

The PC diet increased the uric acid ($P < 0.01$), total protein ($P < 0.05$), total cholesterol ($P < 0.05$), triglyceride ($P < 0.05$), and VLDL cholesterol contents ($P < 0.05$) and reduced the NH_3 content ($P < 0.01$) of serum compared with the other diets.

The NC diet ($P < 0.05$) decreased the serum total protein and uric acid ($P < 0.01$) levels, and increased the serum triglyceride level compared with the NC+ENZY diet ($P < 0.05$). These results agree with those of Law *et al.* (2018), who reported that reducing the crude protein level decreased serum total protein and uric acid significantly, and increased the serum triglyceride level. The decrease in uric acid could be associated with a deficiency in digested and consumed amino acids owing to the LP diet because uric acid is a by-product of protein catabolism (Law *et al.*, 2018). The effects of the diets on the serum biochemistry parameters of broilers are summarized in Table 3.

In the current study, protease supplementation to a diet with low crude protein affected the serum uric acid level of broilers similarly to of broilers fed an LP diet. This finding did not concur with the results of Law *et al.* (2018), who reported that an LP diet supplemented with protease decreased the serum uric acid level of broilers significantly compared with the crude protein diet without protease.

The effects of the experimental diets on the relative weights of internal organs and carcass yields of broilers are given in Table 4.

The PC diet decreased the relative weights of the proventriculus ($P < 0.01$), gizzard ($P < 0.01$), and abdominal fat ($P < 0.05$), and increased the relative weight of the liver ($P < 0.05$) of broilers compared with the other diets (Table 5). The NC+ENZY diet did not influence the relative weight of the livers of broilers significantly compared with the NC diet. This finding agrees with the results of Ndazigaruye *et al.* (2019), who reported that protease supplementation to the LP diet did not significantly affect the relative weight of the livers of broilers compared with the unsupplemented LP diet. Moreover, the treatments did not affect the relative weight of the pancreas. This result concurs with the findings of Ndazigaruye *et al.* (2019), who reported that neither CP nor protease enzyme influenced the relative weight of the pancreas of broilers at 35 days. In addition, in the current study, the NC diet increased the relative weight of abdominal fat significantly

compared with the other diets ($P < 0.05$). This situation may be derived from a higher calorie to protein ratio in low protein diets because excess energy is diverted to abdominal fat deposition (Law *et al.*, 2018). The current result for the relative weight of abdominal fat concurs with the findings of Law *et al.* (2018), who reported that the rate of abdominal fat deposition was significantly higher in birds fed the LP diet compared with the LP diet supplemented with protease. These findings do not agree with Freitas *et al.* (2011), who pointed out that the diet with normal protein did not influence the abdominal fat weight of broilers significantly compared with the diet with reduced crude protein at the level of 4.4% and an LP diet supplemented with protease at 200 ppm.

Table 3 Effects of experimental diets on the serum biochemistry parameters of broilers

Serum constituents	Positive control	Negative control	Negative control with protease	SE	P-value
Uric acid, mg/dl	2.800 ^a	2.614 ^b	2.629 ^b	0.129	0.008
Total protein, g/l	3.220 ^a	2.864 ^b	2.993 ^b	0.070	0.011
Total cholesterol, mg/dl	167.867 ^a	151.643 ^b	152.267 ^b	4.248	0.021
Triglyceride, mg/dl	49.933 ^a	41.214 ^b	37.067 ^c	3.243	0.025
VLDL cholesterol, mg/dl	10.533 ^a	8.214 ^b	7.400 ^c	0.669	0.013
Ammonia, $\mu\text{mol/L}$	346.787 ^c	377.693 ^a	356.593 ^b	16.893	0.008

^{a,b,c} Within a row, means with a common superscript did not differ at $P=0.05$

¹ protease supplemented at 200 mg/kg; VLDL: very low density lipoprotein

Table 4 Effects of experimental diets on the relative weights of internal organs and carcass yields of broilers

Organ, %	Positive control	Negative control	Negative control with protease	SE	P-value
Proventriculus	0.373 ^c	0.446 ^a	0.413 ^b	0.009	0.002
Spleen	0.097	0.109	0.115	0.006	0.448
Gizzard	2.085 ^b	2.571 ^a	2.555 ^a	0.059	0.002
Heart	0.457	0.474	0.467	0.009	0.751
Bursa fabricius	0.242	0.252	0.236	0.009	0.747
Liver	2.310 ^a	2.070 ^b	2.071 ^b	0.064	0.021
Small intestine	3.654	3.641	3.629	0.112	0.996
Pancreas	0.193	0.204	0.201	0.007	0.794
Carcass,					
Abdominal fat	1.835 ^c	2.296 ^a	1.905 ^b	0.077	0.026
Hot yield	72.019 ^a	70.127 ^c	71.215 ^b	0.263	0.010
Cold yield	71.674 ^a	69.633 ^c	70.714 ^b	0.262	0.004

^{a,b,c} Within a row, means with a common superscript did not differ at $P=0.05$

¹ protease supplemented at 200 mg/kg;

The PC diet increased hot ($P < 0.05$) and cold ($P < 0.01$) carcass yields compared with the other diets. Also, the NC+ENZY diet enhanced hot ($P < 0.05$) and cold ($P < 0.01$) carcass yields of broilers significantly compared with the NC diet. The results for the carcass yield of broilers agree with the findings of Law *et al.* (2018), who reported that carcass yield was decreased when the broilers were fed the LP diet. There are conflicting reports on the effect of protease supplementation on the carcass yield of broilers. Abudabos (2012) noticed an increase in carcass yield, whereas Rehman *et al.* (2017) pointed out that there was no significant effect on carcass yield. The improvement in hot and cold carcass yields because of protease

supplementation is associated with the increased utilization and deposition of protein compared with the LP diet (Lauri *et al.*, 2003; Law *et al.*, 2018).

Treatment effects on ileal protein digestibility were highly significant ($P < 0.001$). Broilers fed the PC diet had higher ileal digestibility of crude protein at 42 days of age ($83.6 \pm 0.7\%$) than the birds that were fed the NC diet ($78.6 \pm 0.7\%$). Likewise, ileal protein digestibility by the birds fed the NC+ENZY diet ($82.6 \pm 0.7\%$) was higher in comparison to those fed the NC diet. Supplementation of protease to the LP diet influenced the digestibility of crude protein positively (Borda-Molina *et al.*, 2019). This positive effect might have derived from the ability to target protease inhibitors and the cereal portion of the diet (Liu *et al.*, 2013; Mohammadigheisar & Kim, 2018).

The current findings for ileal CP digestibility concur with those of Mahmood *et al.* (2017), who noted that the diet supplemented with protease improved apparent total tract digestibility for nitrogen significantly. However, this finding did not concur with the results of Angel *et al.* (2011) and Freitas *et al.* (2011), who reported that protease supplementation to an LP diet improved the ileal digestibility of crude protein significantly compared with that of broilers fed a diet with normal crude protein. Mohammadigheisar & Kim (2018) also reported that protease supplementation to the LP diet did not affect the CP digestibility significantly compared with the normal and low CP diets.

The PC diet increased the dry matter ($P < 0.05$), crude protein ($P < 0.001$) and crude ash ($P < 0.01$) contents of the meat (Table 5). However, it decreased the crude fat content of meat ($P < 0.01$) compared with the NC diet. The NC+ENZY diet increased the crude protein content of the meat significantly compared with the NC diet ($P < 0.001$). Similarly the PC diet influenced the dry matter, crude protein and crude fat contents of meat compared with the NC+ENZY diet. The improvement in meat protein content with protease supplementation might have been derived from the increased utilization and deposition of protein (Lauri *et al.*, 2003; Law *et al.*, 2018).

Table 5 Effects of experimental diets on the proximate analysis of meat from broilers

Variable	Positive control	Negative control	Negative control with protease	SE	<i>P</i> -value
Dry matter, %	25.23 ^a	25.03 ^b	25.11 ^{ab}	0.036	0.048
Crude protein, %	21.42 ^a	20.34 ^b	21.25 ^a	0.152	0.000
Crude fat, %	3.01 ^b	3.10 ^a	3.02 ^b	0.016	0.009
Crude ash, %	0.93 ^a	0.84 ^b	0.89 ^b	0.013	0.008

^{a,b} Within a row, means with a common superscript did not differ at $P = 0.05$

¹ protease supplemented at 200 mg/kg;

Conclusion

Protease supplementation at the level of 200 mg/kg may not remove the detrimental effects caused by a 6% reduction in crude protein and digestible amino acids on growth performance, hot and cold carcass yields and ileal digestibility of crude protein in broilers.

Acknowledgments

This work was supported financially completely by Fundamental Research Funds for Tokat Gaziosmanpasa University (grant number. 2015/120).

Authors' Contributions

The authors developed the original hypotheses, designed the experiments, collected the data, conducted the statistical analyses, collaborated in interpreting the results, wrote the initial draft and finalized the manuscript. All authors have read and approved the finalized manuscript.

Conflict of Interest Declaration

There is no conflict of interest.

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