RESEARCH ARTICLE

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Influence of Dietary Protease on Growth Performance and Carcass Yield of Indian River Meat Broiler Chickens

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ABSTRACT

The aim of the present study was to evaluate the effect of a multi-component dietary protease complex on the growth performance and carcass yields of commercial broiler chickens. A total of 630 Indian River broiler DOCs were considered and assigned to five dietary treatments namely: 1) Positive control (PC), a standard diet with no supplemental protease, 2) Negative control (NC), a diet similar to PC but reduced in CP, digestible EAA, and ME based on supplier's nutrient matrix recommendation, 3) PC+125 ppm protease (PC+125), 4) NC+125 ppm protease (NC+125), and 5) NC+200 ppm protease (NC+200). The birds were fed experimental diets for 28 days. Body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and survival rate were recorded weekly. On day 28, three birds per treatment were sacrificed for carcass yield analysis. FI was similar among the treatment groups except in the 2nd week where broilers fed PC+125, NC+125, and NC+200 diets had significantly higher (*P*<0.05) FI than those fed the PC diet. In terms of BW and BWG, there were no differences among the treatment groups at 1st and 2nd weeks, however, at 3rd and 4th weeks, broilers fed the PC+125, NC+125, and NC+200 diets were significantly heavier (*P*<0.05) than those fed the PC and NC diets. A similar trend was also observed in FCR. Among the carcass yield parameters, the percentage weights of thigh, breast, and heart in birds fed the NC+200 diet were significantly increased (*P*<0.05) as compared to those fed the NC diet, while results for PC, PC+125, and NC+125 were intermediate between the two treatment groups. Overall, the results of present study demonstrated that the supplementation of protease complex either on a standard or reduced diet may improve the overall production performance of commercial broiler chickens and offers benefits on certain aspects of carcass yield.

Keywords: broiler chickens, carcass yield, growth performance, protease.

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I. Introduction

Poultry nutritionists throughout the world are exerting great efforts to improve the efficiency of feed utilization to reduce production cost and make poultry farming profitable. Feedstuffs contain certain natural compounds that animals and birds cannot digest or that interfere with their normal digestive process. A frequent reason for such problems is that the animals and birds are unable to produce necessary enzymes at adequate levels to degrade these compounds [1].

Protein is the second major component (next to energy) but the most expensive among all the nutrients in poultry feed. Despite the fact, a considerable portion (18–20%) of dietary protein passes through the gastrointestinal tract without being completely digested and absorbed [2]–[3], which may cause a considerable loss in poultry production. As a result, feed enzymes have become important biotechnological tools to improve the nutritional value of feed ingredients, reduce feed costs, and improve the environment by reducing nutritional wastes; all the while improving overall performance of animals and birds [4].

Since the price of major protein-based feed ingredients have been increased significantly during the last decades, the use of exogenous proteases in poultry diet gained momentum. The first commercial protease was introduced in the poultry feed market back to 1990s in combination with other enzymes, with the aim of increasing energy and protein digestibility of a grain and oilseed meal-based diet [5]. Since then, several published reports evaluated protease enzymes for their ability to improve protein and amino acid digestibility in the diets of animals and birds [6]–[12]. Gitoee *et al*. [13] clearly mentioned that the adding enzymes to corn-soy-based diets allowed the reduction in the energy level without any negative effects on the performance of broiler chickens. Additionally, protease enzymes have several ancillary benefits linked with their prime mode of action on the digestion of dietary proteins. This includes the reduction of proteolytic fermentation and decreasing undigested part of protein in the diet that leads to reduce the overall dietary protein supplementation, which altogether attributes in minimizing the cost of protein feed ingredients in diet formulation [14]–[16].

Further, the use of nutrient matrix values in diet formulation is also one of the ways successfully practiced all across the glove to maximize the functional ability of protease enzymes. Ideally, the particular enzyme manufacturers derive the matrix values based on several digestibility trials, which describe the amount of additional nutrients that are potentially released with dietary protease supplementation. This allows the reduction of overall nutrient levels in poultry diets, resulting in lower feed cost, while maintaining or improving performance [3], [8], [11], [15], [16]. Most proteases enzymes available in the market today are produced from genetically engineered bacterium e.g., *Bacillus licheniformis* or *Bacillus subtilis*. The product is referred to as mono-component protease, a single component targeted protease that usually dominates in the market [17]. Conversely, a protease complex, where none of the components is dominant, can be defined as multi-protease or multi-component protease.

Several studies have been conducted on the effects of mono-component protease in broiler chickens [3], [7], [8], [9], [14], [15], [18], but very few on multi-component protease [6], [16].

In this trial, the effect of a multi-component dietary protease complex on the production performances and carcass yields of commercial broiler chickens was evaluated. The protease was supplemented in the commercial broiler diets either on top level or using a nutrient matrix value as recommended by the supplier.

II. Materials and Method

A. Ethical Approval

The handling of birds, slaughtering and other relevant experimental activities were carried out by following instructions of the Animal Welfare and Ethical Committee of Bangladesh Agricultural University, Mymensingh-2202, Bangladesh (ethical approval no: AWEEC/BAU/2022 (18); date; 17/4/2022).

B. Bird's Management, Immunization and Experimental Groups

The experiment was conducted at the Bangladesh Agricultural University Poultry Farm, Mymensingh, Bangladesh. A total of 630 Indian River Meat Broiler DOCs were collected from a reputed commercial hatchery and allocated into five treatments having seven replications in each treatment and 18 birds per replication. The treatments were: 1) Positive control (PC), a standard diet with no supplemental protease and formulated to meet the nutritional requirements of the birds [19]; 2) Negative control (NC), a diet similar to the PC but reduced in crude protein (CP), digestible essential amino acids (EAA), and metabolizable energy (ME) based on the supplier's nutrient matrix recommendation for the protease complex; 3) PC+125 ppm protease (PC+125); 4) NC+125 ppm protease (NC+125); and 5) NC+200 ppm protease (NC+200). The supplemented enzyme is an alkaline protease complex with multiple protease activity developed and marketed by Jefo Nutrition Inc., Saint-Hyacinthe, Quebec, Canada. The protease complex contains 25,000 U/g, where one unit of protease activity is defined as the amount of enzyme which liberates 1 nmol amino fluorescein per minute from casein iso thiocyanate-conjugated substrate flourescein, pH 9.9. Birds were reared on a gable type open sided poultry house. The chicks were brooded in respective pens using one 200-watt electric bulb in each pen. Birds were vaccinated with Infectious Bronchitis (IB)+Newcastle Disease (ND) vaccine (CEVAC® BIL-contains the Massachusetts B48 strain of IB virus and the Hitchner B1 strain of ND virus in live, freeze-dried form) at 4th day followed by a booster dose on 20th days. The Infectious Bursal Disease (IBD) vaccination (GumboMed™ Vet**,** containing live attenuated IBD/Gumboro virus intermediate strain) was performed at day 11 followed by a booster dose on day 18.

C. Experimental Diets and Feeding

Starter and grower diets were formulated for the experiment (Table I). The starter diet was provided for the first 14 days while the grower diet was provided from 15 to 28 days. The diets were pelleted in a commercial feed mill at 80 °C with the retention time of 35 seconds (Power Fish and Poultry Feed Limited, Gazipur, Dhaka, Bangladesh). Nutrient requirements for ME, CP, calcium (Ca), phosphorus (P), lysine, methionine, and other EAA were satisfied as per recommended specifications for Indian River broiler (Indian River Broiler Nutrition Specifications, 2019). Feed was analyzed for proximate composition following Bates J. AOAC [20]. In all cases, birds were fed *ad-libitum*. Fresh and clean drinking water was made available at all the times.

TABLE I: COMPOSITION OF THE EXPERIMENTAL DIETS, AS FED BASIS

Ingredients	Starter		Grower	
	PC	NC	PC	NC
Corn (8.7% CP)	51.15	53.88	53.34	56.12
Soybean meal (45% CP)	32.80	30.57	29.50	27.16
Full fat soybean (35% CP)	4.00	3.90	4.00	4.00
Soybean oil (8,800 kcal/kg ME)	2.10	1.73	3.95	3.55
Corn distillers dried grains with soluble (27% CP)	2.00	2.00	2.00	2.00
Rice polish (12% CP)	2.00	2.00	0.00	0.00
Fish meal (55% CP)	1.90	1.90	1.90	1.90
De-oiled rice bran (20% CP)	0.00	0.00	1.60	1.60
Limestone (38% Ca)	1.20	1.20	1.12	1.12
Monocalcium phosphate (21% P)	0.90	0.90	0.75	0.75
DL-methionine	0.40	0.38	0.36	0.33
L-threonine	0.15	0.14	0.09	0.08
Salt	0.30	0.30	0.30	0.30
Emulsifier	0.025	0.025	0.025	0.025
Choline chloride	0.07	0.07	0.06	0.06
Vitamin and mineral premix[1]	1.00	1.00	1.00	1.00
Protease[2]	0	0	0	0
Total	100.00	100.00	100.00	100.00
Nutrient composition (%)				
CP, % (calculated)	23.0	22.5	21.5	21.0
CP, % (analyzed)	23.2	22.6	21.8	21.2
ME, kcal/kg	3000	2975	3100	3075
Ca, % (calculated)	0.96	0.96	0.87	0.87
Ca, % (analyzed))	1.01	0.99	0.89	0.86
Available P, % (calculated)	0.48	0.48	0.44	0.44
Total P, % (analyzed)	0.72	0.72	0.69	0.65
Digestible lysine, % (calculated)	1.28	1.25	1.15	1.12
Digestible methionine, % (calculated)	0.51	0.50	0.47	0.46
Digestible methionine + cysteine, % (calculated)	0.95	0.93	0.87	0.85
Digestible threonine, % (calculated)	0.86	0.84	0.77	0.75
Digestible tryptophan, % (calculated)	0.20	0.19	0.18	0.17

[1]Provided per kg of diet: 13,500 IU of vitamin A; 4,500 IU of vitamin D3; 80 IU of vitamin E; 4 mg of vitamin K (menadione); 4 mg of thiamine; 9 mg of riboflavin; 4 mg of pyridoxine; 0.02 mg of vitamin B12; 0.30 mg of biotin; 2 mg of folic acid; 60 mg of niacin; 16mg of calcium pantothenate; 150 mg of ethoxyquin (antioxidant); 20 mg of Fe; 100 mg of Zn; 15 mg of Cu; 120 mg of Mn; 1.25 mg of I; 0.25 mg of Se.
[2]Included at 125 or 200 g/t of feed.

D. Data Collection

Throughout the experimental period, body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), and survival rate were recorded and calculated weekly. Temperature and relative humidity were recorded daily. At the end of experiment (day 28), three birds were randomly selected from each treatment group, weighed, slaughtered, and processed to determine the carcass yield of birds. Percentages of dressed, thigh, drumstick, breast meat, wing, head, liver, gizzard, heart, and shank weight were also recorded.

E. Analysis of Dietary Protease Activity

The activity of protease complex in the formulated feed sample was determined using the Protease Fluorescent Detection Kit (Sigma-Aldrich, Missouri, USA). This assay detects protease activity using casein labeled with fluorescein isothiocyanate (FITC) as the substrate and was based on the slightly modified procedure of Twining [21]. In brief, protease activity results in the cleavage of the FITC-labeled casein substrate into smaller fragments, which do not precipitate under acidic conditions. After incubation of the protease sample and substrate, the reaction is acidified with addition of trichloroacetic acid. The mixture is then centrifuged with undigested substrate, forming a pellet and the smaller, acid-soluble fragments remaining in solution. The supernatant is neutralized, and fluorescence of the FITC-labeled fragments is measured. One unit of protease activity equals to the amount of enzyme which liberates 1 nmol amino fluorescein per minute from FITC-labeled casein substrate.

F. Statistical Analysis

All recorded and calculated data were statistically analyzed using one-way analysis of variance (ANOVA) in SPSS, Statistical Computer Package Program [22] (SPSS Inc., Chicago, IL). Tukey's honestly significance difference test was used to compare variations among the treatments where ANOVA showed significant differences. The level of significance was considered at 95%.

III. RESULTS

In this study, the birds consumed all experimental diets very well and there was no sign of suppressed feed intake. Temperature in the broiler house recorded within the range between 22 °C to 32 °C during the experimental period. The survival rate was also very good, with overall recorded survivability of 99.2%. Only five birds from different treatment groups died during the entire experimental period.

A. Live Body Weight

Live body weights of the broiler chickens under different treatment groups are presented in Table II. No significant differences were observed during the 1st and 2nd week in average BW among the treatment groups. However, at 3rd and 4th week, the average BW was significantly higher (P<0.05) in birds fed the protease supplemented diets (PC+125, NC+125, and NC+200) as compared to those fed the non-supplemented diets (PC and NC). Moreover, the average BW of birds fed NC+200 diet was significantly higher (P<0.05) as compared to those fed PC+125 and NC+125 diets at 3rd week, but this difference was diminished at 4th week of age.

TABLE II: WEEKLY BODY WEIGHT (G/BIRD) OF BROILER CHICKENS AT DIFFERENT TREATMENT GROUPS

Age (Weeks)	Body weight (g/bird)					P-value
	PC	NC	PC+125	NC+125	NC+200	
Initial	45.93	45.56	46.56	45.59	46.33	0.910
1	203.8	203.4	208.1	204.8	205.6	0.070
2	584.4	583.8	592.3	588.1	597.6	0.063
3	1142.8^{c}	1137.4^{c}	1183.2^{b}	1181.7^{b}	1200.2^{a}	0.000
4	1715.5^{b}	1701.2^{b}	1790.0^{a}	1781.8^{a}	1814.6^{a}	0.003

^{a,b,c} Data within a row that do not share superscripts are significantly different (P<0.05).

B. Body Weight Gain

Weekly body weight gains for broiler chickens are shown in Table III. Similar to BW, there were no significant differences in average BWG among the treatment groups at 1st and 2nd week. However, the average BWG of birds fed protease supplemented diets (PC + 125, NC + 125, and NC + 200) were significantly increased (P < 0.05) than those fed the PC and NC diets at 3rd and 4th weeks of age.

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TABLE III: WEEKLY BODY WEIGHT GAIN (G/BIRDS) OF BROILERS AT DIFFERENT TREATMENT GROUPS

Age (Weeks)	Body weight gain, (g/bird)					P-value
	PC	NC	PC+125	NC+125	NC+200	
1	157.8	157.5	161.5	159.2	163.3	0.323
2	380.6	378.9	384.2	383.3	388.0	0.346
3	561.8^{b}	558.6^{b}	590.9^{a}	586.6^{a}	602.6^{a}	0.000
4	572.6^{b}	563.8^{b}	606.7^{a}	600.1^{a}	614.3^{a}	0.045
Final weight gain	1669.5^{b}	1655.6^{b}	1743.4^{a}	1736.2^{a}	1768.3^{a}	0.005

[a,b,c] Data within a row that do not share superscripts are significantly different (*P*<0.05).

C. Feed Intake

The average weekly FI in all treatment groups increased with age Table IV. The only significant difference among the treatment groups was observed at 2[nd] week (*P*<0.05) when broilers fed different levels of protease supplemented diets had significantly higher FI than those fed the PC diet.

TABLE IV: WEEKLY FEED INTAKE (G/BIRDS) OF BROILERS AT DIFFERENT TREATMENT GROUPS

Age (Weeks)	Feed intake, (g/bird)					P-value
	PC	NC	PC+125	NC+125	NC+200	
1	177.1	180.7	179.9	176.8	184.2	0.455
2	469.5^{b}	473.1^{ab}	477.2^{a}	477.6^{a}	476.3^{a}	0.033
3	770.9	797.2	780.0	793.4	790.4	0.346
4	1007.8	1025.2	1015.4	1004.0	1006.7	0.889
Total	2425.4	2476.2	2452.5	2451.9	2457.7	0.653

[a,b,c] Data within a row that do not share superscripts are significantly different (*P*<0.05).

D. Feed Conversion Ratio (FCR)

Table V represents the weekly average FCR value of different treatment groups. There was no significant difference among the treatment groups in the 1st and 2[nd] weeks. However, at 3[rd] and 4[th] weeks, the birds fed diets supplemented with protease complex have significantly better FCR than those fed the NC diet (*P*<0.05) but were similar to the birds fed PC diet.

TABLE V: WEEKLY AVERAGE FCR OF BROILERS AT DIFFERENT TREATMENT GROUPS

Age (Weeks)	Feed conversion ratio					P-value
	PC	NC	PC+125	NC+125	NC+200	
1	1.122	1.145	1.114	1.111	1.156	0.825
2	1.234	1.244	1.242	1.246	1.215	0.834
3	1.381^{ab}	1.440^{a}	1.320^{b}	1.337^{b}	1.312^{b}	0.012
4	1.760^{ab}	1.818^{a}	1.673^{b}	1.673^{b}	1.639^{b}	0.024
Average	1.453^{ab}	1.496^{a}	1.407^{b}	1.412^{b}	1.390^{b}	0.009

[a,b,c] Data within a row that do not share superscripts are significantly different (*P*<0.05).

E. Carcass Yields

Results on carcass yield of broilers are presented in Table VI. The dressed carcass, drumstick, wing, head, liver, gizzard, neck, and shank percentage were similar among the treatment groups. However, the thigh, breast meat, and heart were significantly increased (*P*<0.05) in birds that were fed NC+200 as compared to NC group, while results for PC, PC+125, and NC+125 were intermediate between those fed the NC and NC+200 diets.

TABLE VI: MEAT YIELD CHARACTERISTICS OF BROILERS FED STANDARD OR REDUCED DIETS AND SUPPLEMENTED WITHOUT AND WITH A PROTEASE COMPLEX

Response[1]	PC	NC	PC + 125	NC + 125	NC + 200	P-value
Dressed carcass, %	71.37	70.93	71.32	72.19	74.68	0.090
Thigh, %	18.40^{ab}	17.53^{b}	18.63^{ab}	18.60^{ab}	20.35^{a}	0.014
Drumstick, %	13.13	13.46	13.16	12.74	13.26	0.390
Breast, %	37.77^{ab}	36.46^{b}	38.38^{ab}	38.12^{ab}	38.60^{a}	0.016
Wing, %	11.44	12.10	12.12	11.98	11.28	0.221
Head, %	1.49	1.45	1.42	1.47	1.27	0.089
Liver, %	3.87	3.96	3.61	3.58	3.69	0.468
Gizzard, %	1.86	1.70	2.09	2.03	2.27	0.714
Heart, %	0.75^{ab}	0.65^{b}	0.82^{ab}	0.80^{ab}	0.85^{a}	0.024
Neck, %	5.98	6.88	6.05	6.32	6.06	0.124
Shank, %	4.60	5.42	5.69	5.19	5.13	0.098

[a,b,c] Data within a row that do not share superscripts are significantly different (*P*<0.05).
[1] Data are presented as least square means.

IV. DISCUSSION

Earlier studies conducted by Yu et al. [6] and Cardinal *et al*. [16] observed similar improvements as present study in the production performance of broilers fed either a standard or a reduced diet supplemented with the same protease complex. Ghazi *et al*. [23] and Kamel *et al*. [9], however reported the same observation when different protease was added on top of a standard corn and soybean meal-based broiler diet. In both studies, however the effect of dietary protease supplementation was evident during the starter period. Previous works by Yan *et al*. [24] and Mohammadigheisar and Kim [11] reported a clear benefit of using protease enzyme during the starter period as compared to finisher, suggesting that the young animals may be more responsive to protease supplementation. Moreover, Liu *et al*. [10] noted that the effectiveness of protease was correlated to the protein level of diet since it can influence the degree of available protein substrates for the enzyme to exert its proteolytic activity. Broiler starter diets contain comparatively higher CP but lower ME levels than broiler grower diets. Indeed, an interaction between protein and protease was observed by Freitas *et al*. [7] in which digestibility of CP was greater when protease was added to high-protein diets as compared with the low-protein diets. However, the same authors also reported that another interaction between energy and protease was associated with a greater increase in energy digestibility when protease was added to high-energy diets, as compared with the low-energy diets. Since nutrients in most feed ingredients are present in a complex matrix, it is therefore not surprising that feed enzymes like protease can exert wide influence on nutrient digestibility beyond their targeted substrates. For instance, the disruption of protein matrix surrounding starch granules due to protease had been shown to improve energy digestibility in broiler diets [14]. Therefore, the improvement of production parameters observed in current study could be due to the sustained positive effects of protease complex on both the protein and energy digestibility, which indeed was started from starter period and becoming much more evident in the grower period. This result however specifically highlights the significance of using an exogenous protease throughout the whole production cycle in broilers to achieve the most desirable production performance.

The use of nutrient matrix values in diet formulation derived from a series of digestibility trials that ensured the amount of additional nutrients to the birds which are being potentially released with enzyme supplementation using matrix values of nutrients, is considered as cutting-edge nutritional biotechnology that have been successfully practiced in all over the world to maximize the efficacy of protease enzyme on nutrients digestibility. In general, matrix values for CP, digestible EAA, and ME are applied in protease supplemented livestock diets resulting to a reduction in nutrient levels and thereby lowering feed cost while maintaining or improving animal’s performance. In current study, application of nutrient matrix in the NC diet did not result to significant losses in performance as compared to birds fed the PC diet, indicating that the reduction of nutrient density in NC diet was not enough to elicit losses in broiler performance. This was supported by a similar level of FI in birds fed the PC and NC diets. Several studies done previously reported an increase in FI when dietary CP level is reduced [25]–[28]. Broilers fed reduced CP diets needed to increase their FI to fulfill the required amino acid level to achieve their genetically determined growth potential. Since overall, FI was similar among the treatment groups, the observed differences in broiler performance support the effect of the protease complex on the bird’s capacity to better utilize ingested nutrients through enhanced nutrient digestibility. Furthermore, since better overall performance was observed in birds fed the NC+125 and NC+200 diets as compared to those belonging in the PC group, the results of present study also indicated that the nutrient matrix applied on PC diet could be lower than the actual nutrient potential of supplemented protease complex. In general, enzyme suppliers set their nutrient matrix values so that animal diets can be reduced in nutrient levels to achieve a reduction on feed cost while maintaining a constant animal performance based on those observed when birds are fed their standard nutritional requirement. However, in situations where birds are provided suboptimal nutrition or are raised in poor rearing conditions, a more conservative approach on nutrient matrix level may provide safety allowances to prevent significant losses in performance. There were no significant differences among the treatment groups in terms of dressed carcass, drumstick, wing, head, liver, gizzard, neck, and shank percentage. This result is in agreement with the findings of Espino *et al.* [25] and Al-juboori [29], who both reported no significant increase in the carcass yield of broilers fed diets containing a cocktail of enzyme (protease, amylase, and lipase) and a protease, respectively. On the other hand, an improvement in the carcass yield of broilers fed protease supplemented corn and soybean meal-based diets was reported by Kamel *et al*. [9]. In another study by Hartman [30], the author reported a significant increase in the dressing percentage of broilers fed wheat-based diets supplemented with a commercial protease. In the present study, a significant improvement of thigh and breast meat yield was observed in birds fed NC+200 diet as compared to those fed standard and reduced diets without a protease complex. Although, the overall production performance of the birds fed the NC + 200 diet was similar to those fed the NC+125 diet, this result indicates the potential of a protease complex to improve certain aspects of carcass characteristics when supplemented at a higher dose rate. This can be of great benefit particularly in markets that deal with portioned poultry meat.

V. Conclusion

Overall, under the condition of present study, results demonstrated that the supplementation of a protease complex either on a standard or a reduced diet may improve the overall production performance of commercial broiler chickens. In addition, the use of protease complex at a higher dosage may improve certain carcass characteristics. As feed cost represents a significant portion of the total investment in broiler production, the use of protease complex in low density diets may offer an opportunity for commercial poultry producers to optimize feed cost, while avoiding potential losses in chicken performance and making poultry farming more profitable.

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Conflict of Interest

There is no conflict of interest regarding to financial matter mentioned.

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