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Applied Research Note: Exogenous protease supplementation to reduced-energy, reduced-protein, and reduced-amino acid diets for broiler chickens from days 1 to 42

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Primary Audience: Nutritionists, Poultry Industry

SUMMARY

Supplementing exogenous proteases in poultry diets was shown to facilitate the digestion of dietary proteins. However, there is limited information on the effects of proteases inclusion on performance of broiler chickens fed reduced energy, protein, and essential amino acid (EAA) diets. This study aimed to address this gap in knowledge. A total of 720 one-day-old Cobb 500 male broilers were randomly allocated to 6 treatments and 6 replicates of 20 birds. The treatments included: positive control (PC; formulated as per the breeder guideline), negative control (NC; reduction in nutrition levels by 100 kcal/kg ME; 1% CP, and 5% EAAs), and NC diets supplemented with 50 g/t, 100 g/t, 200 g/t, 400 g/t of Vitazyme Pro protease (NC 50, NC 100, NC 200, and NC 400). From d 1 to 14 and 14 to 28, all nutrient reduction groups, except for NC 100, had higher FCR ($P < 0.05$) than PC. From d 28 to 42, the NC group had higher ($P < 0.01$) FI and FCR than PC. However, protease supplementation from 100 to 400 g/t maintained the higher FI ($P < 0.01$) while increased BWG ($P < 0.01$) compared to PC. For the entire 42-d period, PC and NC 100 had lower ($P < 0.01$) FCR than NC and NC 50. In conclusion, the results suggested that protease supplementation could mitigate the negative impacts of reductions in dietary nutrition levels on growth performance. The inclusion of Vitazyme PRO protease at 100 g/t seemed to be optimal for improving growth performance in diets with reduced energy, protein, and EAAs.

Key words: broiler, protease, protein, metabolizable energy

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DESCRIPTION OF PROBLEM

Proteases are known to catalyze the hydrolysis of peptide bonds, breaking down proteins into smaller peptides and amino acids that can be absorbed by the intestinal epithelium and utilized by animals for energy and other biological functions (Cowieson and Roos, 2016). While it is widely believed that poultry can secret sufficient amounts of endogenous proteases for digesting the dietary CP, recent studies have shown that incompletely digested and absorbed protein and amino acids can be found in the ileum and excreta of birds during the initial 3 wk of life (Attia, 2001; Cowieson and

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Roos, 2016). These findings suggest that there is an opportunity to improve protein and amino acid digestibility in poultry diets by supplementing exogenous proteases.

In recent years, the rising costs of feed ingredients have led to the exploration of strategies for reducing the cost of feed production. One approach is to reduce the levels of metabolizable energy (ME) and crude protein (CP) in the diet, as these are the most expensive components (Attia et al., 2020). In addition, reducing protein or energy contents in the diet could reduce nitrogen excretion and lower ammonia emissions, potentially lending to lessening the environmental impact (Liu et al., 2023). However, such reductions in nutritional levels can lead to deterioration in growth performance of the chickens. As previous study reported (Attia et al., 2020), exogenous enzymes can enhance the digestibility of nutrients and reduce nutrient loss. They are also capable of removing antinutritional factors. Supplementing these enzymes in the diets might ameliorate the negative impacts on growth performance caused by the reduction of nutritional levels. Indeed, previous studies have investigated the beneficial effects of supplementing enzyme preparations in diets with reduced nutritional levels (Zhou et al., 2009). Nevertheless, there is limited literature examining the effects of supplementing protease alone in poultry diets with reduced ME, CP, and essential amino acids (EAAs).

To fill the gap in knowledge, the current study was conducted with the objective to evaluate the effects of supplementing exogenous protease at different levels in diets with a reduction in ME, CP, and EAA on the growth performance of broilers from 1 to 42 d of age.

MATERIALS AND METHODS

Birds and Management

An experiment was conducted with the approval of the Institutional Animal Care and Use Committee of the University of Georgia (Athens, GA). The birds were housed in 3 rooms, each with 18 identical floor pens. Each pen was equipped with one nipple drinker and one feeder, providing the chicks with ad libitum access to water and feed for the duration of the experiment. The temperature and lighting programs followed the Cobb 500 Broiler Management Guide (Cobb-Vantress, 2018b).

Dietary Treatments and Measurements

A randomized complete block design was used to distribute a total of 720 one-day-old male Cobb chicks into 6 dietary treatments, each with 6 replicates of 20 birds. The dietary treatments included: positive control (PC; formulated as per the breeder guideline (Cobb-Vantress, 2018a)), negative control (NC; reduction in nutrition levels by 100 kcal/kg ME; 1% CP, and 5% EAAs), and NC diets supplemented with 50 g/t, 100 g/t, 200 g/t, 400 g/t of protease (NC 50, NC 100, NC 200, and NC 400). The inclusion levels of protease were chosen after considering the results from the previous study (Rehman et al., 2018). The protease sample of Vitazyme Pro (enzyme activity: 20,000 U/g) was provided by Vitech Ultra Bioscience Corp., Orange, CA. The enzyme is derived from *Bacillus licheniformis* fermentation. One unit of protease activity refers to the amount of enzyme that releases 1 mg of tyrosine per minute from casein substrate at 40°C and pH value of 10.5 (Rehman et al., 2018). The birds were fed mashed diets formulated with corn, soybean meal, and distillers dried grains with solubles for 42 d (Table 1). The dietary phases were as follows: starter (d 1–14), grower (d 14–28), and finisher (d 28–42). On d 14, 28, and 42, body weight and feed consumption per pen were weighed to calculate body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). Mortality was recorded daily and used to adjust the FCR calculations.

Statistical Analysis

Prior to conducting statistical analysis, the data were tested to ensure homogeneity of variances and normality of studentized residuals. The PROC GLM procedure of SAS (SAS University Edition, version 1.8.0_181; SAS Institute Inc., Carry, NC) was used to perform a one-way ANOVA. Room was treated as the blocking factor and Tukey's test was utilized as the post hoc comparison test ($P < 0.05$).

Table 1. Composition and calculated nutrient content of diets.

	Starter (d 1–14)		Grower (d 14–28)		Finisher (d 29–42)	
Ingredients (%)	PC	NC	PC	NC	PC	NC
Corn	52.66	58.05	56.35	60.40	59.48	63.20
Soybean meal -48%	29.57	26.65	25.46	22.74	23.77	21.15
DDGS	10	10	10	10	10	10
Soybean oil	2.78	0.45	3.39	1.50	3.45	1.60
Dicalcium phosphate	1.47	1.50	1.34	1.35	1.13	1.16
Limestone	1.23	1.23	1.18	1.19	1.10	1.09
Common salt	0.30	0.30	0.35	0.35	0.30	0.30
Mineral premix[1]	0.08	0.08	0.08	0.08	0.08	0.08
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.27	0.24	0.23	0.22	0.17	0.16
L-lysine	0.31	0.32	0.30	0.31	0.18	0.18
L-Threonine	0.08	0.08	0.07	0.07	-	0.01
Product space/sand	1.00	0.85	1.00	1.54	0.05	0.82
Nutrients (%) and energy						
ME, kcal/kg	3010	2910	3090	2990	3150	3050
Protein	21.33	20.33	19.61	18.61	19	18
Lysine	1.31	1.24	1.19	1.13	1.05	0.99
Methionine	0.63	0.59	0.57	0.54	0.50	0.47
TSAA	0.97	0.92	0.89	0.85	0.82	0.78
Threonine	0.85	0.81	0.78	0.74	0.71	0.67
Calcium	0.90	0.90	0.84	0.84	0.76	0.76
Available P	0.45	0.45	0.42	0.42	0.38	0.38

Abbreviations: DDGS, distillers dried grains with soluble; NC, negative control; PC, positive control.

[1]Vitamin premix: Supplemented per kg of diet: thiamin mononitrate, 2.4 mg; nicotinic acid, 44 mg; riboflavin, 4.4 mg; D-Ca pantothenate, 12 mg; vitamin B12 (cobalamin), 12.0 g; pyridoxine HCl, 4.7 mg; D-biotin, 0.11 mg; folic acid, 5.5 mg; menadione sodium bisulfite complex, 3.34 mg; choline chloride, 220 mg; cholecalciferol, 27.5 g; transretinyl acetate, 1,892 g; a tocopheryl acetate, 11 mg; ethoxyquin, 125 mg.

[2]Mineral premix: Supplemented as per kg of diet: manganese ($MnSO_4.H_2O$), 60 mg; iron ($FeSO_4.7H_2O$), 30 mg; zinc (ZnO), 50 mg; copper ($CuSO_4.5H_2O$), 5 mg; iodine (ethylene diaminedihydroiodide), 0.15 mg; selenium ($NaSeO_3$), 0.3 mg.

RESULTS AND DISCUSSION

In the starter phase (d 1−14), all diets with reduced nutritional levels except for NC 100 had significantly higher FCR than the PC group (Table 2). Notably, NC and NC 400 groups had higher FCR than the NC 100 group ($P = 0.010$). For the grower phase (d 14−28), all diets with reduced nutritional levels except for NC 100 had significantly higher FCR than the PC group. Moreover, the NC 50 group had higher FCR than the NC 100 group ($P = 0.021$). No significant differences were found for FI and BWG at these 2 phases.

In the finisher phase (d 28−42), all nutrition-reduced diets had significantly higher ($P = 0.003$) FI than the PC group (Table 2). The NC 100 group had higher BWG than the PC and NC groups. The NC 200 group had higher BWG than PC, NC, and NC 50 groups. The NC 400 group had higher BWG than the PC group ($P = 0.004$). The NC group had significantly better FCR than all groups except for the NC 100 group ($P = 0.009$). For the whole experiment period (d 1−42), PC and NC 100 had worse FCR than NC and NC 50 groups ($P = 0.010$). No significant differences were observed for FI and BWG.

The reduction in nutrition levels by 100 kcal/kg in ME (approximately 3%), 1% in CP, and 5% in EAA negatively affected the growth performance of the birds with the most significant effect observed in a worsening FCR. Similarly, Kamran et al. (2008) found that feeding a diet with reduction in nutrition levels by 5% in ME and 1% in CP could lead to lower BWG from d 27 to 35 and better FCR throughout the experiment period. Another study also found that reductions in dietary CP and ME levels would worsen the FCR from d 14 to 35 (Chrystal et al., 2020). It is interesting to note

Table 2. Feed intake (FI, kg), body weight gain (BWG, kg), and feed conversion ratio (FCR) of birds fed different dietary treatments.

	Treatments[1]							
	PC	NC	NC 50	NC 100	NC 200	NC 400	*P*-value	SE
Starter (d 1–14)								
BWG	0.364	0.317	0.333	0.347	0.319	0.317	0.981	0.005
FI	0.639	0.628	0.644	0.632	0.625	0.631	0.079	0.005
FCR	1.76^{c}	1.98^{a}	1.94^{ab}	1.82^{bc}	1.97^{ab}	1.99^{a}	0.010	0.021
Grower (d 14–28)								
BWG	1.11	1.03	1.07	1.08	1.07	1.07	0.116	0.010
FI	1.84	1.82	1.91	1.83	1.89	1.87	0.215	0.008
FCR	1.66^{c}	1.76^{ab}	1.79^{a}	1.70^{bc}	1.77^{ab}	1.74^{ab}	0.021	0.012
Finisher (d 28–42)								
BWG	1.73^{d}	1.77^{cd}	1.80^{bcd}	1.86^{ab}	1.89^{a}	1.83^{abc}	0.003	0.02
FI	2.63^{b}	2.84^{a}	2.81^{a}	2.84^{a}	2.92^{a}	2.82^{a}	0.004	0.0135
FCR	1.52^{b}	1.61^{a}	1.56^{ab}	1.52^{b}	1.54^{b}	1.54^{b}	0.009	0.007
Overall (d 1–42)								
BWG	3.20	3.12	3.20	3.29	3.28	3.22	0.055	0.027
FI	5.11	5.29	5.37	5.30	5.43	5.32	0.378	0.021
FCR	1.60^{b}	1.70^{a}	1.68^{a}	1.61^{b}	1.65^{ab}	1.65^{ab}	0.010	0.008

[a,b,c]Means followed by superscript letters are different as per Tukey's test ($P < 0.05$).

[1]PC, positive control; NC, negative control; NC 50, a NC diet supplemented with 50 g/t of protease; NC 100, a NC diet supplemented with 100 g/t of protease; NC 200, a NC diet supplemented with 200 g/t of protease; NC 400, a NC diet supplemented with 400 g/t of protease.

that during the starter and grower phases, the reductions of nutrition levels in NC did not affect FI of the birds while it lowered the BWG, although not significantly, leading to the worsened FCR. However, in the finisher phase, FI of the NC group was significantly higher than the PC group while BWG was not different, which contributed to the increase in FCR. The increase in FI in the later growing phase of the broiler chickens fed diets with reduced nutrition levels have also been reported by previous studies (Kamran et al., 2008; Chrystal et al., 2020). The observed variations in the impact of diets on FI during different phases may be attributed to physical limitations. During the earlier phase, birds could not increase their FI because of the physical constraints. However, as the birds grew larger, the physical limitation likely diminished and they were able to compensate the lower nutritional density of the diets by consuming more feed (Hidalgo et al., 2004).

We observed that supplementing the NC diet with 100 g/t of protease could improve FCR in all 3 phases. Similar results were reported by Rehman et al. (2018), who fed the birds diets with 0.5% CP and 7% EAA reductions, and supplemented with proteases. Rehman et al. (2018) also observed higher nitrogen retention in protease supplemented groups than the nutrient reduction diet. Since proteases are responsible for catalyzing protein hydrolysis, supplementing exogenous proteases in diets could improve the digestion of protein by breaking down the proteins to small peptides or amino acids which are readily absorbed by the epithelial cells (Cowieson and Roos, 2016). Moreover, exogenous proteases may facilitate the digestion of plant-based proteins which are otherwise difficult to digest by the birds due to the presence of antinutritional factors in the diets (Adeola and Cowieson, 2011). Therefore, supplementing the nutrient-reduced diets with protease could potentially increase the availability of protein and resulting in improved FCR. However, further research is needed to determine why the improvement in FCR was only observed at 100 g/t of protease supplementation and not at higher levels. Interestingly, we also found that, during the finisher phase, supplementing the NC diet with 100 g/t, 200 g/t, and 400 g/t of protease increased BWG without affecting FCR compared to the PC group. One possible explanation for the observation is that the birds were compensating for the decrease in dietary protein by increasing their FI, as was observed in this study. However, protease supplementation improved protein digestion and absorption, the availability of protein in the supplemented diets was comparable to the PC diet. As a result, the birds were able to achieve higher BWG due to the increased FI. Additionally, the effect of proteases in reducing the impacts of antinutritional factors reported by Cowieson and Roos (2016) could further contribute to this increase in BWG. Further studies are necessary to investigate and gain a better understanding of the mechanisms underlying these findings.

In summary, reducing the dietary nutrient levels by 100 kcal/kg ME, 1% CP, and 5% EAAs negatively affected the growth performance of the broiler chickens. However, supplementation of such diets with the Vitazyme PRO protease at 100 g/t has potential to mitigate the negative impacts on growth performance.

DISCLOSURES

There is no conflict of interest.

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