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Effect of Protein Level and Avizyme Supplementation on Performance, Carcass Characteristics and Nitrogen Excretion of Broiler Chicks

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Abstract: An experiment was carried out to evaluate the effect of reducing dietary protein level without and with Avizyme® (xylanase, protease and amylase) supplementation on performance, carcass characteristics and nitrogen excretion of broiler chicks from 1 to 40 d of age. Three dietary regimens were formulated being high, medium and low crude protein (HCP, MCP and LCP). Protein levels of such regimens were: 23, 21 and 19% CP for the starting; 21, 19 and 18% CP for the growing and 19, 18 and 17% CP for the finishing period. These diets were fed without or with 1g avizyme /kg diet representing six treatments. Every dietary treatment was fed to 5 replicates of 10 one-day old ROSS 308 broiler chicks each. During the starting period, birds fed LCP diet gained significant ($P<0.001$) less weight and consumed more feed. Addition of avizyme to all of these diets significantly ($P<0.001$) increased body weight gain (BWG) and improved feed conversion ratio (FCR). Avizyme supplementation was of no significant effect upon feed intake (FI) during the growing period. During the finishing period decreasing dietary CP level significantly decreased BWG ($P<0.05$), increased FI ($P<0.001$) and impaired FCR ($P<0.001$). Avizyme supplementation did not significantly affect BWG but it did significantly ($P<0.01$) decrease FI and improve ($P<0.05$) FCR. The results of the overall experimental period indicated that BWG decreased ($P<0.001$) with decreasing dietary CP and FCR improved with increasing dietary CP level ($P<0.001$). Addition of avizyme significantly ($P<0.001$) improved FCR. Adding avizyme to all diets slightly improved BWG without significant effect. However, carcass characteristics measurements were not affected by adding avizyme. Reducing dietary protein level significantly ($P<0.001$) decreased N excretion in starting, growing and finishing periods. Avizyme supplementation decreased ($P<0.001$) N excretion by 8.33, 7.60 and 7.97% in starting, growing and finishing periods, respectively. In conclusion, feeding broiler low protein diets adversely affected the growth performance. Addition of avizyme to low protein diets if not enhance gain in body weight, it improves efficiency of feed utilization. Lowering dietary protein level proved to be of beneficial effect on reducing the impact of intensive poultry production upon environmental pollution.

Key words: Broiler performance · Protein · Enzyme · Nitrogen excretion

INTRODUCTION

The excretion of nitrogen (N) originating from intensive livestock and poultry operation is one of the greatest environmental concerns. In addition to polluting the air and water, N in poultry excreta or litter is converted to volatile ammonia through microbial fermentation and can affect the health of birds and farm workers. Approximately, 70-75% of the N consumed by animals is lost or excreted due to the inefficiencies associated with protein digestion and or absorption [1]. Schutte *et al.* [2] reported that for each percentage of N decrease in the feed, N excretion is reduced by 10%. In response, dietary means to decrease N excretion by feeding low crude protein (CP) diets to poultry has been reported [3]. However, feeding low protein diets affected growth performance and carcass yield of broiler chickens [4]. Researchers showed that low-protein diets failed to support equal growth performance to that of high-protein control diets. Controversial results have been reported on the effect of feeding low CP diet compared to the standard 23% CP diet to broilers. Reduction in growth and an increase in feed conversion ratio in birds fed low protein diets were reported [4-6]. However, Waldroup [7] and Sterling *et al.* [8] reported that growth performance was not affected as the CP level was decreased below 20%.

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Feeds that are not completely digested by poultry are passed through the gastrointestinal tract and excreted into the litter. The result is loss of nutrients and money. Improving the digestibility of the diet will improve nutrient utilization by the bird. Ferket *et al.* [9] found that using enzymes improved digestibility and absorption of nutrients and consequently the performance and minimizing nutrient wastage. Environmental pollution, through excretion of nitrogen, can occur in higher or lower level, depending on the utilization capacity of protein, which is improved with exogenous enzymes addition [10]. Torres *et al.* [11] evaluated the enzymes influence on broiler performance, verified that lower protein levels, supplemented with enzymes, provided the broilers a similar performance of those fed diets with normal nutrient levels without effect on performance and reducing costs. Silva *et al.* [12] suggested that the manipulation of protein levels in diets supplemented with amino acids can reduce N excretion, minimizing environmental pollution. Nguyen *et al.* [13] found that dietary protein level of 21-19% were sufficient for the best performance and carcass quality of the Betong chicken during growing period.

The objective of this study was to determine the impact of dietary protein level and avizyme supplementation on performance, carcass characteristics and nitrogen excretion of broiler chicks.

MATERIALS AND METHODS

Three feed regimens were applied, a control high crude protein (HCP), medium crude protein (MCP) and low crude protein (LCP). Every dietary regimen was offered for the three phase feeding starter, grower and finisher. The starting period lasted from 1 to 11, the growing period from 12 to 25 and the finishing period from 26 to 40 days of age. The dietary CP content of the starter diets were 23, 21 and 19% for HCP, MCP and LCP, respectively (Table 1). For the growing period the dietary CP contents were 21, 19 and 18% for HCP, MCP and LCP, respectively (Table 2). The corresponding values for the finishing period were 19, 18 and 17% CP, respectively (Table 3). The experimental diets were formulated to meet the nutrient requirements for ROSS 308 broiler strain. These diets were fed without or with avizyme (1g/Kg diet) supplementation. Avizyme® contained 300 U/g xylanase, 4000 U/g protease and 400 U/g amylase (produced by Danisco Animal Nutrition, United Kingdom). Avizyme allows reducing the apparent metabolizable energy by 3 to 4% (100 Kcal/Kg) in feed formulas [14, 15]. Thus a number of 6 diets (per phase) were formulated.

A number of 300 one-day old (ROSS 308) broiler chicks were used in this experiment. Every dietary treatment was fed to 5 replicates of 10 chicks each.

Table 1: Formulation and nutrient composition of the starter (1-11 days) diets

Ingredients %	HCP	MCP	LCP	HCP+ avizyme	MCP+ avizyme	LCP+ avizyme
Yellow corn	50.26	58.65	66.76	52.53	60.97	66.53
Soybean meal (44%)	36.50	29.00	22.00	36.00	28.50	25.00
Corn gluten meal (60%)	5.00	5.00	5.00	5.00	5.00	3.00
Soybean oil	3.70	2.29	0.80	1.83	0.35	0.00
Dicalcium phosphate	1.87	2.00	2.00	1.87	2.00	2.00
Limestone	1.37	1.37	1.37	1.37	1.37	1.37
Vit and Min. mix(1)	0.30	0.30	0.30	0.30	0.30	0.30
NaCL	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.33	0.55	0.77	0.33	0.56	0.70
DL-methionine	0.30	0.37	0.43	0.30	0.37	0.44
Threonine	0.07	0.17	0.27	0.07	0.18	0.26
Avizyme(2)	0.00	0.00	0.00	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrients composition % (3)						
Crude protein %	23.10	21.01	19.07	23.10	21.02	19.05
ME (Kcal/Kg)	3030.00	3031.00	3030.00	2930.00	2932.00	2939.00
Lysine %	1.42	1.42	1.42	1.42	1.42	1.42
Methionine %	0.69	0.72	0.75	0.69	0.72	0.75
Methionine + Cystine %	1.07	1.07	1.07	1.07	1.07	1.07
Threonine %	0.94	0.94	0.94	0.94	0.94	0.94
Calcium %	1.05	1.05	1.05	1.05	1.05	1.05
Nonphytate P %	0.50	0.50	0.50	0.50	0.50	0.50

(1)Vitamin - mineral mixture supplied per Kg of diet: Vit A, 12000 IU; Vit D_3, 2200 IU; Vit E, 10 mg; Vit K_3, 2 mg; Vit B_1, 1mg; Vit B_2, 4mg; Vit B_6, 1.5mg; Vit B_{12}, 10µg; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 µg; Choline chloride, 500 mg; Copper, 10 mg; Iodine, 1mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 mg and Selenium, 0.1 mg. (2)1Kg Avizyme/Kg diet allows to reduce the ME by 100 Kcal/Kg, [15]. (3)According to NRC [33]

Table 2: Formulation and nutrient composition of the grower (12-25 days) diets

Ingredients %	HCP	MCP	LCP	HCP+ avizyme	MCP+ avizyme	LCP+ avizyme
Yellow corn	56.10	64.21	68.21	58.35	66.43	70.38
Soybean meal (44%)	30.47	23.35	19.80	30.00	22.90	19.40
Corn gluten meal (60%)	5.00	5.00	5.00	5.00	5.00	5.00
Soybean oil	4.43	3.00	2.30	2.55	1.12	0.42
Dicalcium phosphate	1.68	1.75	1.80	1.68	1.75	1.80
Limestone	1.15	1.15	1.15	1.15	1.15	1.15
Vit and Min. mix(1)	0.30	0.30	0.30	0.30	0.30	0.30
NaCL	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.29	0.50	0.61	0.29	0.51	0.62
DL-methionine	0.23	0.29	0.33	0.23	0.29	0.33
Threonine	0.05	0.15	0.20	0.05	0.15	0.20
Avizyme(2)	0.00	0.00	0.00	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrients composition % (3)						
Crude protein %	21.00	19.00	18.00	21.00	19.00	18.00
ME (Kcal/Kg)	3150.00	3150.00	3150.00	3050.00	3050.00	3050.00
Lysine %	1.24	1.24	1.24	1.24	1.24	1.24
Methionine %	0.59	0.62	0.64	0.59	0.62	0.64
Methionine + Cystine %	0.95	0.95	0.95	0.95	0.95	0.95
Threonine %	0.83	0.83	0.83	0.83	0.83	0.83
Calcium %	0.90	0.90	0.90	0.90	0.90	0.90
Nonphytate P %	0.45	0.45	0.45	0.45	0.45	0.45

(1)Vitamin - mineral mixture supplied per Kg of diet: Vit A, 12000 IU; Vit D_3, 2200 IU; Vit E, 10 mg; Vit K_3, 2 mg; Vit B_1, 1mg; Vit B_2, 4mg; Vit B_6, 1.5mg; Vit B_{12}, 10μg; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 μg; Choline chloride, 500 mg; Copper, 10 mg; Iodine, 1mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 mg and Selenium, 0.1 mg. (2)1Kg Avizyme/Kg diet allows to reduce the ME by 100 Kcal/Kg, [15]. (3)According to NRC [33]

Table 3: Formulation and nutrient composition of the finisher (26-40 days) diets

Ingredients %	HCP	MCP	LCP	HCP+ avizyme	MCP+ avizyme	LCP+ avizyme
Yellow corn	62.66	66.90	68.71	64.76	68.87	70.92
Soybean meal (44%)	24.50	20.85	20.80	24.10	20.50	20.35
Corn gluten meal (60%)	5.00	5.00	3.00	5.00	5.00	3.00
Soybean oil	4.00	3.22	3.36	2.20	1.50	1.50
Dicalcium phosphate	1.62	1.62	1.62	1.62	1.62	1.62
Limestone	1.10	1.10	1.10	1.10	1.10	1.10
Vit and Min. mix(1)	0.30	0.30	0.30	0.30	0.30	0.30
NaCL	0.30	0.30	0.30	0.30	0.30	0.30
L-lysine HCl	0.28	0.40	0.42	0.28	0.40	0.42
DL-methionine	0.20	0.22	0.26	0.20	0.22	0.26
Threonine	0.04	0.09	0.13	0.04	0.09	0.13
Avizyme(2)	0.00	0.00	0.00	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrients composition % (3)						
Crude protein %	19.00	18.00	17.00	19.00	18.00	17.00
ME (Kcal/Kg)	3200.00	3200.00	3200.00	3100.00	3100.00	3100.00
Lysine %	1.09	1.09	1.09	1.09	1.09	1.09
Methionine %	0.54	0.54	0.55	0.54	0.54	0.55
Methionine + Cystine %	0.86	0.86	0.86	0.86	0.86	0.86
Threonine %	0.74	0.74	0.74	0.74	0.74	0.74
Calcium %	0.85	0.85	0.85	0.85	0.85	0.85
Nonphytate P %	0.42	0.42	0.42	0.42	0.42	0.42

(1)Vitamin - mineral mixture supplied per Kg of diet: Vit A, 12000 IU; Vit D_3, 2200 IU; Vit E, 10 mg; Vit K_3, 2 mg; Vit B_1, 1mg; Vit B_2, 4mg; Vit B_6, 1.5mg; Vit B_{12}, 10μg; Niacin, 20 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50 μg; Choline chloride, 500 mg; Copper, 10 mg; Iodine, 1mg; Iron, 30 mg; Manganese, 55 mg; Zinc, 50 mg and Selenium, 0.1 mg. (2)1Kg Avizyme/Kg diet allows to reduce the ME by 100 Kcal/Kg, [15]. (3)According to NRC [33]

The average initial live body weights of all replicates were nearly similar. Replicates were randomly allocated in batteries of three-tier system divided into 30 compartments (5 replicates x 6 dietary treatments). Birds were raised in a warmed fumigated brooder house and fed the dietary treatments. Gas heaters were used during the first two weeks of age to keep the required temperature for the brooding period while light was provided 23 hr daily throughout the experimental period.

Feed and water were allowed for *ad libitum* consumption throughout 40-day-experimental period. After fasting overnight, birds were individually weighed and feed consumption was recorded per replicate at 11, 25 and 40 days of age. Body weight gain and feed conversion ratio were calculated.

At 9-11, 23-25 and 38-40 days of age, samples of excreta were collected from all treatments after spraying with 1% boric acid solution to trap the released ammonia to determine nitrogen in excreta. Feathers and any scattered feed were taken out. The collected excreta were dried in an air-draft oven at 60°C for 24 hr then left in room temperature to equilibrate with atmosphere moisture. The dried excreta from each replicate for the successive 3 days collection period were pooled; finely ground, well mixed and placed in a screw-top glass jar for nitrogen determination.

At 40 days of age, five birds per treatment with body weight close to the group average were randomly taken to study the carcass characteristics as percentage of live body weight. Chicks were fasted for approximately 12 hr before slaughtering and then individually weighed, slaughtered, feathered and eviscerated [16]. Weights of carcass, heart, liver and gizzard were recorded. The abdominal fat surrounding the gizzard and leaf fat attached to the abdominal wall and around the vent area were removed and weighed. The percentage (% LBW) of abdominal fat, heart, liver and gizzard for the individual chicks were calculated. Birds were vaccinated against avian influenza, New Castle, IB and IBD. After such medical treatments a dose of vitamins (AD_3E) was offered in the drinking water for the successive 3 days. Nitrogen content was determined in samples of excreta using Macro-Kjeldahle method according to the Official Methods of Analysis [17].

Data were statistically analyzed for analysis of variance as 3 × 2 factorial arrangement using the General Liner Model of SAS [18]. Significant differences among treatment means were separated by Duncan's new multiple rang test [19] with a 5% level of probability.

RESULTS AND DISCUSSION

The effect of reducing dietary crude protein and avizyme supplementation on growth performance during the starting and growing periods is summarized in Table 4. During the starting period, birds fed LCP diet recorded significant (P<0.001) less BWG compared to those fed HCP diet and MCP diet. Reducing CP from 23 to

Table 4: Effect of dietary treatments on growth performance of broiler during starting and growing periods

Item		Starting (1-11 days)			Growing (12-25 days)		
Dietary treatments							
CP %	Avizymeg g/kg	BWG (g)	FI (g)	FCR (g/g)	BWG (g)	FI (g)	FCR (g/g)
HCP (23/21)	0	198^{b}	280^{c}	1.41^{b}	703^{a}	1110^{a}	1.58^{bc}
MCP (21/19)	0	189^{c}	293^{b}	1.55^{a}	652^{b}	1070^{ab}	1.64^{b}
LCP (19/18)	0	187^{c}	297^{b}	1.59^{a}	546^{d}	1007^{b}	1.85^{a}
HCP (23/21)	1	213^{a}	291^{b}	1.37^{c}	719^{a}	1095^{a}	1.52^{c}
MCP (21/19)	1	216^{a}	306^{a}	1.42^{b}	659^{b}	1058^{ab}	1.61^{bc}
LCP (19/18)	1	198^{b}	307^{a}	1.55^{a}	591^{c}	1051^{ab}	1.78^{a}
SE of means		±2.17	±2.03	±0.02	±12.05	±9.75	±0.02
Mean values of CP %							
HCP (23/21)		206^{a}	286^{b}	1.39^{c}	711^{a}	1103^{a}	1.55^{b}
MCP (21/19)		202^{a}	300^{a}	1.48^{b}	655^{b}	1064^{ab}	1.62^{b}
LCP (19/18)		193^{b}	302^{a}	1.57^{a}	569^{c}	1029^{b}	1.81^{a}
Mean values of Avizyme g/Kg							
0		191^{b}	290^{b}	1.52^{a}	633^{b}	1062	1.69^{a}
1		209^{a}	302^{a}	1.44^{b}	657^{a}	1068	1.63^{b}
Significances							
CP		***	***	***	***	**	***
Avizyme		***	***	***	**	NS	*
CP × Avizyme		***	***	***	***	*	***

a-d Mean within each column with no common superscript differ significantly (p < 0.05)
*** P<0.001 ** P<0.01 * P<0.05 NS: not significant

21% significantly ($P<0.001$) reduced BWG by 4.55% and reducing CP from 23 to 19% reduced body weight gain (BWG) by 5.55%. No significant differences were detected between birds fed MCP and those fed LCP without avizyme on BWG. Addition of avizyme to the HCP, MCP and LCP diets significantly ($P<0.001$) increased BWG by 7.58 %, 14.29 % and 5.88 %, respectively. The best value of BWG was recorded with birds fed MCP with avizyme. Feed intake (FI) differ ($P<0.001$) among treatments during the starting period. However, birds fed MCP and LCP diets consumed significant ($P<0.001$) more feed than those fed HCP. No significant differences were detected on FI between birds fed MCP and those fed LCP diets. Addition of avizyme to HCP, MCP and LCP diets significantly ($P<0.001$) increased FI by 3.93%, 4.44% and 3.37%, respectively. The calculated values of feed conversion ratio (FCR) showed significant differences ($P<0.001$) among treatments. Regarding the effect of CP level, FCR values were 1.39, 1.48 and 1.57 for birds fed HCP, MCP and LCP, respectively. However, addition of avizyme improved FCR from 1.52 to 1.44. Addition of avizyme improved ($P<0.001$) FCR (from 1.41 to 1.37 and from 1.55 to 1.42 for the HCP and MCP diet, respectively) and did not significantly improve FCR with birds fed LCP ($P<0.005$). No significant differences were observed between birds fed HCP without avizyme (1.42) and birds fed MCP with avizyme (1.41).It could be concluded that reducing dietary CP levels in the starting period impaired growth performance. Avizyme supplementation to HCP or MCP diets gave more BWG, increased FI and improved FCR.

The results of the growing period followed almost the same trend as the starting period except that avizyme supplementation was of no significant effect on feed intake. Level of CP showed significant effect on BWG ($P<0.001$), FI ($P<0.01$) and FCR ($P<0.001$). Avizyme supplementation affected BWG ($P<0.01$) and FCR ($P<0.05$). Also, significant effects ($P<0.05$ - $P<0.001$) were detected for the interaction (CP X Avizyme). Reducing CP from 21 to 19% (MCP) decreased BWG by 7.25% and reducing CP from 21 to 18% (LCP) decreased BWG by 22.33%. Avizyme supplementation to the HCP diet improved BWG by 2.27%. While adding avizyme to LCP diet significantly ($P<0.001$) increased BWG by 8.32%. Reducing dietary CP levels slightly ($P>0.05$) decreased FI with that of birds fed MCP and significantly ($P<0.05$) decreased with birds fed LCP compared to the HCP diet. Addition of avizyme did not significantly affect FI. Reducing dietary protein levels significantly ($P<0.001$) impaired FCR. Such values varied from 1.58 for birds fed the HCP diet to 1.64 and 1.85 for birds fed the MCP and LCP diet, respectively. The worst FCR value was recorded with birds fed LCP without avizyme (1.85). Addition of avizyme improved FCR (from 1.58 to 1.52, from 1.64 to 1.61 and from 1.85 to 1.78 for the HCP, MCP and LCP diet, respectively. These results indicated that reducing CP levels impaired growth performance and avizyme supplementation gave more BWG and slightly improved FCR ($P<0.05$).

Table 5 represents the resulted performance during the finishing and the overall experimental periods. Decreasing dietary CP level decreased BWG ($P<0.05$), increased FI ($P<0.001$) and impaired FCR ($P<0.001$). Avizyme supplementation did not significantly affect BWG during the finishing period ($P>0.05$). It did decrease FI ($P<0.01$) and improve ($P<0.05$) FCR. FI increased ($P<0.001$) with birds fed LCP diet without avizyme addition. Addition of avizyme to LCP diet significantly ($P<0.01$) decreased FI. No significant differences were detected between birds fed HCP or MCP with or without avizyme supplementation on FI. The values of FCR showed that reducing dietary protein levels to 17% impaired FCR ($P<0.001$). No significant differences on FCR were observed between birds fed HCP or MCP with and without avizyme supplementation. Addition of avizyme to 17% CP diet improved FCR ($P<0.001$). These results indicated that reducing CP levels from HCP to MCP showed no adverse effect on growth performance.

Data of the overall experimental period showed significant ($P<0.001$) effect of CP level on BWG, FI and FCR. BWG decreased with decreasing dietary CP level and FCR improved with increasing dietary CP level. Addition of avizyme slightly enhanced ($P>0.05$) BWG and significantly ($P<0.001$) improved FCR. Birds fed LCP diet recorded the lowest BWG compared with those fed HCP or MCP diet. No significant differences were observed between birds fed HCP and MCP diets. Adding avizyme to all diets slightly improved BWG without significant effect. FI increased ($P<0.01$) with birds fed LCP diet. Addition of avizyme showed no significant effect on FI, except for birds fed LCP diet ($P<0.05$). Reducing dietary protein levels (LCP) significantly ($P<0.001$) impaired FCR. Addition of avizyme to MCP or LCP diets significantly ($P<0.001$) improved FCR (from 1.72 to 1.68 and from 2.02 to 1.91, respectively). Addition of avizyme to HCP diet improved FCR from 1.68 to 1.63 without significant effect. Therefore, the performance results clearly showed that addition of avizyme to low CP diets enhanced BWG and FCR of broiler chicks only during starting period.

Table 5: Effect of dietary treatments on growth performance of broiler chicks during finishing and overall periods

Item: Dietary treatments		Finishing (26- 40 days)			Overall (1-40 days)		
CP %	Avizyme g/kg	BWG (g)	FI (g)	FCR (g/g)	BWG (g)	FI (g)	FCR (g/g)
HCP (19)	0	896[a]	1618[c]	1.81[c]	1797[ab]	3008[bc]	1.68[cd]
MCP (18)	0	901[a]	1638[c]	1.82[c]	1741[b]	3001[bc]	1.72[c]
LCP (17)	0	856[ab]	1904[a]	2.23[a]	1589[c]	3208[a]	2.02[a]
HCP (19)	1	911[a]	1620[c]	1.78[c]	1844[a]	3007[bc]	1.63[d]
MCP (18)	1	870[ab]	1563[c]	1.80[c]	1744[b]	2928[c]	1.68[cd]
LCP (17)	1	834[b]	1742[b]	2.09[b]	1623[c]	3101[b]	1.91[b]
SE of means		±8.48	±23.26	±0.03	±19.16	±21.32	±0.03
Mean values of CP %							
HCP (23/21/19)		904[a]	1619[b]	1.79[b]	1820[a]	3008[b]	1.65[b]
MCP (21/19/18)		885[ab]	1601[b]	1.81[b]	1743[b]	2965[b]	1.70[b]
LCP (19/18/17)		845[b]	1823[a]	2.16[a]	1606[c]	3154[a]	1.97[a]
Mean values of Avizyme g/Kg							
0		884	1720[a]	1.95[a]	1709	3072[a]	1.81[a]
1		872	1642[b]	1.89[b]	1737	3012[b]	1.74[b]
Significances							
CP		*	***	***	***	***	***
Avizyme		NS	**	*	NS	*	***
CP × Avizyme		*	***	***	***	***	***

a-d Mean within each column with no common superscript differ significantly (p < 0.05)
*** P<0.001 ** P<0.01 * P<0.05 NS: not significant

Table 6: Effect of dietary treatments on carcass characteristics (% live body weight) of broiler chicks at 40 days of age

Item: Dietary treatments					Giblets		
CP%	Avizyme g/kg	Carcass weight (g)	Dressing %	Abdominal fat %	Liver %	Heart %	Gizzard %
HCP (23/21/19)	0	1376[a]	73.05	2.60[ab]	2.59	0.69	1.67
MCP (21/19/18)	0	1310[ab]	73.93	2.09[b]	2.6	0.59	1.73
LCP (19/18/17)	0	1288[ab]	73.48	2.41[ab]	2.81	0.6	1.74
HCP (23/21/19)	1	1422[a]	73.14	2.11[b]	2.55	0.59	1.72
MCP (21/19/18)	1	1291[ab]	74.45	2.98[a]	2.77	0.69	1.73
LCP (19/18/17)	1	1179[b]	72.61	2.25[ab]	2.82	0.65	1.76
SE of means		±23.23	±0.32	±0.10	±0.05	±0.02	±0.03
Mean values of CP %							
HCP (23/21/19)		1399[a]	73.09	2.36	2.57	0.64	1.7
MCP (21/19/18)		1300[ab]	74.19	2.53	2.68	0.64	1.73
LCP (19/18/17)		1233[b]	73.04	2.33	2.81	0.63	1.75
Mean values of Avizyme g/Kg							
0		1324	73.49	2.37	2.66	0.63	1.71
1		1297	73.4	2.45	2.71	0.65	1.74
Significances							
CP		**	NS	NS	NS	NS	NS
Avizyme		NS	NS	NS	NS	NS	NS
CP × Avizyme		*	NS	*	NS	NS	NS

a-d Mean within each column with no common superscript differ significantly (p < 0.05)
** P<0.01 * P<0.05 NS: not significant

During the finishing and overall experimental period BWG, FI and FCR did not significantly (P<0.001) differ when birds fed HCP or MCP diets. These results indicated that reducing CP did significantly affect growth performance during the finishing period. Addition of avizyme improved FCR (P<0.05%) (growing period). Using the lowest level of protein resulted in slow growth and less efficient use of feed.

The results of carcass characteristics are given in Table 6. Carcass weight followed the same trend as body weight gain. The highest dressing percentage value was recorded for birds fed the HCP diet supplemented with avizyme and the highest abdominal fat percentage value was recorded for birds fed the low protein diet supplemented with avizyme. Nitrogen (N) excretion as affected by dietary protein level and avizyme

Table 7: Effect of dietary treatments on nitrogen excretion of broiler chicks

Item Dietary treatments CP%	Avizyme g/kg	Nitrogen excretion % of starting period	Nitrogen excretion % of growing period	Nitrogen excretion % of finishing period
HCP	0	4.90^{a}	4.52^{a}	4.22^{a}
MCP	0	4.54^{b}	4.23^{b}	3.88^{b}
LCP	0	4.23^{c}	3.87^{c}	3.57^{c}
HCP	1	4.53^{b}	4.22^{b}	3.87^{b}
MCP	1	4.24^{c}	3.86^{c}	3.59^{c}
LCP	1	3.77^{d}	3.58^{d}	3.29^{d}
SE of means		±0.08	±0.08	±0.07
Mean values of CP %				
HCP (23/21/19)		4.71^{a}	4.37^{a}	4.04^{a}
MCP (21/19/18)		4.39^{b}	4.04^{b}	3.73^{b}
LCP (19/18/17)		4.00^{c}	3.73^{c}	3.43^{c}
Mean values of Avizyme g/Kg				
0		4.56^{a}	4.21^{a}	3.89^{a}
1		4.18^{b}	3.89^{b}	3.58^{b}
Significances				
CP		***	***	***
Avizyme		***	***	***
CP × Avizyme		***	***	***

a-d Mean within each column with no common superscript differ significantly ($p < 0.05$)

** * P<0.001

supplementation is shown in Table 7. Reducing dietary protein level significantly (P<0.001) decreased N excretion in starting, growing and finishing periods. Also, avizyme supplementation decreased (P<0.001) N excretion. In the starting period reducing CP from 23 to 21% (MCP) significantly (P<0.001) reduced N excretion by 6.79% and reducing CP from 23 to 19% (LCP) reduced N excretion by 15.07%. Addition of avizyme significantly (P<0.001) decreased N excretion by 8.33%. In the growing period reducing CP from 21 to 19% significantly (P<0.001) reduced N excretion by 7.55 % and reducing CP from 21 to 18 % (LCP) reduced N excretion by 14.65 %. Addition of avizyme significantly (P<0.001) decreased N excretion by 7.60%. In the finishing period reducing CP from 19 to 18 % significantly (P<0.001) decreased N excretion by 7.67 % and reducing CP from 19 to 17 % (LCP) decreased N excretion by 15.10 %. Addition of avizyme significantly (P<0.001) decreased N excretion by 7.97%. The results of performance are in agreement with many of the previous studies. Leeson *et al.* [20] reported that birds consume feed to primarily meet their energy requirements. The feed intake during the starting and growing period remained unchanged. In the starting period, birds might have had a physical limitation when trying to consume the low-density diets due to which feed intake was not altered during this period. Bregendahl *et al.* [4] found that chicks fed low-protein diets (19% CP) grew slower, used feed less efficiently and retained less N and more ether extract than chicks fed the control diets of 23% CP (P<0.05). Low protein diets failed to support equal growth performance to that of high-protein control diets. Yamazaki *et al.* [21] fed low crude protein diets (19% CP) with commercial enzyme complex (cellulase, protease and pectinase) and a control diet (21% CP) to 6-day-old male broiler chicks for 14 days. BWG, FI and FCR were not affected by the dietary treatments. The authors concluded that dietary CP content could be reduced from 21% to 19% without affecting performance of chick and supplementation of the enzyme complex to the 19% CP diet increases the AMEn level of the diet. Hidalgo *et al.* [22] found that birds fed low dietary CP had increased their feed intake during the finishing and overall experimental period, whereas it was unaffected during the starting period. BWG and FCR were adversely affected when the broilers were fed diets formulated to contain suboptimum concentrations of CP. Kamran *et al.* [23] suggested that dietary protein level of broilers could be reduced from 23 to 20% with beneficial effects on growth performance and carcass characteristics. Rezaei *et al.* [24] reported that reducing dietary protein decreased weight gain in starting, growing and total period of broiler chicks down to 6.0, 4.6 and 5.6%, respectively (P<0.05). Yamazaki *et al.* [25] suggested that CP content in the broiler diet can be reduced by 20 g/kg without lowering performance by the supplementation of crystalline amino acids. Zhuye *et al.* [26] indicated that the optimal dietary CP requirement is 21-22% of broilers from 1-21 days of age. Abdel-Maksoud *et al.* [27] suggested that maximum body weight could be obtained with a 21% low-CP plus EAAs supplementation which was the same as that of the chicks fed high protein diet (23% CP). Nguyen *et al.* [13] found that dietary protein level of 21-19% were sufficient for the best performance and carcass quality of the Betong chicken during growing period.

Inconsistent results had been published on the effect of dietary protein level and enzyme supplementation on carcass characteristics. Increased dressing percentage and abdominal fat by the addition of enzymes has been reported by Jamroz *et al.* [28] and Alam *et al.* [29]. They attributed the increased carcass yield by addition of enzymes in diet to higher fat deposition in carcass and also for increased meat yield. Alam *et al.* [29] found that addition of three different enzymes to broiler diets increased (P<0.01) dressing and liver weight. Gracia *et al.* [30] reported that weight of gizzard and liver as a percentage of BW decreased (P>0.05) with á-amylase supplementation to broiler diets based on corn-soybean meal. Hidalgo *et al.* [22] also reported no differences in carcass yield, breast meat yield and abdominal fat pad in broilers fed low-CP diets. On the other hand, Rezaei *et al.* [24] reported that decreasing dietary protein had no significant effect on breast meat yield, but increased abdominal fat percentage (P<0.05) of broiler chicks. Yamazaki *et al.* [25] found that abdominal fat deposition of broiler chicks was not affected by dietary CP level. Cellulase supplementation of a low-CP diet slightly lowered abdominal fat deposition.

The results of the present study proved that reducing dietary protein level and/or using avizyme supplementations reduced N excretion. This would help to decrease diet cost and environmental pollution. Nutritional strategies employed to address N losses to the environment have focused on dietary CP content and amino acids (AAs) composition. Reducing CP content of broiler diets by less than 2 percentage units, or 13% reduction in N intake, resulted in greater than 18% decrease in litter N content [31]. Parks *et al.* [32] demonstrated that amino acid supplemented diets with CP levels at 90% of NRC [33] reduced N excretion by 16.4% without adversely affecting growth performance. Ferguson et al. [34] indicated that reducing broiler starter diet CP by 16 g/kg, reduced litter N concentration by 16.3% with the low CP diets. Yamazaki *et al.* [21] reported that excreted nitrogen was lower for chicks fed low-protein diets compared to the control diet, however, no significant effect of enzyme supplementation was observed. Rezaei *et al.* [24] showed that with decreasing dietary protein level in broiler diet N excretion reduced (P<0.05). Nahm [1] indicated that use of amino acid and CP digestibilities values in formulating diets can reduce N excretion down to 40% and a 25% increase in N digestibility can be achieved with enzyme supplementation in broiler diets. Powers *et al.* [35] observed a 15% reduction in NH_3 emissions as a result of reducing dietary crude protein by 2 percentage units. Yamazaki *et al.* [25] found that birds given low-protein diets supplemented with 1000 U/kg of cellulase excreted less nitrogen. Results suggested that when CP content in the broiler diet reduced by 20 g/kg nitrogen excretion reduced by about 25%.

In conclusion, feeding broiler low CP die adversely affected the growth performance. Addition of avizyme if did not enhance gain in body weight, it improved efficiency of feed utilization. Reducing dietary protein level with adding 1 g avizyme/kg diet in starter and grower diets and reducing one percent of CP in finisher diet without avizyme could be suitable for improving body weight gain, feed efficiency and reducing nitrogen excretion for ROSS broiler chickens under the conditions of this study.

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