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Effect of Low Levels of Dietary Crude Protein with Constant Metabolizable Energy on Nitrogen Excretion, Litter Composition and Blood Parameters of Broilers

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ABSTRACT

A study was conducted to determine the effect of lowering dietary crude protein (CP) with optimum limiting amino acids (AA) levels on nitrogen (N) excretion, litter composition and blood parameters of broilers from 1 to 35 days of age. Four isocaloric broiler diets were formulated. Diet A served as control with 23, 22 and 20% CP, while in diets B, C and D, the CP was reduced to 22, 21 and 20%; 21, 20 and 19% and 19, 18 and 17% with 2925, 3075 and 3125 kcal/kg energy for starter, grower and finisher periods, respectively. A total of 1760, one day-old Hubbard broiler chicks were randomly divided into 16 experimental units, 110 chicks in each unit and each diet was offered to four experimental units at random. Results suggested that N excretion decreased ($P<0.05$), while N retention and its excretion as percent of N intake were not different among treatments. Low CP diets significantly reduced ($P<0.05$) the moisture and N content of the litter; although ash content was not altered. The plasma uric acid concentration decreased ($P<0.05$) and triglycerides concentration increased significantly by lowering the dietary CP content. However plasma glucose, triiodothyronine and thyroxine concentrations were unaffected. In conclusion, lowering dietary CP resulted in reduced N excretion without any adverse effect on N retention, which was also expressed in the levels of plasma metabolites and hormones.

Key Words: Low protein diets; Broilers; Nitrogen excretion; Litter composition; Blood parameters

INTRODUCTION

There has been a reasonable interest in reducing nitrogen (N) excreted in litter and the amount of ammonia produced in recent years (Morse, 1995). The use of low crude protein (CP) diets with amino acids (AA) supplementation is an effective way to reduce the N excretion by broilers. It therefore, decreases the disposal problems and pollution potential of the resulting litter (Moran *et al.*, 1992). However the balance of AA is important, while reducing CP because birds need AA in certain ratios to ensure optimum performance. The most N losses through excreta are due to the inability of dietary CP to meet the AA requirements and particularly the imbalances between the different AA. Dietary CP also has profound effects on metabolism and endocrine functioning of broiler chickens (Collin *et al.,* 2003). These changes in metabolism and body composition are reflected in blood plasma levels of intermediary metabolites and hormones. Some of these easy-to-measure factors can serve as bio-indicators for more complex processes, such as AA degradation and N excretion (Swennen *et al.,* 2005).

Much work has been done on the use of low CP diets in broilers. N excretion is reduced in most of the cases but there are contradictory results in terms of broiler performance (Parr & Summers, 1991; Moran & Stilborn, 1996; Aletor *et al*., 2000; Bregendahl *et al*., 2002; Sterling *et al.*, 2005; Waldroup *et al.*, 2005). The reason for contradictory results still needs to be determined. This is because limited information is available about the metabolism and endocrine functioning that regulate the effect of diet on animal performance. Therefore the present study was undertaken to determine the effect of lowering dietary CP on N excretion and retention, litter composition and blood parameters of broilers from 1 to 35 days of age.

MATERIALS AND METHODS

Birds and housing: Seventeen hundred and sixty, one day-old straight-run Hubbard broiler chicks were randomly divided into 16 experimental units, 110 chicks in each unit and each diet was offered to four experimental units at random. Vaccination for Newcastle disease was done on day 6 and 24, for Hydropericardium Syndrome on day 15

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and for Gumboro disease on day 10 and 20. The birds were kept under standard management conditions as recommended in Hubbard Management Guide 2004. Fresh water and feed were provided *ad libitum* throughout the experimental period.

Experimental diets: Before the formulation of experimental diets, the ingredients were analyzed in triplicate for their dry matter (DM), CP, ether extract, crude fiber (AOAC, 1990) and AA contents (Degussa AG, Germany). The N content was analyzed in triplicate by the Kjeldahl procedure and CP was calculated as N × 6.25. The fat content was determined in triplicate as ether extract using a Soxhlet apparatus. Four experimental broiler diets were formulated. Diet A served as control with 23, 22 and 20% CP in starter (1 to 10 days), grower (11 to 26 days) and finisher (27 to 35 days) periods, respectively. In diets B, C and D, the CP was reduced to 22, 21 and 20%; 21, 20 and 19% and 19, 18 and 17% for starter (Table I), grower (Table II) and finisher (Table III) periods, respectively. All the diets were isocaloric (2925, 3075 & 3125 kcal/kg metabolizable energy (ME) within starter, grower and finisher periods, respectively). Digestible lysine was maintained at 1.10, 1.02 and 0.90% of the diet in starter, grower and finisher periods, respectively and remaining limiting AA like methionine, threonine and tryptophan were included according to Hubbard recommendations. The nutrient composition of control and other diets either met or exceeded the Hubbard recommendations for broiler diets except CP, which was reduced in other diets. Analyzed dietary values for CP and AA closely matched with the calculated values. The experimental diets were fed up to 35 days of age.

Data collection: The apparent N excretion was calculated as the difference between N intake and its retention (Bregendahl *et al.*, 2002). N retention was calculated by computing the difference between the whole body N content at 26 days of age and the corresponding whole body baseline content. For whole body baseline content, ten chicks with a body weight (40±0.74 g) close to overall mean were selected and killed at the start of experiment to determine the baseline whole body N content. Litter samples were taken from centre and mid way between centre and four corners of each pen at the end of experiment. Hence five samples were taken from each pen and then combined and homogenized in plastic bags to make one sample/pen and were refrigerated until the moisture contents were determined by placing in an oven at 105°C for 24 h. The dried litter was ground and then N and ash contents were measured using standard AOAC (1990) procedures. At the last day of experiment, five birds per pen were selected for blood sampling. The blood samples were taken from wing vein with a syringe and heparine was used as anti-coagulant. Plasma was separated after centrifugation and was stored refrigerated for further analysis. Plasma triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured by radioimmunoassay, while plasma glucose (enzymatic UV test using hexokinase), triglycerides (colorimetric enzymatic test using glycerol-3-phosphate oxidase) and uric acid (enzymatic photometric test using TBHBA) concentrations were measured with an automated apparatus (DiaSys International Diagnostic Systems) as described by Swennen *et al.* (2005).

Statistical analysis: The results were analyzed by General Linear Model (P<0.05) and Tukey's significant difference test was used to compare means (Minitab 13.1, Minitab Inc., State College, PA). Linear and quadratic regression analyses were also done to estimate the effect of various CP levels (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

There was a decrease (P<0.05) in N excretion with reduction in dietary CP content (Table IV). However N retention and excretion as percent of N intake were not different among treatments (Table IV). Litter moisture and N contents were reduced (P<0.05) with low CP diets; however ash content remained un-changed (Table V). Blood plasma uric acid concentration decreased (P<0.05) and plasma triglycerides concentration increased (P<0.05) by lowering the dietary CP content. However, plasma glucose, T_3 and T_4 concentrations were un-affected by the dietary treatments (Table VI).

Chicks contained 1.46 g N on an average at the start of

Table I: Ingredient and nutrient composition of broiler starter diets with different levels of CP (1 to 10 days)

Ingredients Composition (%)	A (23% CP)	B (22% CP)	C (21% CP)	D (20% CP)
Corn	46.53	50.01	53.48	57.00
Rice polishings	5.00	5.00	5.00	5.00
Rapeseed meal	2.00	2.00	2.00	2.00
Canola meal	12.01	12.00	12.00	12.00
Guar meal	2.00	2.00	2.00	2.00
Soybean meal	21.01	17.90	14.79	11.60
Fish meal 60%	5.00	5.00	5.00	5.00
Limestone	0.70	0.70	0.70	0.70
Di-calcium phosphate	1.52	1.55	1.57	1.59
L-Lysine	0.08	0.17	0.25	0.34
DL-Methionine	0.12	0.15	0.17	0.19
L-Threonine	-	-	0.06	0.11
Sodium chloride	0.19	0.18	0.18	0.18
Sodium bicarbonate	0.08	0.08	0.08	0.08
Vegetable oil	3.26	2.73	2.22	1.71
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient Composition (%)				
ME, kcal/kg	2925	2925	2925	2925
CP[2]	23.0 (22.79)	22.0 (21.85)	21.0 (20.69)	20.0 (19.75)
Energy:Protein ratio	127	133	139	146
Crude fiber	4.87	4.75	4.63	4.51
Ether extract	6.68	6.24	5.83	5.41
Total ash	7.30	7.19	7.07	6.95
Calcium	1.01	1.01	1.01	1.00
Available phosphorus	0.50	0.50	0.50	0.50
Lysine[3]	1.10	1.10	1.10	1.10
Methionine[3]	0.49	0.49	0.49	0.49
Cystine[3]	0.34	0.34	0.34	0.34
Threonine[3]	0.73	0.73	0.73	0.73
Tryptophan[3]	0.21	0.21	0.21	0.21

Table II: Ingredient and nutrient composition of broiler grower diets with different levels of CP (11 to 26 days)

Ingredients Composition (%)	A (22% CP)	B (21% CP)	C (20% CP)	D (19% CP)
Corn	47.40	51.00	54.56	58.12
Rice polishings	5.00	5.00	5.00	5.00
Canola meal	12.10	11.99	11.99	12.00
Guar meal	2.00	2.00	2.00	2.00
Soybean meal	20.00	16.90	13.79	10.60
Fish meal 60%	5.00	5.00	5.00	5.00
Limestone	0.70	0.70	0.70	0.70
Di-calcium phosphate	1.56	1.59	1.61	1.63
L-Lysine	0.14	0.23	0.32	0.41
DL-Methionine	0.13	0.14	0.15	0.16
L-Threonine	-	-	-	-
Sodium chloride	0.19	0.19	0.19	0.19
Sodium bicarbonate	0.08	0.08	0.08	0.08
Vegetable oil	5.21	4.67	4.13	3.60
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient Composition (%)				
ME, kcal/kg	3075	3075	3075	3075
CP[2]	22.0 (21.65)	21.0 (20.95)	20.0 (19.63)	19.0 (18.76)
Energy:Protein ratio	140	146	154	162
Crude fiber	4.62	4.50	4.38	4.26
Ether extract	8.58	8.14	7.69	7.26
Total ash	7.13	7.01	6.90	6.79
Calcium	1.01	1.00	1.00	1.00
Available phosphorus	0.50	0.50	0.50	0.50
Lysine[3]	1.02	1.02	1.02	1.02
Methionine[3]	0.46	0.46	0.46	0.46
Cystine[3]	0.35	0.35	0.35	0.35
Threonine[3]	0.69	0.69	0.69	0.69
Tryptophan[3]	0.18	0.18	0.18	0.18

Table III: Ingredient and nutrient composition of broiler finisher diets with different levels of CP (27 to 35 days)

Ingredients Composition (%)	A (20% CP)	B (19% CP)	C (18% CP)	D (17% CP)
Corn	55.18	58.61	62.14	65.50
Rice polishings	5.00	5.00	5.00	5.00
Canola meal	9.00	9.00	9.01	9.00
Guar meal	2.00	2.00	2.00	2.00
Soybean meal	16.60	13.50	10.31	7.20
Fish meal 60%	5.00	5.00	5.00	5.00
Limestone	0.80	0.80	0.80	0.90
Di-calcium phosphate	1.05	1.07	1.09	1.11
L-Lysine	0.04	0.13	0.22	0.31
DL-Methionine	0.10	0.11	0.13	0.14
L-Threonine	-	0.04	0.08	0.12
Sodium chloride	0.13	0.13	0.13	0.13
Sodium bicarbonate	0.09	0.09	0.09	0.09
Vegetable oil	4.52	4.01	3.50	3.00
Vitamin/mineral premix[1]	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient Composition (%)				
ME, kcal/kg	3125	3125	3125	3125
CP[2]	20.0 (20.15)	19.0 (18.65)	18.0 (17.91)	17.0 (16.81)
Energy:Protein ratio	156	164	174	184
Crude fiber	4.17	4.06	3.94	3.81
Ether extract	8.07	7.66	7.25	6.83
Total ash	6.43	6.32	6.20	6.14
Calcium	0.89	0.88	0.88	0.92
Available phosphorus	0.41	0.41	0.41	0.41
Lysine[3]	0.90	0.90	0.90	0.90
Methionine[3]	0.44	0.44	0.44	0.44
Cystine[3]	0.34	0.34	0.34	0.34
Threonine[3]	0.64	0.64	0.64	0.64
Tryptophan[3]	0.16	0.16	0.16	0.16

[1]Supplied per kilogram of diet: vitamin A (as retinyl acetate), 14,000 IU; vitamin D_3 (as cholecalciferol), 3,500 IU; vitamin K (menadione sodium bisulfite), 2.8 mg; vitamin E (as d-*á*-tocopherol), 42 IU; biotin, 0.07 mg; folic acid, 1.7 mg; niacin, 35 mg; calcium pantothenate, 12.32 mg; pyridoxine, 3.36 mg; riboflavin, 7 mg; thiamin, 1.7 mg; vitamin B_{12}, 12.1 µg; Fe, 98 mg; Mn, 112 mg; Cu, 9.8 mg; Se, 0.07 mg ; Zn, 70 mg; choline chloride, 550 mg; salinomycin (as Phibro coccidiostat), 60 mg; zinc bacitracin (as Albac 10%), 50 mg.
[2]The values in parenthesis are analyzed values; [3]Digestible (ileal)

the experiment. It was observed that although, the birds fed low CP diets retained lesser N, the difference was, however non-significant. Some studies have shown that carcass composition and quality becomes inferior with reduced CP diets (Pinchasov *et al*., 1990; Aletor *et al*., 2000). However in the present study, there was no adverse effect on the N retention of the chicks fed with various low CP diets. This is likely because AA particularly the limiting AA lysine, methionine, threonine and tryptophan, were probably in suitable balance; thus there was no adverse effect on the N retention of chicks. It was further observed that chicks fed low CP diets excreted lesser N than those fed high CP diets. However N excretion as a fraction of N intake was similar among treatments, as evident from a positive correlation between N excretion and N intake ($r=0.99$; $P<0.01$). This signifies the importance of low CP diets in that by decreasing the excess of non-essential AA and supplementing essential AA, N excretion can be significantly reduced (Moran *et al*., 1992; Ferguson *et al.*, 1998; Bregendahl *et al*., 2002; Rezaei *et al*., 2004).

Reduction in dietary CP significantly reduced the N and moisture content of the litter, while ash content was not affected. A significant positive correlation ($r=0.96$; $P<0.05$) was found between the dietary CP level and the moisture content of the litter. Dietary protein in excess of requirements causes an increased heat increment and water intake, which results in an elevated litter moisture content (Alleman & Leclercq, 1997). So the litter moisture was linearly decreased with a reduction in dietary CP content. Reducing dietary CP also significantly reduced the N content of the litter by decreasing N excretion. These results are consistent with the findings of Ferguson *et al.* (1998) but contrarily, Moran *et al.* (1992) and Khajali and Moghaddam (2006), who although noted a significant decrease in N content but recorded no change in the associated moisture and ash contents.

Blood plasma analysis showed that lowering the dietary CP content did not affect the plasma glucose concentration, thus indicating that carbohydrate metabolism was not affected by the diet (Swennen *et al*., 2005). The plasma triglycerides level was increased with low CP diets. It was expected, because increased ME:CP ratio increases the process of lipogenesis in the body (Rosebrough & Steele, 1985). These findings implied that the birds fed on low CP diets preferably used carbohydrates as an energy

Table IV: Nitrogen retention and excretion of broilers as influenced by different dietary CP levels from 1 to 26 days of age[1]

Item	N retention[2] (g/chick)	N excretion[3] (g)	(% of N intake)
Diets[4]			
A	22.9	38.0[a]	62.3
B	22.1	36.2[a]	61.9
C	22.4	32.9[ab]	59.5
D	21.5	30.2[b]	58.4
SEM	0.84	1.31	1.69
ANOVA		Probability	
CP	0.581	0.001	0.179
Linear	0.288	<0.001	0.060
Quadratic	0.962	0.757	0.835

[a-b]Means within a column with different letters differ significantly (P <0.05)
[1]Means of 4 replicates with 5 birds from each replicate
[2]Chicks contained 1.46 g nitrogen on an average at the start of experiment
[3]Calculated as nitrogen intake - retention
[4]Diet A contained 23 & 22% CP; diet B contained 22 and 21% CP; diet C contained 21 and 20% CP and diet D contained 20 and 19% CP for starter and grower periods, respectively

Table V: Litter analysis as influenced by different dietary CP levels from 1 to 35 days of age[1]

Item	Moisture (%)	Nitrogen (% DM)	Ash (% DM)
Diets[2]			
A	59.8[a]	4.0[a]	9.00
B	59.3[a]	3.6[b]	8.95
C	57.4[b]	3.6[b]	9.00
D	57.0[b]	3.3[c]	9.15
SEM	0.26	0.04	0.39
ANOVA		Probability	
CP	<0.001	<0.001	0.985
Linear	<0.001	<0.001	0.762
Quadratic	0.877	0.757	0.794

[a-c]Means within a column with different letters differ significantly (P < 0.05)
[1]Means of four replicates, five samples were taken from each replicate and then combined and homogenized to make one sample at the end of experiment.
[2]Diet A contained 23, 22 and 20% CP; diet B contained 22, 21 and 19% CP; diet C contained 21, 20 and 18% CP and diet D contained 20, 19 and 17% CP for starter, grower and finisher periods, respectively

source rather than free fatty acids resulting in higher plasma triglycerides levels. Swennen *et al.* (2005) also confirmed these findings in a study using iso-energetic broiler diets with the substitutions of protein and fat. Yeh and Leveille (1969) reported that the dietary CP level and *in vitro* lipogenesis are inversely related. They reported that a higher dietary CP content decreased the utilization of glucose and increased its synthesis from substrates that were used for fat synthesis previously.

Considerably lower uric acid levels were observed in the plasma of the birds fed low CP diets, which confirmed the previous findings of Collin *et al*. (2003). Swennen *et al.* (2005) also reported that in low CP fed chickens, AA oxidation rate is decreased in order to spare more protein, in addition to their increased ability of protein retention. Since birds fed low CP diets consumed less protein, as a reaction of this, they improved their efficiency of protein retention. It is likely that reduced protein degradation/AA oxidation

Table VI: Blood parameters of broilers as influenced by different dietary CP levels from 1 to 35 days of age[1]

Item	Glucose (mg/dL)	Triglycerides (mg/dL)	Uric acid (mg/dL)	T3 (nmol/L)	T4 (nmol/L)
Diets[2]					
A	241.5	40.8[b]	5.7[a]	2.70	7.42
B	226.0	40.8[b]	5.1[ab]	1.93	10.37
C	236.0	46.5[a]	3.6[ab]	2.84	7.25
D	242.5	44.0[a]	3.5[b]	1.68	8.22
SEM	8.07	3.15	0.52	0.32	1.03
ANOVA			Probability		
CP	0.480	<0.001	0.024	0.067	0.179
Linear	0.729	0.261	0.015	0.221	0.891
Quadratic	0.190	0.040	0.545	0.621	0.417

Means within a column with different letters differ significantly (P < 0.05)
[1]Means of 4 replicates with 5 birds from each replicate
[2]Diet A contained 23, 22 and 20% CP; diet B contained 22, 21 and 19% CP; diet C contained 21, 20 and 18% CP and diet D contained 20, 19 and 17% CP for starter, grower and finisher periods, respectively

contributed to more efficient retention of dietary protein as a compensatory mechanism for a reduced protein intake. In a study, Swennen *et al.* (2006) observed that chickens fed low CP diets had significantly increased plasma triglycerides and decreased uric acid levels, while the glucose levels were un-affected by the dietary treatments. The increased plasma triglycerides level in that study was due to increased energy to protein ratio and ultimately increased de novo lipogenesis.

Plasma T_3 and T_4 levels were un-affected by the dietary treatments. The plasma thyroid hormones concentrations are changed with the change in dietary protein resulting in differences in growth (Rosebrough & McMurtry, 1998). Generally plasma T_3 concentrations are increased and T_4 concentrations are decreased with low CP diets (Swennen *et al*., 2005, 2006). However in the present study, the plasma concentrations of T_3 and T_4 were not changed. This was expressed as equal N retention by broilers fed different dietary treatments.

In conclusion, lowering dietary CP content resulted in reduced N excretion without any adverse effect on N retention and it was also expressed in the levels of plasma metabolites and hormones.

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