

Nutrients retention, nitrogen excretion, litter composition and plasma biochemical profile in broilers fed low crude protein diets with constant metabolizable energy to crude protein ratio

Z. Kamran^{a†}, M. Sarwar^b, M. Nisa^b, M. A. Nadeem^c, S. Ahmad^a, H. Nawaz^b, K. Koutoulis^e, M. U. Sohail^d, Z. Iqbal^b, M. I. Shahzad^a

^aUniversity College of Veterinary and Animal Sciences, The Islamia University of Bahawalpur-63100, Pakistan; ^bInstitute of Animal Science, University of Agriculture, Faisalabad-38040, Pakistan; ^cAnimal Nutrition, Animal Sciences Institute, National Agriculture Research Centre, Park Road, Islamabad-45500, Pakistan; ^dDepartment of Physiology, Government College University, Faisalabad-38000, Pakistan; ^eDepartment of Avian Medicine, Faculty of Veterinary Science, School of Health Sciences, University of Thessaly, Karditsa-43100, Greece

SUMMARY

An experiment was carried out to investigate the effect of low CP diets with constant ME:CP ratio on nutrients retention, nitrogen (N) excretion, litter composition and plasma biochemical profile in broilers from 1 to 35 d of age. Four dietary treatments having 4 levels of CP and ME were formulated during starter (23, 22, 21 and 20% CP, and 3,036, 2,904, 2,772 and 2,640 kcal/kg ME), grower (22, 21, 20 and 19% CP, and 3,146, 3,003, 2,860 and 2,717 kcal/kg ME), and finisher (20, 19, 18 and 17% CP, and 3,100, 2,945, 2,790 and 2,635 kcal/kg ME) periods. In total, 1,760 day-old Hubbard broiler chicks were distributed to 16 experimental units of 110 chicks each at random and each dietary treatment was randomly fed to 4 experimental units. Results suggested that 26 d N intake, fat retention, N retention, N excretion and N excretion as percent of N intake were not different among treatments. Similarly, 35 d litter moisture, N and ash contents were not affected by reducing dietary CP and ME levels. However, plasma glucose and triglycerides concentrations increased ($P < 0.05$) without any treatment effect on plasma uric acid, triiodothyronine and thyroxine concentrations. In conclusion, dietary treatments resulted in similar N retention without any significant increase in fat retention. However, N excretion and litter N contents were not decreased due to similar N intake among treatments, also reflected by plasma parameters.

† Corresponding author e-mail: zahid.kamran@iub.edu.pk

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INTRODUCTION

Reducing the CP level of diet has been found to reduce nitrogen (N) excretion in broilers (Ferguson et al., 1998; Kamran et al., 2010; Hernandez et al., 2012). This, in turn, decreases the litter N, ammonia production and pollution potential of litter (Moran et al., 1992; Ferguson et al., 1998). However, dietary CP reduction is associated with decrease in protein and increase in fat retention in carcass (Neto et al., 2000; Si et al., 2001), along with contradictory results in terms of growth performance (Bregendahl et al., 2002; Waldroup et al., 2005; Kamran et al., 2011). With the increasing feed cost, efficiency of nutrients retention for the production of lean meat is as important as other performance parameters. In this regard, limiting amino acids (AA) supplementation of low CP diets helps to reduce fat retention, N excretion and improve N retention efficiency in broilers (Chung and Baker, 1992; Si et al., 2001). However, it is interesting to know whether keeping a constant energy to protein ratio in low CP diets can result in reduced fat retention along with reduced N excretion. It is now well recognized that diet composition and nutrient ratios greatly affect the carcass composition of broilers (Collin et al., 2003; Swennen et al., 2006). Diet has a significant effect on metabolism and endocrine functioning which causes changes in plasma concentrations of metabolites and hormones. These changes are reflected in the processes like fat and N retention and N excretion (Swennen et al., 2005; Kamran et al., 2010).

Not much work has been done in broilers to find out the effects of low CP diets with constant ME:CP ratio on nutrients retention and N excretion (Zhao et al., 2009). In addition, some previously conducted studies have shown poor results in terms of broiler performance (Hidalgo et al., 2004; Kamran et al., 2008). No conclusive explanation has been found yet for these results. This is because little is known about the metabolism, endocrine functioning and underlying mechanisms that regulate the effect of diet on animal performance. Therefore, the aim of the present work was to investigate the effects of low CP diets with constant ME:CP ratio on nutrients retention, N excretion, litter composition and plasma biochemical profile in broilers from 1 to 35 d of age.

MATERIAL AND METHODS

Birds and housing

A sum of 1,760 day-old Hubbard broiler chicks was distributed to 16 experimental units of 110 chicks each at random. Vaccination for Newcastle Disease was done on d 6 and 24, for Infectious Bursal Disease on

d 10 and 20 and for Hydropericardium Syndrome on d 15. The management of temperature, ventilation and light was done as recommended by Hubbard Management Guide (2004). Ad libitum quantities of fresh water and feed were offered during the whole experiment.

Table 1. Ingredient and nutrient composition of broiler starter diets containing low protein with constant energy to protein ratio (1 to 10 d)

Ingredients	A	B	C	D
Composition (%)	(23/3036)	(22/2904)	(21/2772)	(20/2640)
Corn	43.96	50.51	49.78	49.04
Rice polishings	5.00	5.00	12.20	7.01
Wheat bran	-	-	-	8.41
Canola meal	11.99	12.00	12.00	13.01
Guar meal	2.00	2.00	2.00	2.00
Soybean meal	21.58	17.80	13.59	9.81
Fish meal 60%	5.00	5.00	5.00	4.00
Limestone	0.70	0.70	0.70	0.80
Di-Calcium Phosphate	1.53	1.54	1.48	1.48
L-Lysine HCl	0.06	0.17	0.27	0.37
DL-Methionine	0.12	0.14	0.17	0.19
L-Threonine	-	0.02	0.07	0.11
Sodium chloride	0.19	0.18	0.17	0.19
Sodium bicarbonate	0.08	0.08	0.08	0.08
Vegetable oil	5.30	2.35	-	-
Vitamin/mineral premix ¹	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient Composition (%)				
ME, kcal/kg	3036	2904	2772	2640
CP ²	23.0 (22.87)	22.0 (22.18)	21.0 (20.77)	20.0 (19.85)
ME:CP ratio	132	132	132	132
Crude fiber	4.84	4.75	5.38	5.90
Ether extract	8.61	5.88	4.49	3.98
Calcium	1.01	1.01	0.98	0.99
Available phosphorus	0.50	0.50	0.49	0.49
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

¹Supplied per kilogram of diet: vitamin A (as retinyl acetate), 14,000 IU; vitamin D3 (as cholecalciferol), 3,500 IU; vitamin K (as menadione sodium bisulfite), 2.8 mg; vitamin E (as d- α -tocopherol), 42 IU; biotin, 0.07 mg; folic acid, 1.7 mg; niacin, 35 mg; calcium pantothenate, 12.3 mg; pyridoxine, 3.4 mg; riboflavin, 7 mg; thiamin, 1.7 mg; vitamin B₁₂, 12.1 μ g; Fe, 98 mg; Mn, 112 mg; Cu, 9.8 mg; Se, 0.07 mg; Zn, 70 mg; choline chloride, 550 mg; salinomycin, 60 mg; zinc bacitracin (as Albac 10%), 50 mg.

²The values in parenthesis are analyzed values; ³Digestible

Experimental diets

The dietary ingredients analysis was done in triplicate for their dry matter, CP, ether extract, crude fiber (AOAC, 1990) and AA composition. Four dietary treatments having 4 levels of CP and ME were formulated during starter (23, 22, 21 and 20% CP, and 3,036, 2,904, 2,772 and 2,640 kcal/kg ME; 1 to 10 d), grower (22, 21, 20 and 19% CP, and 3,146, 3,003, 2,860 and 2,717 kcal/kg ME; 11 to 26 d), and finisher (20, 19, 18 and 17% CP, and 3,100, 2,945, 2,790 and 2,635 kcal/kg ME; 27 to 35 d) periods. Dietary energy to protein ratio of 132, 143 and 155, and digestible Lys level of 1.10, 1.02 and 0.90% were kept during starter, grower and finisher phases, respectively (Tables 1, 2 and 3). Other essential AA like Met, Thr and Trp were kept as recommended by Hubbard. Each dietary treatment was randomly assigned to 4 replicates up to 35 d of age.

Table 2. Ingredient and nutrient composition of broiler grower diets containing low protein with constant energy to protein ratio (11 to 26 d)

Ingredients	A	B	C	D
Composition (%)	(22/3146)	(21/3003)	(20/2860)	(19/2717)
Corn	45.56	52.67	59.56	55.47
Rice polishings	5.00	5.00	5.00	5.00
Wheat bran	-	-	-	6.90
Canola meal	11.99	11.99	12.01	11.99
Guar meal	2.00	2.00	2.00	3.00
Soybean meal	19.98	16.59	12.71	6.50
Fish meal 60%	5.00	5.00	5.00	5.00
Limestone	0.70	0.70	0.70	1.30
Di-Calcium Phosphate	1.57	1.58	1.59	1.47
L-Lysine HCl	0.13	0.24	0.34	0.45
DL-Methionine	0.13	0.14	0.15	0.16
L-Threonine	-	-	-	-
Sodium chloride	0.19	0.19	0.18	0.18
Sodium bicarbonate	0.08	0.08	0.08	0.08
Vegetable oil	6.58	3.34	0.17	-
Vitamin/mineral premix ¹	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient Composition (%)				
ME, kcal/kg	3146	3003	2860	2717
CP ²	22.0 (21.95)	21.0 (20.87)	20.0 (20.09)	19.0 (18.79)
ME:CP ratio	143	143	143	143
Crude fiber	4.00	3.50	4.00	4.50
Ether extract	11.67	7.33	4.67	5.00
Calcium	1.01	1.01	0.98	0.99
Available phosphorus	0.50	0.50	0.49	0.49
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

¹Supplied per kilogram of diet: vitamin A (as retinyl acetate), 14,000 IU; vitamin D3 (as cholecalciferol), 3,500 IU; vitamin K (as menadione sodium bisulfite), 2.8 mg; vitamin E (as d- α -tocopherol), 42 IU; biotin, 0.07 mg; folic acid, 1.7 mg; niacin, 35 mg; calcium pantothenate, 12.3 mg; pyridoxine, 3.4 mg; riboflavin, 7 mg; thiamin, 1.7 mg; vitamin B₁₂, 12.1 μ g; Fe, 98 mg; Mn, 112 mg; Cu, 9.8 mg; Se, 0.07 mg; Zn, 70 mg; choline chloride, 550 mg; salinomycin, 60 mg; zinc bacitracin (as Albac 10%), 50 mg.

²The values in parenthesis are analyzed values; ³Digestible

Table 3. Ingredient and nutrient composition of broiler finisher diets containing low protein with constant energy to protein ratio (27 to 35 d)

Ingredients	A	B	C	D
Composition (%)	(20/3100)	(19/2945)	(18/2790)	(17/2635)
Corn	55.72	64.10	59.99	53.09
Rice polishings	5.00	5.00	5.00	8.00
Wheat bran	-	-	5.40	10.70
Canola meal	9.00	6.00	12.00	9.00
Guar meal	2.00	2.00	2.00	2.00
Sunflower meal	-	-	-	6.00
Soybean meal	16.50	14.60	8.10	4.40
Fish meal 60%	5.00	5.00	4.00	3.00
Limestone	0.80	0.90	1.20	1.30
Di-Calcium Phosphate	1.05	1.07	1.09	1.08
L-Lysine HCl	0.04	0.14	0.24	0.37
DL-Methionine	0.10	0.12	0.12	0.14
L-Threonine	-	0.05	0.08	0.14
Sodium chloride	0.13	0.13	0.19	0.20
Sodium bicarbonate	0.09	0.09	0.09	0.09
Vegetable oil	4.06	0.30	-	-
Vitamin/mineral premix ¹	0.50	0.50	0.50	0.50
Total	100	100	100	100
Nutrient Composition (%)				
ME, kcal/kg	3100	2945	2790	2635
CP ²	20.0 (19.68)	19.0 (18.82)	18.0 (17.88)	17.0 (16.93)
ME:CP ratio	155	155	155	155
Crude fiber	4.18	3.84	4.90	6.75
Ether extract	7.64	4.13	3.85	4.10
Calcium	0.89	0.91	1.01	1.00
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

¹Supplied per kilogram of diet: vitamin A (as retinyl acetate), 14,000 IU; vitamin D3 (as cholecalciferol), 3,500 IU; vitamin K (as menadione sodium bisulfite), 2.8 mg; vitamin E (as d- α -tocopherol), 42 IU; biotin, 0.07 mg; folic acid, 1.7 mg; niacin, 35 mg; calcium pantothenate, 12.3 mg; pyridoxine, 3.4 mg; riboflavin, 7 mg; thiamin, 1.7 mg; vitamin B₁₂, 12.1 μ g; Fe, 98 mg; Mn, 112 mg; Cu, 9.8 mg; Se, 0.07 mg; Zn, 70 mg; choline chloride, 550 mg; salinomycin, 60 mg; zinc bacitracin (as Albac 10%), 50 mg.

²The values in parenthesis are analyzed values; ³Digestible

Data collection

At 26 d, five birds from each replicate were selected and whole body analysis was done for the determination of fat and N retention. Fat and N retention were calculated by computing the difference between the whole body ether extract and N contents at 26 d of age and the corresponding whole body baseline content. For whole body baseline content, 10 chicks with a body weight close to overall mean (40 ± 0.74 g) were selected and killed for further analysis at the start of experiment. Total ME intake and 26 d N intake were also calculated for various dietary treatments. The apparent N excretion was calculated as the difference between N intake and retention (Kamran et al., 2010). At the end of experiment, 5 litter samples were taken from each pen and combined and homogenized in plastic bags to make 1 sample per pen. The moisture content was determined by placing litter samples in an oven at 105°C for 24 h. The dried litter was ground and N and ash contents were measured using standard AOAC (1990) procedures. At the last d of experiment, 5 birds per pen were selected and blood samples were taken from wing vein using heparin as anti-coagulant. After centrifugation, plasma was separated and refrigerated for further analysis of hormones and metabolites. Plasma triiodothyronine (T₃) and thyroxine (T₄) concentrations were measured by RIA, whereas, 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 using an automated apparatus (DiaSys International Diagnostic Systems; Kamran et al., 2010).

Statistical analysis

The data analysis was done by General Linear Model ($P < 0.05$) and means were separated by Tukey's test (Minitab 13.1, Minitab Inc., State College, PA). Analysis for various regressions was also completed to approximate the effect of various diets.

RESULTS

Reducing the dietary CP and ME levels did not affect the fat retention, N retention and N intake of birds (Table 4). The N excretion and N excretion as percent of N intake were also similar among treatments (Table 4). Likewise, litter moisture, N and ash contents were not affected by reducing the dietary CP and ME levels (Table 5). However, plasma glucose and triglycerides concentrations increased ($P < 0.05$), whereas, no treatment effect was noted on plasma uric acid, T₃ and T₄ concentrations (Table 6). In spite of reducing ME along with CP in low nutrient density diets, the total ME intake during 35 day growth period was not different

among various treatments. The experimental average for total ME intake was 10,050 kcal/bird (data not shown).

Table 4. Fat retention, N¹ retention, intake and excretion of broilers fed different diets containing low protein with constant energy:protein ratio during 1 to 26 days of age²

Item	Fat retention ³ (g/chick)	N retention ³ (g/chick)	N intake (g/chick)	N excretion ⁴ (% of N intake)	N excretion
Diets ⁵					
A	71.6	21.8	62.3	40.5	65.0
B	73.6	22.4	60.5	38.1	63.0
C	78.7	21.6	59.9	38.3	64.0
D	80.7	20.4	58.9	38.5	65.4
SEM	3.11	0.80	0.86	0.98	1.35
ANOVA			Probability		
Diet	0.188	0.398	0.088	0.334	0.609
Linear	0.026	0.175	0.010	0.207	0.731
Quadratic	0.993	0.282	0.231	0.214	0.214

¹Nitrogen; ²Means of 4 replicates with 5 birds from each replicate; ³Chicks contained 1.46 g nitrogen and 4.6 g fat on average at the start of the experiment; ⁴Calculated as nitrogen intake - retention; ⁵Diet A contained 23 and 22% CP and 3,036 and 3,146 kcal/kg ME; diet B contained 22 and 21% CP and 2,904 and 3,003 kcal/kg ME; diet C contained 21 and 20% CP and 2,772 and 2,860 kcal/kg ME and diet D contained 20 and 19% CP and 2,640 and 2,717 kcal/kg ME for starter and grower periods, respectively

Table 5. Litter moisture, nitrogen and ash contents as influenced by different diets containing low protein with constant energy:protein ratio during 1 to 35 days of age¹

Item	Moisture, (%)	Nitrogen, (% DM)	Ash, (% DM)
Diets ²			
A	59.0	3.81	9.30
B	59.1	3.78	9.15
C	58.9	3.79	9.53
D	58.6	3.76	9.08
SEM	0.60	0.06	0.33
ANOVA		Probability	
Diet	0.925	0.926	0.778
Linear	0.541	0.572	0.835
Quadratic	0.720	0.965	0.653

¹Means of 4 replicates. Five samples were taken from each replicate and combined and homogenized to make one sample at the end of experiment

²Diet A contained 23, 22 and 20% CP and 3,036, 3,146 and 3,100 kcal/kg ME; diet B contained 22, 21 and 19% CP and 2,904, 3,003 and 2,945 kcal/kg ME; diet C contained 21, 20 and 18% CP and 2,772, 2,860 and 2,790 kcal/kg ME and diet D contained 20, 19 and 17% CP and 2,640, 2,717 and 2,635 kcal/kg ME for starter, grower and finisher periods, respectively

DISCUSSION

Although, fat and N retention were similar among treatments, the birds fed diets containing reduced CP and ME levels tended to retain more fat in a linear fashion. This tendency of increased body fat might be due to the fact that birds fed low nutrient density diets ate more to maintain their ME intake, resulting in this trend of fat retention. Moreover, due to low heat increment, the net energy of low CP diets may be higher than the conventional broiler diets (Emmans, 1994). This increased energy availability may also be the reason of this increasing trend. However, this increase could not become significant as ME:CP ratio was kept constant in all diets. Namroud et al. (2008, 2010) reported an increased fat deposition with reduced CP diets. The N retention was similar among treatments without any adverse effect. This might partly be due to similar ME and hence, N intake for various dietary treatments (Kamran et al., 2008) and partly due to similar efficiency of N utilization resulting from limiting AA supplementation of low CP and low ME diets. Bregendahl et al. (2002) reported significantly less N and more fat retention with the lowest dietary regimen as compared to control group. Similarly, Zhao et al. (2009) reported higher fat and lower carcass protein contents in Arbor Acres broilers (breed × diet interaction) fed low CP diets having constant energy to protein ratio. On the other hand, increased N retention was reported with lower dietary CP level by Kermanshahi et al. (2011); and by Hernandez et al. (2012) during starter and finisher phase.

Table 6. Plasma biochemical profile of broilers as influenced by different diets containing low protein with constant energy:protein ratio during 1 to 35 days of age¹

Item	Glucose (mg/dL)	Triglycerides (mg/dL)	Uric acid (mg/dL)	T3 (nmol/L)	T4 (nmol/L)
Diets ²					
A	211.2 ^b	37.0 ^b	4.4	1.64	10.8
B	210.2 ^b	42.8 ^{ab}	3.8	1.38	8.6
C	236.8 ^{ab}	46.8 ^{ab}	5.2	1.86	11.6
D	246.2 ^a	59.2 ^a	5.8	2.56	8.4
SEM	8.17	4.08	0.80	0.34	1.92
ANOVA			Probability		
Diet	0.018	0.014	0.377	0.144	0.578
Linear	0.003	0.001	0.136	0.054	0.614
Quadratic	0.539	0.411	0.476	0.170	0.813

^{a-b}Means within a column with different superscripts differ significantly ($P < 0.05$)

¹Means of 4 replicates with 5 birds from each replicate

²Diet A contained 23, 22 and 20% CP and 3,036, 3,146 and 3,100 kcal/kg ME; diet B contained 22, 21 and 19% CP and 2,904, 3,003 and 2,945 kcal/kg ME; diet C contained 21, 20 and 18% CP and 2,772, 2,860 and 2,790 kcal/kg ME and diet D contained 20, 19 and 17% CP and 2,640, 2,717 and 2,635 kcal/kg ME for starter, grower and finisher periods, respectively

The N excretion, N excretion as percent of N intake, litter N, moisture and ash contents were not different among various treatment groups. Decreasing dietary CP may decrease heat increment and thus water intake, along with reducing N excretion. This results into decreased litter moisture and N contents (Alleman and Leclercq, 1997; Ferguson et al., 1998). However, in spite of reducing dietary CP, the birds maintained their N intake resulting in similar N excretion and litter composition among various treatments in the present study. This fact was evident from positive correlations between N intake and N excretion ($r = 0.81$) and between N intake and litter N ($r = 0.92$). The results regarding N excretion and litter composition were not in accordance with the observations of Ferguson et al. (1998) and Kamran et al. (2010) who reported significant decrease in N excretion, litter N and moisture content with decrease in dietary CP. Similarly, Moran et al. (1992), Bregendahl et al. (2002), Namroud et al. (2008) and Hernandez et al. (2012) reported a decrease in N excretion with reduction in dietary CP. Moran et al. (1992) and Khajali and Moghaddam (2006) also observed reduced litter N content, whereas, no effect was noted on litter moisture. In the present study, the main reason for observed differences was the reduced ME content (to maintain a constant ME:CP ratio), resulting in similar N intake and excretion among treatments, although the dietary CP was reduced.

Blood plasma analysis showed a linear increase in plasma glucose concentrations. Zhao et al. (2009) reported increased plasma glucose levels with low CP and low ME diets. Recently, Hernandez et al. (2012) reported increased plasma glucose concentrations by lowering the dietary CP of finishing broilers. The increased glucose level with the use of reduced CP and ME diets might be due to increased intake of nutrients on one hand (Lorenz and Cornelius, 1993), and decreased insulin sensitivity (Boden et al., 1994) affecting the transport and utilization of glucose, on the other hand. However, Swennen et al. (2005, 2006) and Kamran et al. (2010) reported that carbohydrate metabolism and glucose levels were not affected by the diet composition. A linear increase in plasma triglycerides concentrations was observed which was unexpected as ME:CP ratio was kept constant in all the diets. Because the fat content of reduced CP and ME diets was low, the increased plasma triglycerides levels indicated enhanced de novo lipogenesis. In this respect, it can be assumed that the low ME and CP contents of diets resulted in increased intake for these nutrients which probably enhanced lipogenesis and plasma triglycerides levels. Increased lipogenesis and plasma triglyceride levels due to high dietary ME:CP ratios have been reported previously (Swennen et al., 2005, 2006; Kamran et al., 2010). In the present study, plasma triglyceride levels matched to carcass fat retention to some extent. This was evident from a strong positive correlation ($r = 0.93$) between plasma triglycerides and fat retention.

Although, plasma triglycerides levels were not measured at 26 d of age, it can be speculated from the carcass fat retention that lipogenesis in birds fed low nutrient density diets was less at early age as compared to later growth period.

Contrary to the expectations, the plasma uric acid levels were not affected by various dietary CP and ME levels. These results were in contrast to the findings of Collin et al. (2003), Swennen et al. (2005, 2006), Namroud et al. (2008) and Kamran et al. (2010) who found a decrease in plasma uric acid levels with the reduction in dietary CP content. Zhao et al. (2009) also found reduced plasma uric acid levels in Arbor Acres broilers fed low CP and low ME diets. These different results might be due to similar N intake for all diets in the present study as compared to those studies. Plasma T₃ and T₄ concentrations were not altered by various dietary CP and ME levels. These observations confirmed our earlier results showing that thyroid hormones concentrations were not changed by the dietary CP (Kamran et al., 2010). The plasma T₃ and T₄ concentrations may be affected by the protein nutrition of the chicken (Rosebrough et al., 1999). Increased plasma T₃ and decreased T₄ concentrations have been reported with lower CP diets (Swennen et al., 2005, 2006). These changes in thyroid hormones have very important role in metabolism and hence, growth performance. Moreover, importance of T₄ for predicting carcass fatness in pullets has also been shown previously (Sun et al., 2006). Therefore, reduction in dietary CP may increase fat accumulation and affect growth performance partly by altering thyroid hormones concentrations. Namroud et al. (2010) found reduced plasma T₄ concentrations, whereas, no effect was noted on T₃ concentrations with low CP diets. However, there was no change in plasma T₃ and T₄ levels in the present study which was probably due to similar N intake among various treatments groups. The equal plasma T₃ and T₄ concentrations were reflected as equal N and fat retention and N excretion in birds fed different dietary treatments.

CONCLUSIONS

In conclusion, low CP diets with constant ME:CP ratio resulted in similar N retention without significant increase in fat retention. However, N excretion and litter N contents were not decreased due to similar N intake among treatments. These results were also reflected from the plasma levels of metabolites and hormones. Further research is needed on nutrient retention, N excretion and plasma metabolites and hormones to better understand and utilize the idea of low CP diets with constant ME:CP ratio in broilers.

REFERENCES

- Alleman, F. and Leclercq, B., 1997. Effect of dietary protein and environmental temperature on growth performance and water consumption of male broiler chickens. *Br. Poult. Sci.* 38, 607-610.
- AOAC, 1990. Official methods of analysis. 16th ed. Association of official analytical chemists, Arlington, VA.
- Boden, G., Chen, X., Ruiz, J., White, J.V. and Rossetti, L., 1994. Mechanisms of fatty acid-induced inhibition of glucose uptake. *J. Clin. Invest.* 93, 2438-2446.
- Bregendahl, K., Sell, J.L. and Zimmerman, D.R., 2002. Effect of low protein diets on growth performance and body composition of broiler chicks. *Poult. Sci.* 81, 1156-1167.
- Chung, T.K. and Baker, D.H., 1992. Ideal amino acid pattern for 10-kilogram pigs. *J. Anim. Sci.* 70, 3102-3111.
- Collin, A., Malheiros, R.D., Moraes, V.M.B., Van As, P., Darras, V.M., Taouis, M., Decuypere, E. and Buyse, J., 2003. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. *Br. J. Nutr.* 90, 261-269.
- Emmans, G.C., 1994. Effective energy: A concept of energy utilization applied across species. *Br. J. Nutr.* 71, 801-821.
- Ferguson, N.S., Gates, R.S., Taraba, J.L., Cantor, A.H., Pescatore, A.J., Ford, M.J. and Burnham, D.J., 1998. The effect of dietary crude protein on growth, ammonia concentration, and litter composition in broilers. *Poult. Sci.* 77, 1481-1487.
- Hernandez, F., Lopez, M., Martinez, S., Megias, M.D., Catala, P. and Madrid, J., 2012. Effect of low protein diets and single sex on production performance, plasma metabolites, digestibility and nitrogen excretion in 1- to 48- day- old broilers. *Poult. Sci.* 91, 683-692.
- Hidalgo, M.A., Dozier III, W.A., Davis, A.J. and Gordon, R.W., 2004. Live performance and meat yield responses to progressive concentrations of dietary energy maintained at a constant metabolizable energy-to-crude protein ratio. *J. Appl. Poult. Res.* 13, 319-327.
- Hubbard, 2004. Management guide for broiler. Hubbard L. L. C., Duluth GA 30096 - U. S. A.
- Kamran, Z., Nisa, M., Nadeem, M.A., Sarwar, M., Amjid, S.S., Pasha, R.H. and Nazir, M.S., 2011. Effect of low crude protein diets with constant metabolizable energy on performance of broiler chickens from one to thirty-five days of age. *Indian J. Anim. Sci.* 81, 79-100.
- Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M.A. and Mahmood, S., 2010. Effect of low levels of dietary crude protein with constant metabolizable energy on nitrogen excretion, litter composition and blood parameters of broilers. *Int. J. Agric. and Biol.* 12, 401-405.

- Kamran, Z., Sarwar, M., Nisa, M., Nadeem, M.A., Mahmood, S., Babar, M.E. and Ahmed, S., 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. *Poult. Sci.* 87, 468-474.
- Kermanshahi, H., Ziaei, N. and Pilevar, M., 2011. Effect of dietary crude protein fluctuation on performance, blood parameters and nutrient retention in broiler chicken during starter period. *Global Veterinaria.* 6, 162-167.
- Khajali, F. and Moghaddam, H.N., 2006. Methionine of low protein broiler diets: Influence upon growth performance and efficiency of protein utilization. *Int. J. Poult. Sci.* 5, 569-573.
- Lorenz, M.D. and Cornelius, L.M., 1993. *Small Animal Medical Diagnosis.* 2nd ed. Lippincott, Williams and Wilkins, Philadelphia, PA.
- Moran, E.T., Jr., Bushong, R.D. and Bilgili, S.F., 1992. Reducing dietary crude protein for broilers while satisfying amino acids requirements by least cost formulations: Live performance, litter composition and yield of fast food carcass cuts at six weeks. *Poult. Sci.* 71, 1687-1694.
- Namroud, N.F., Shivazad, M. and Zaghari, M., 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poult. Sci.* 87, 2250-2258.
- Namroud, N.F., Shivazad, M., Zaghari, M. and Zare Shahneh, A., 2010. Effects of glycine and glutamic acid supplementation to low protein diets on performance, thyroid function and fat deposition in chickens. *South Afric. J. Anim. Sci.* 40, 238-244.
- Neto, M.G., Pesti, G.M. and Bakalli, R.I., 2000. Influence of dietary protein level on the broiler chicken's response to methionine and betaine supplements. *Poult. Sci.* 79, 1478-1484.
- Rosebrough, R.W., McMurtry, J.P. and Vasilatos-Younken, R., 1999. Dietary fat and protein interactions in the broiler. *Poult. Sci.* 78, 992-998.
- Si, J., Fritts, C.A., Burnham, D.J. and Waldroup, P.W., 2001. Relationship of dietary lysine level to the concentration of all essential amino acids in broiler diets. *Poult. Sci.* 80, 1472-1479.
- Sun, J.M., Richards, M.P., Rosebrough, R.W., Ashwell, C.M., McMurtry, J.P. and Coon, C.N., 2006. The relationship of body composition, feed intake, and metabolic hormones for broiler breeder females. *Poult. Sci.* 85, 1173-1184.
- Swennen, Q., Janssens, G.P.J., Collin, A., Bihan-Duval, E.L., Verbeke, K., Decuyper, E. and Buyse, J., 2006. Diet-induced thermogenesis and glucose oxidation in broiler chickens: Influence of genotype and diet composition. *Poult. Sci.* 85, 731-742.

-
- Swennen, Q., Janssens, G.P.J., Millet, S., Vansant, G., Decuypere, E. and Buyse, J., 2005. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: Endocrine functioning and intermediary metabolism. *Poult. Sci.* 84, 1051-1057.
- Waldroup, P.W., Jiang, Q. and Fritts, C.A., (2005. Effect of supplementing broiler diets low in crude protein with essential and nonessential amino acids. *Intr. J. Poult. Sci.* 4, 425-431.
- Zhao, J.P., Chen, J.L., Zhao, G.P., Zheng, M.Q., Jiang, R.R. and Wen, J., 2009. Live performance, carcass composition and blood metabolite responses to dietary nutrient density in two distinct broiler breeds of male chickens. *Poult. Sci.* 88, 2575-2584.