Effects of glycine and glutamic acid supplementation to low protein diets on performance, thyroid function and fat deposition in chickens

N.F. Namroud#, M. Shivazad, M. Zaghari and A. Zare Shahneh
Department of Animal Science, University of Tehran, Karaj, Tehran, Iran
Department of Animal Science, University of Tehran, University Street, Karaj, Iran

Abstract

Consumption of low crude protein (CP) diets causes elevation in fat accumulation in chickens, and this effect is independent of dietary essential amino acid levels. Thyroid hormones, because of their metabolic regulatory characteristics, might be an effective factor in lipogenesis. Therefore, a study was conducted to investigate the influence of low CP diets on hormonal function of the thyroid. Two hundred and sixteen male broiler chicks, 10 - 25 days of age, were fed experimental diets that contained 230, 210, 190 and 170 g CP/kg with adequate amounts of total and digestible essential amino acids at or above NRC (1994) recommendations. Two other diets were the same as the 190 and 170 g CP/kg diets but contained additional glycine and glutamic acid. Reduction in CP below 190 g/kg led to a decline in body weight and feed intake and an increase in fat deposition in body, as would be expected. Plasma T_4 concentration decreased significantly in the birds on the 170 g CP/kg diet and supplementation of glycine and glutamic acid had no effect on hormonal levels of the thyroid despite the reduction in whole-body and abdominal cavity fat deposition. Plasma T_3 concentration was not affected. Therefore, it is concluded that a reduction in circulatory levels of T_4 that occurs in broiler chicks fed diets containing below 190 g CP/kg though with adequate essential amino acids, may be an effective method of increasing fat deposition.

Keywords: Crude protein, nonessential amino acids, T_3, T_4, lipid deposition
Corresponding author. E-mail: nebonidnamroud@hotmail.com

Introduction

Distinct effects of dietary protein on thyroid gland function especially circulating levels of triiodothyronine (T_3) and thyroxine (T_4) have become obvious in many investigations (Buyse *et al.*, 1992; Carew & Alster, 1997). Protein restricted chickens are characterized by a reduced plasma T_4 and increased plasma T_3 level (Buyse *et al.*, 2001). Carew *et al.* (1983; 1997) reported that dietary deficiencies of some individual essential amino acids may affect thyroid function in chicks. Carew *et al.* (1997) have categorized the mechanisms by which protein and essential amino acid deficiencies alter thyroid function in several animal species. The correlation between thyroid hormone levels and many biological factors has been noted. The concentrations of T_3 and T_4 in plasma have a direct correlation with growth of broiler cockerels (Chaisson *et al.*, 1978). A low concentration of T_4 induced by propylthiouracil reduced body weight, muscle weight and bone length (King & King, 1973). Thyroid hormones are involved in the regulation of growth, metabolism, heat production, gonadal development, moulting, migration and hatching in birds (McNabb, 2000). Hypothyroidism promotes fatness significantly (Decuypere *et al.*, 1987). Direct actions of T_3 have also been described on lipolysis (Harden & Oscar, 1993) and malic enzyme activity (Goodridge *et al.*, 1989). Several studies assumed that the depressing influence of malnutrition on thyroid secretion is due to an alteration of the amount of thyroid stimulating hormone (TSH) secretion (Penn & Huston 1968; Abdullah & Falconer, 1977; Poczopko *et al.*, 1977). The TSH is the primary regulating factor which controls production and secretion of T_4. Triiodothyronine is believed to originate mainly from the peripheral (liver, kidneys, and intestines) deiodination of T_4.

Although many studies have determined the minimal crude protein (CP) level of diets with adequate amounts of essential amino acids for maximum performance (Namroud *et al.*, 2008), there is no acceptable explanation for the reason for increased lipid deposition in chicks fed diets with low CP, but adequate essential amino acid levels in comparison with diet containing higher levels of CP. The present experiment was carried out to investigate the effect of different dietary CP levels with almost equal amounts of essential digestible amino acid levels on growth, thyroid hormonal responses and fat deposition of growing broiler chicks. Furthermore, the effect of supplementing low CP diets with glycine and glutamic acid as sources of nonessential amino acids was examined. Nonessential amino acid supplementation was done on the assumption that in low CP diets, deficiency of nonessential amino acids may become a limiting factor in protein synthesis which could lead to fattening (Yamazaki *et al.*, 1998). This study was conducted to investigate whether dietary CP and not essential amino acid levels could reduce the hormonal function of the thyroid and elevate fat accumulation in the body.

Materials and Methods

Three hundred and seventy two day-old male Ross 308 broiler chicks obtained from a local hatchery were housed in electrically heated battery cages (0.197 m^2 per bird) and had free access to water and a commercial starter diet for 10 days. On the tenth day, 216 birds (215 ± 20 g) were selected and allotted to one of the six feeding treatments on the basis of body weight (BW). Each dietary treatment was applied randomly to six replicates of six chicks. The experimental birds were given *ad libitum* access to water and diet. The ambient temperature was gradually decreased from 34 °C to 24 °C over the period of 1 to 28 d of age. The birds were exposed to a 23 : 1, light : dark cycle. All procedures were approved by the Animal Care and Welfare Committee of Tehran University.

Maize, dehulled soyabean meal and maize gluten meal were sampled prior to diet formulation to determine their CP levels (Kjeldahl-N × 6.25), moisture and total amino acids content (Degussa AG, Germany.), after which the content of true digestible amino acids was calculated from standardized ileal digestibility coefficients listed by Lemme *et al.* (2004). The concentrations of calcium (method 968.08), phosphorus (method 965.17), potassium (method 966.03), sodium (method 966.03) and chloride (method 943.01) were analyzed in triplicate by the AOAC (1995) procedures for all feed ingredients. The dietary electrolyte balance was set at 280 mEq/kg. All diets were formulated to be isoenergetic (13.29 MJ ME_n/kg). The amount of dietary calcium, available phosphorus and sodium were maintained constant in all treatments (Table 1). Four levels of CP were used in this study, *viz.* 230, 210, 190 and 170 g/kg with almost equal ileal digestible amounts of essential amino acids. Total essential amino acid levels in all treatments were maintained at or above NRC (1994) recommended levels (Table 2). Furthermore, one series of 190 and 170 g CP/kg diets were supplemented with Gly and Glu to reach the amounts in the high CP diets. Diets were supplemented with complete vitamin and trace mineral premixes. The L-Lys.HCl, DL-Met and L-Thr used in the diets were feed grade, whereas all other crystalline amino acids as well as K_2CO_3 were reagent grade (minimum 99.9% purify) and purchased from Degussa Iran AG.

At 25 days of age, three blood samples were taken per replicate from the brachial vein of the birds and placed into evacuated heparinized tubes. Samples were put on ice immediately and processed within 1.5 h of collection. Plasma was obtained by centrifuging the blood samples at 3000 × *g* for 15 min at 4 °C and kept frozen at –20 °C for later analysis. Plasma T_3 and T_4 concentrations were determined by radioimmunoassay. RIA was performed using the Coat-A-Count Free T_4 and T_3 RIA kit validated for use with chicken blood according to the manufacturer's protocol. Intra- and inter-assay coefficients of variation were 6.9 and 4.7% for T_4 and 10.9 and 6.1 for T_3, respectively. A different assay was used in an attempt to assess plasma levels of TSH, the human RIA immunoradiometric assay from Diagnostic Products Corporation (USA) but the TSH assay did not prove to be effective in measuring chicken TSH.

At the end of the experimental period (Day 28), two birds per replicate (with a BW close to the replicate mean), were slaughtered by cervical dislocation. The birds were killed 20 h after their last meal (with free access to water). One of the slaughtered birds was used to determine carcass characteristics including abdominal fat. The other euthanized chick per replicate was stored in airtight polyethylene bags at -22 °C for later determination of whole-body composition. The whole body of chicks were thawed overnight at room temperature, homogenized with a Waring blender (Waring Products Division, New Hartford, CT.) for 2.5 min, and sampled according to procedures described by Barker & Sell (1994). Whole-body DM (Barker & Sell, 1994), nitrogen (N) content, and fat content (EE) were analyzed in triplicate subsequently. Whole-body CP was calculated as Kjeldahl N × 6.25.

Data were analyzed using the general linear models ANOVA with $P \leq 0.05$ in a completely randomized design (SAS, 2004). Means were compared using Duncan's multiple range test.

Table 1 Ingredient composition of experimental diets (%)

Diet number	1	2	3	4	5	6
Crude protein (g/kg)	230	210	190	170	190	170
Amino acids status[1]	100%	100%	100%	100%	100% + Gly, Glu	100% + Gly, Glu
Maize	50.83	54.91	59.85	63.86	58.70	60.99
Soyabean meal(CP:48%)	38.99	35.21	30.15	25.22	30.31	25.87
Maize gluten meal	0.52	0	0	0	0	0
Maize oil	5.61	5.46	4.98	4.88	5.28	5.52
Dicalcium phosphate	1.95	1.97	2.01	2.05	2.01	2.18
Calcium carbonate	1.06	1.05	1.02	0.99	1.02	0.99
Sodium chloride	0.35	0.35	0.35	0.35	0.35	0.35
Potassium carbonate	0	0.19	0.48	0.78	0.48	0.77
DL-Methionine	0.19	0.24	0.29	0.32	0.29	0.35
L-Lysine HCl	0	0.07	0.22	0.37	0.22	0.39
L-Threonine	0	0.05	0.10	0.17	0.10	0.17
L-Arginine	0	0	0.05	0.20	0.05	0.22
L-Tryptophan	0	0	0	0.02	0	0.02
L-Isoleucine	0	0	0	0.19	0	0.19
L-Valine	0	0	0	0.10	0	0.10
L-Glutamic acid	0	0	0	0	0.49	1.12
Glycine	0	0	0	0	0.20	0.26
Trace mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin premix[3]	0.25	0.25	0.25	0.25	0.25	0.25

[1] Amino acids status: 100% total essential amino acids maintained at or above NRC 1994; Gly - glycine; Glu - glutamic acid; EAA - essential amino acids.
[2] Trace mineral premix (per kg diet): 111 mg Mn; 110 mg Zn; 50 mg Fe; 8.3 mg Cu; 1.38 mg I; 0.3 mg Se; 0.1 mg Co; 0.05 mg Mo; a minimum of 6.98 mg of Ca, and a maximum of 8.02 mg of Ca per kg diet. The carrier was calcium carbonate, and the premix contained less than 0.7% mineral oil.
[3] Vitamin premix (per kg of diet): 11.023 IU vitamin A (retinyl acetate); 118 IU vitamin D (cholecalciferol); 23.54 IU vitamin E (DL-α-tocopheryl acetate); 1.47 mg menadione (menadione dimethylpyrimidinol); 0.0151 mg B_{12}; 5.895 mg riboflavin; 42.93 mg niacin; 12.11 mg D-pantothenic acid; 477.7 mg choline (choline chloride); 1.15 mg folic acid; 4.17 mg pyridoxine (pyridoxine hydrochloride); 1.23 mg thiamin (thiamin mononitrate); 0.075 mg d-biotin.

Result

The body weights of the birds on the 230, 210 and 190 g dietary protein/kg diets were not significantly different, but was significantly reduced with 170 g/kg ($P \leq 0.05$). Supplementing low CP diets with Gly and Glu did not improve performance significantly (Table 3).

Abdominal fat and whole-body fat were affected by dietary CP (Table 4). High CP diets produced carcasses that contained lower levels of abdominal and total fat than the low CP diets. Fortifying low CP diets with Gly and Glu led to a reduction in whole-body and abdominal cavity fat content. No difference in percentage of whole-body CP was observed between different treatments.

Figure 1 shows that T_4 concentration was affected ($P \leq 0.05$) by CP level. Below the 19 g CP/kg level, plasma T_4 concentration with and without nonessential amino acids supplementation was lower. Plasma concentration of T_3 was not influenced significantly.

Discussion

Although effects of individual amino acid deficiencies on hormonal function of thyroid gland were studied previously, the specific effects of CP and nonessential amino acid were not determined. This study has indicated that not only amino acid levels and pattern (Refetoff *et al.*, 1970; Carew *et al.*, 1997,) but also

Table 2 Nutritional composition of experimental diets

Diet number	1	2	3	4	5	6
Crude protein (CP, g/kg)	230	210	190	170	190	170
Amino acid status[1]	100%	100%	100%	100%	100% + Gly, Glu	100% + Gly, Glu
AME_n (MJ/kg)[2]	13.29	13.29	13.29	13.29	13.29	13.29
CP (N × 6.25) (g/kg)	231.2	210.0	190.0	170.0	195.6	181.9
Ca (g/kg)	9	9	9	9	9	9
Available P (g/kg)[2]	4.5	4.5	4.5	4.5	4.5	4.5
Na (g/kg)	1.6	1.6	1.6	1.6	1.6	1.6
Na + K - Cl (meq/kg)	280	280	280	280	280	280
Standardized ileal digestible amino acids[3] (%)						
Lysine	1.11	1.09	1.09	1.09	1.09	1.09
Methionine	0.50	0.53	0.55	0.57	0.55	0.57
Methionine + Cysteine	0.81	0.81	0.81	0.81	0.81	0.81
Arginine	1.28	1.23	1.23	1.23	1.23	1.23
Threonine	0.72	0.71	0.71	0.71	0.71	0.71
Leucine	1.70	1.60	1.59	1.57	1.57	1.57
Isoleucine	0.82	0.79	0.78	0.78	0.78	0.78
Valine	0.82	0.80	0.79	0.79	0.79	0.79
Histidine	0.55	0.52	0.49	0.45	0.48	0.44
Phenylalanine	0.98	0.92	0.87	0.86	0.86	0.85
Tryptophan	0.23	0.22	0.21	0.21	0.21	0.21
Tyrosine	0.94	0.92	0.91	0.89	0.90	0.88
Total amino acid concentrations (%)						
Lysine	1.32	1.28	1.25	1.22	1.24	1.22
Methionine	0.55	0.58	0.60	0.62	0.60	0.62
Methionine + Cysteine	0.94	0.92	0.91	0.88	0.90	0.89
Arginine	1.50	1.49	1.47	1.44	1.46	1.42
Threonine	0.84	0.83	0.83	0.82	0.83	0.82
Leucine	1.94	1.83	1.79	1.77	1.77	1.77
Isoleucine	0.99	0.91	0.87	0.86	0.86	0.85
Valine	0.92	0.91	0.89	0.88	0.89	0.89
Histidine	0.58	0.56	0.52	0.45	0.50	0.45
Phenylalanine	1.06	1.00	0.95	0.92	0.93	0.90
Tryptophan	0.27	0.25	0.24	0.23	0.24	0.23
Tyrosine	1.03	1.02	0.99	0.95	0.96	0.94
Glycine (Gly) + Serine	1.96	1.86	1.60	1.44	1.90	1.90
Glutamic acid (Glu)	5.98	5.76	5.46	4.93	5.93	5.92

[1] Amino acids status: 100% total essential amino acids maintained at or above NRC (1994).
[2] AME_n – apparent metabolizable energy (N-balanced) and available P were calculated amount.
[3] Includes amino acids from intact protein and crystalline sources. Calculated, using standardized ileal digestibility coefficients from Lemme *et al.* (2004). Crystalline amino acids were assumed to have a 100% true digestible.

dietary CP by itself may reduce plasma thyroid hormone concentrations. In agreement with Namroud *et al.* (2008), in the present experiment a minimum CP level was recorded below which retardation in growth and appetite were experienced. Glycine and Glu supplementation to low CP diets did not improve performance except by reducing fat deposition in the abdominal cavity and whole-body. Namroud *et al.* (2008) reported that lower CP levels may increase fattening quantitatively. Triiodothyronine is the main metabolic stimulating hormone (McNabb & King, 1993; Gabarrou *et al.*, 1997). Plasma T_3 and T_4 concentrations are associated strongly with metabolism regulation and they are important growth promoters in chickens

Table 3 Effects of dietary crude protein (CP) level and supplementation with nonessential amino acids (glycine and glutamic acid) on performance during 10 to 25 days of age[1]

Diet: CP (g/kg)	Amino acids Status[2]	25-d Body weight (g)	10 to 25 d Feed intake (g)	10 to 25 d FCR (g/g)
1: 230	100%	940[a]	1276[a]	1.360[ab]
2: 210	100%	939[a]	1257[a]	1.338[b]
3: 190	100%	932[a]	1266[a]	1.358[ab]
4: 170	100%	747[b]	1027[b]	1.372[a]
5: 190	100%+Gly,Glu	941[a]	1266[a]	1.345[b]
6: 170	100%+Gly,Glu	760[b]	1038[b]	1.368[a]
Significant level		0.05	0.05	0.05
s.e.m.		12	19	0.017

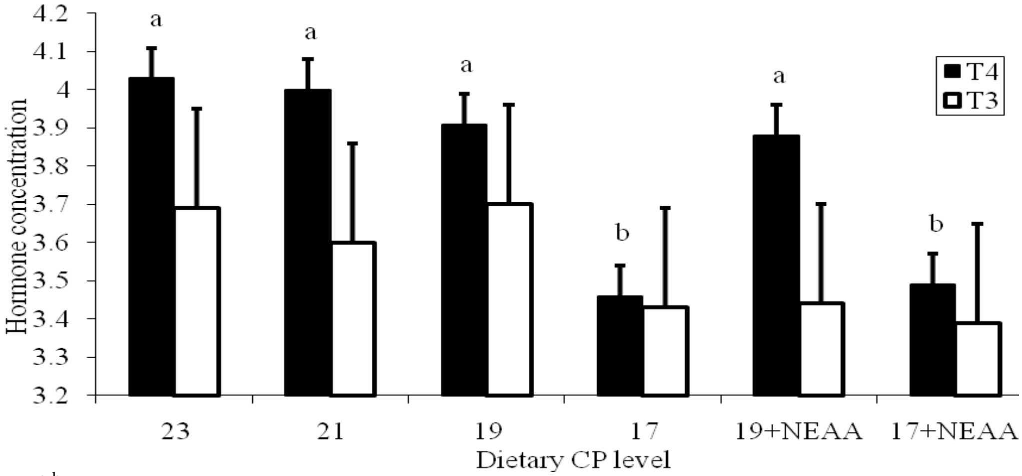
[a-f] Means within columns without a common superscript differ significantly, ($P < 0.05$).
[1] Results are means of six replicates (6 chicks per replicate) per treatment.
[2] Amino Acids Status: 100% total essential amino acids maintained at or above NRC 1994;
FCR - Feed conversion ratio (total feed consumed/weight gain of birds).

Table 4 Effects of dietary crude protein (CP) level and supplementation with nonessential amino acids (glycine and glutamic acid) on whole-body composition (28 d of age)

Diet: CP (g/kg)	Amino acids status[2]	Whole-body composition[1]			Abdominal fat (%)
		DM (%)	Fat (%)	Protein (%)	
1: 230	100%	28.10[c]	8.60[e]	16.40	1.65[d]
2: 210	100%	28.70[b]	8.95[d]	16.32	1.72[cd]
3: 190	100%	29.08[a]	9.34[c]	16.34	1.91[b]
4: 170	100%	29.15[a]	10.10[a]	16.24	2.09[a]
5: 190	100% + Gly, Glu	28.82[b]	8.79[de]	16.41	1.77[c]
6: 170	100% + Gly, Glu	29.09[a]	9.62[b]	16.29	1.94[b]
Significant level		0.05	0.01	NS	0.01
s.e.m.		0.30	0.23	0.17	08

[a-d] Means within columns without a common superscript differ significantly ($P < 0.05$); NS - Nonsignificant.
[1] Results are means of six replicates (one chick was slaughtered per replicate) per treatment.
[2] Amino acids status: 100% total essential amino acids maintained at or above NRC 1994;

(McNabb & King, 1993; Carew *et al.*, 1998; Gonzales *et al.*, 1999; Yahav, 2000). Sun *et al.* (2006) demonstrated that T_4 is the most important hormone for predicting the percentage carcass fat in *ad libitum*-fed pullets. Furthermore, the low increase of circulating T_4 does not appear to modify body composition, heart rate, metabolism rate or muscular function in the human (Dubois *et al.*, 2008). The circulating concentration of T_3 and probably T_4 are positively correlated with feed intake (Yahav *et al.*, 1996; 1998). Nevertheless, the low body weight of the birds on the 170 g CP/kg diet could not be the consequence of low hormonal thyroid actions because over a fairly wide range of thyroid activities, growth rate is independent of that, and affected mainly by feed intake and composition. However, just under severe conditions of hypo- or hyperthyroidism, growth retardation may occur. On the other hand, elevated fat deposition could not be the consequence of reduced dietary CP solely in view of the fact that all diets contained the same levels of digestible essential amino acids. Our conclusion is that reducing dietary CP increases fat accumulation partly by altering thyroid hormones metabolism. In the present study, although reducing dietary CP with a constant profile of essential amino acids could decrease circulating T_4 concentration, even the fortifying of the low CP diet with nonessential amino acids could not compensate for this reduction. Despite the fact that nonessential



[a-b] Concentrations within T4 columns without a common letter differ significantly.
[1]Results are means of six replicates (three chicks were sampled per replicate) per treatment.
[2]Amino acids status: 100% total essential amino acids maintained at or above NRC (1994); NEAA – nonessential amino acids, glycine and glutamic acid.

Figure 1 Effects of dietary crude protein (CP) level and supplementation with nonessential amino acids on plasma concentrations of T_3 (ng/mL) and T_4 (µg/mL) in 25-day old male broiler chickens.

amino acid (Gly and Glu) supplementation could reduce fat deposition, its effect on increasing the thyroid hormones (T_3) concentration was not significant. Therefore, dietary Gly and Glu supplementation could not have an important effect on the hormone secretion of the thyroid. Unfortunately, we did not succeed in measuring plasma TSH concentration. Therefore it is not clear if a reduction in thyroid activity is solely the result of changes in T_3 and T_4 metabolism, or if thyrotrophs of the anterior pituitary interfered in that. Further research is needed to investigate the mechanism(s) of how dietary CP affects the thyroid.

Acknowledgments

The authors gratefully acknowledge the Degussa AG and its technical service manager in Iran, Ali Afsar, for conducting the amino acid analyses of our feedstuffs and providing crystalline amino acids.

References

Abdullah, R. & Falconer, L.R., 1977. Responses of thyroid activity to feed restriction in the goat. Aust. J. Boil. Sci. 30, 207-215.

AOAC, 1995. Official Methods of Analysis. (16th ed.). Association of Official Agricultural Chemists, Inc., Arlington, Virginia, USA.

Barker, D.L. & Sell, J.L., 1994. Dietary carnitine did not influence performance and carcass composition of broiler chickens and young turkeys fed low or high-fat diets. Poult. Sci. 73, 281-287.

Buyse, J., Decuypere, E., Berghman, L., Kühn, E.R. & Vandesande, F., 1992. Effect of dietary protein content on episodic growth hormone secretion and on heat production of male broiler chickens. Br. Poult. Sci. 33, 1101-1109.

Buyse, J., Darras, V.M., Vleurick, L., Kühn, E.R. & Decuypere, E., 2001. Nutritional regulation of the somatotrophic axis and intermediary metabolism in the chicken. In: Avian Endocrinology. Eds Dawson, A. & Chaturvedi, C.M., New Dehli, India. pp. 33-313.

Carew, L.B. & Alster, F.A., 1997. Dietary carbohydrate and fat do not alter the thyroid response to protein deficiency in chicks. Proc. Soc. Exp. Biol. Med. 215, 82-86.

Carew, L.B., Alster, F.A., Foss, D.C. & Scanes, C.G., 1983. Effect of a tryptophan deficiency on thyroid gland, growth hormone and testicular functions in chickens. J. Nutr. 113, 1756-1765.

Carew, L.B., Evarts, K.G. & Alster, F.A., 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. Poult. Sci. 76, 1398-1404.

Carew, L.B., Evarts, K.G. & Alster, F.A., 1998. Growth, feed intake, and plasma thyroid hormone levels in chicks fed dietary excesses of essential amino acids. Poult. Sci. 77, 295-298.

Chiasson, R.V., Sharp, P.J., Klandorf, H., Scanes, C.G. & Harvey, S., 1978. The effect of rapeseed meal and methimazole on levels of plasma hormones in growing broiler cockerels. Poult. Sci. 58, 1575-1583.

Decuypere, E., Buyse, J., Scanes, C.G., Huybrechts, L.M. & Kühn, E.R., 1987. Effect of hyper- or hypothyroid status on growth, adiposity, and on levels of growth hormone, somatomedin-C and thyroid metabolism in broiler chickens. Reprod. Nutr. Dev. 27, 555-565.

Dubois, S., Abraham, P., Rohmer, V., Rodien, P., Audran, M., Dumas, J.F. & Ritz, P., 2008. Thyroxine therapy in euthyroid patients does not affect body composition or muscular function. Thyroid 18, 13-19.

Gabarrou, J.F., Duchump, C., Williams, J. & Geraert, P.A., 1997. A role of thyroid hormones in the regulation of dietinduced thermogenesis in birds. Br. J. Nutr. 78, 963-973.

Gonzales, E., Buyse, J., Sartori, J.R., Loddi, M.M. & Decuypere, E., 1999. Metabolic disturbances in male broilers of different strains. 2. Relationship between the thyroid and somatotropic axes with growth and mortality. Poult. Sci. 78, 516-521.

Goodridge, A.G., Crish, J.F., Hillgartner, F.B. & Wilson, S.B., 1989. Nutritional and hormonal regulation of the gene for avian malic enzyme. J. Nutr. 119, 229-308.

Harden, R.L. & Oscar, T.P., 1993. Thyroid hormone and growth hormone regulation of broiler dipocyte lipolysis. Poult. Sci. 72, 669-672.

King, D.B. & King, C.R., 1973. Thyroidal influence on early muscle growth of chickens. Gen. Comp. Endocr. 36, 517-529.

Lemme, A., Ravindran, V. & Bryden, W.L., 2004. Ileal digestibility of amino acids in feed ingredients for broilers. Wld's Poult. Sci. J. 60, 423-437.

McNabb, F.M.A., 2000. Thyroids. In: Sturkie's Avian Physiology. Ed. Whittow, G.C., San Diego: Academic Press. pp. 461-471.

McNabb, F.M.A. & King, D.B., 1993. Thyroid hormones effects on growth development and etabolism. In: The Endocrinology of Growth Development and Metabolism. Vertebrates. Eds Schreibman, M.P., Scanes, C.G. & Pang, P.K.T., Academic Press, New York, USA. pp. 393-417.

Namroud, N.F., Shivazad, M. & 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.

NRC, 1994. Nutrient Requirement of Poultry. (9th ed.). National Academy Press, Washington, D.C., USA.

Penn, R.P. & Huston, T.M., 1968. The influence of feed and water restriction and pitressin administration upon thyroxin secretion in domestic fowl. Poult. Sci. 47, 1432-1437.

Poczopko, P., Keller, J., Piekarzewska, A. & Witkowska, H., 1977. The effect of feeding level on the thyroid gland in chicken. Bull. Acad. Pol. 423-427.

Refetoff, S., Robin, N.I. & Fang, V.S., 1970. Parameters of thyroid function in serum of 16 selected vertebrate species: A study of PBI, serum T_4, free T_4, and the pattern of T_4 and T_3 binding to proteins. Endocrinology 86, 793-805.

SAS, 1985. Statistical Analysis Systems user's guide (5th ed.). SAS Institute Inc., Raleigh, North Carolina, USA.

Sun, J.M., Richards, M.P., Rosebrough, R.W., Ashwell, C.M., McMurtry, J.P. & Coon, C.N., 2006. The relationship of body composition, feed intake, and metabolic hormones for broiler breeder females. Poult. Sci. 85, 1173-1184.

Yahav, S., 2000. Relative humidity at moderate ambient temperatures: its effect on male broiler chickens and turkeys. Br. Poult. Sci. 41, 94-100.

Yahav, S., Straschnow, A., Plavnik, I. & Hurwitz, S., 1996. Effects of diurnally cycling versus constant temperatures on chickens growth and food intake. Br. Poult. Sci. 37, 43-54.

Yahav, S., Plavnik, I., Rusal, M. & Hurwitz, S., 1998. Response of turkeys to relative humidity at high ambient temperature. Br. Poult. Sci. 39, 340-345.

Yamazaki, M., Murakami, H. & Takemasa, M., 1998. Effects of ratios of essential amino acids to nonessential amino acids in low protein diet on nitrogen excretion and fat deposition of broiler chicks. Jap. Poult. Sci. 35, 19-26.