ISSN 1682-8356
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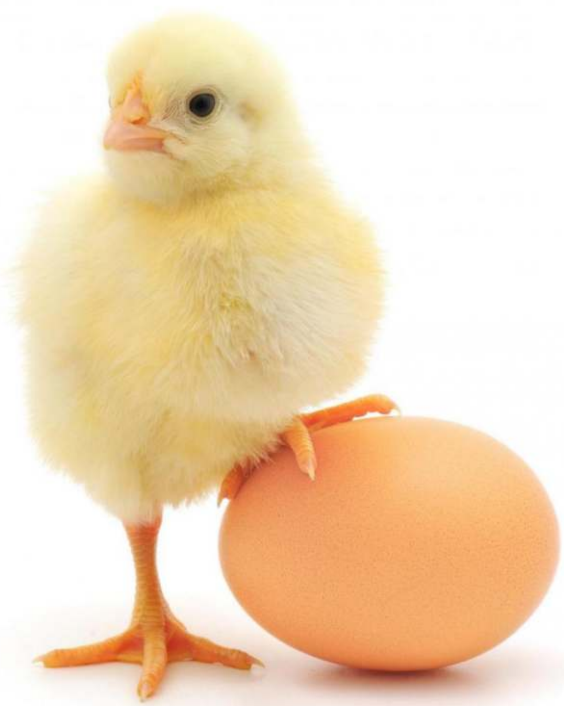

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

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International Journal of Poultry Science 5 (5): 457-462, 2006
ISSN 1682-8356

Plasma Concentrations of Thyroid Hormone and Growth Hormone in Lohmann Male Broilers Fed on Different Dietary Energy and Protein Levels

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Abstract: An experiment on Lohmann male broilers was conducted to evaluate the effect of different energy and protein levels on broiler performance and plasma concentrations of growth hormone (GH), thyroxine (T4), and triiodothyronine (T3). 600 day-old broilers were fed on either 2800(low-energy) or 3200(high-energy) Kcal ME/Kg diets with different protein levels during three periods of starter, grower and finisher. Blood samples were collected at the end of each period from wing vein. Plasma levels were assayed for GH, T4 and T3 by radioimmunoassay (RIA). Body weight (BW) and feed conversion rate (FCR) were also measured at the end of each period. FCR and mean concentrations of plasma GH and T3 increased in broilers fed on low-energy diets compared with those fed on high-energy diets during different periods, but BW and mean concentrations of plasma T4 decreased in the three periods in birds fed on low-energy diets compared with those fed on high-energy diets. Birds fed on different protein levels showed significant differences with respect to BW, FCR and mean concentrations of plasma for GH, T3 and T4 during starter, grower and finisher periods. At the two energy levels and all periods, FCR and mean concentrations of plasma GH and T3 were decreased in broilers fed on high-protein diets compared with those fed on low-protein diets. However, higher BW and plasma T4 concentrations were observed in broilers fed on high-protein diets compared with those fed on low-protein diets. The results of this experiment demonstrate that low-energy intake could increase FCR and mean plasma concentrations of GH and T3 but could decrease BW and mean plasma concentrations of T4. Different protein levels during starter, grower and finisher periods in broilers may change the plasma concentrations of GH, T4 and T3 but nearly similar protein levels were not changed (P>0.05) those hormones.

Key words: Broiler, energy, protein, growth hormone, thyroxine, triiodothyronine

Introduction

Previous investigations suggested that fasting and re-feeding have considerable effects on GH and thyroid hormone metabolism (Geyten *et al.*, 1999). For example, in chicken, fasting decreased plasma T3 and TSH levels and increased plasma T4 concentrations. Starvation decreases circulating T3 but increase T4 (Gabarrou *et al.*, 1997; Klandorf *et al.*, 1981). The rise of plasma T3 in re-fed chickens is proportional to the amount of food consumed and to the metabolisable energy (ME) content of the diet (Klandorf *et al.*, 1981). In a previous study, Armstrong *et al.* (Armstrong and Britt, 1987) have shown that change in energy and protein levels in diets are associated with increased or decreased levels of the concentrations of GH, T4 and T3 in the blood serums of mammals. As with the mammals, birds are also susceptible to imbalanced metabolic hormones levels associated with changes of energy and protein content in their diets. It is well known that thyroid activity is important in controlling metabolic rate (Harvey *et al.,* 1983; Kittok *et al.*, 1982; Reyns *et al.*, 2002). Increasing the energy content of the diet also results in increasing metabolic rate, more oxygen consumption and decreasing the feed conversion rates (Khazali and Moravej, 2003). There are a number of studies (Burke and Marks, 1982; Darras *et al.*, 2000; Harvey *et al.*, 1983; Khazali and Moravej, 2003; March *et al.*, 1964; Rosebrough and Mcmurtry, 1993; Vasilatos-Younken *et al.*, 1999) that show the effect of different energy and protein levels on mean plasma concentrations of GH, T3 and T4 in broilers. It seems that with attention to the following cases, it is essential to do more studies about the effect of different energy and protein levels on mean plasma concentrations of metabolic hormones in broilers. Case 1) the results of these studies vary and there is no consensus on mean plasma concentrations of those hormones in broilers. Case 2) previous investigations were limited to a few broiler strains and there is not any report on Lohmann male broilers. The objective of this study, considering all previous findings, was set to determine whether different dietary energy and protein levels could affect the plasma concentrations of GH, T4 and T3 in the Lohmann mail broilers.

Materials and Methods

600 day-old male from Lohmann strain were randomly divided into ten groups. Broilers were assigned randomly to either 2800 or 3200 Kcal ME/Kg diets during

Table 1: The effect of different energy to protein ratios on BW and FCR in broilers

period	Protein g/kg diet	2800 Kcal ME/Kg		3200 Kcal ME/Kg	
		BW (g)	FC (g feed/g gain)	BW (g)	FC (g feed/g gain)
Starter	230	237[a]	1.18[a]	248[a]	0.96[a]
	220	235[ab]	1.21[b]	245[ab]	0.98[ab]
	210	230[b]	1.26[b]	240[b]	1.02[b]
	200	226[bc]	1.34[bc]	235[bc]	1.06[bc]
	190	220[c]	1.40[c]	230[c]	1.10[c]
±s.e.		7.0	0.05	8.0	0.02
Grower	220	1280[a]	1.74[a]	1350[a]	1.40[a]
	210	1258[b]	1.81[a]	1320[ab]	1.44[ab]
	200	1245[b]	1.84[ab]	1300[b]	1.48[b]
	190	1230bc	1.90[b]	1290[bc]	1.55[bc]
	180	1215[c]	1.94[b]	1235[c]	1.64[c]
±s.e.		28	0.08	30	0.07
Finisher	190	2530[a]	2.05[a]	2635[a]	1.66[a]
	180	2520[a]	2.06[a]	2620[ab]	1.66[ab]
	170	2500[ab]	2.11[ab]	2580[b]	1.72[b]
	160	2480b	2.15[b]	2530[bc]	1.76[bc]
	150	2400[c]	2.23[c]	2490[c]	1.80[c]
±s.e.		40.0	0.08	45	0.06

[abcd] Mean with common superscripts within columns do not differ (p>0.05).

starter, grower and finisher periods. Proteins were set to 230, 220, 210, 200 and 190 g/Kg diet for starters, 220, 210, 200, 190 and 180 g/Kg diet for growers and 190, 180, 170, 160 and 150 g/Kg diet for finishers. The diets were formulated using corn grain, soybean meal, fish meal, wheat bran, barley grain, vegetable oil, mineral and vitamin supplements, which met or exceeded the recommendations of the Lohmann nutrition broiler manual (2004). The experimental design was therefore a 2 by 5 factorial design, yielding 10 different diets. There were three replicates (cages) per treatment. BW and FCR were measured at the days 10, 28 and 42[nd] of the experiment. Blood samples were collected at the end of starter, grower and finisher periods. Having taken blood samples, they were kept at 4°C until centrifugation. A saturated sodium citrate solution (40 µl of sodium citrate solution/ml blood) was added to the samples before centrifugation to prevent clotting during storage. Plasmas were stored at -20°C until assayed for GH, T4 and T3 by RIA. Plasma levels of GH, T4 and T3 were measured by a homologous double-antibody (PEG-separation method) RIA. For GH assay, cGH (Tabeshyarnoor Co., Tehran, Iran) were used for iodination. The rabbit anti cGH was prepared by Tabeshyarnoor Co., Tehran, Iran. A seven-point standard curve ranging from 0.50 to 100 ng GH/mL was used. An average assay binding of 40% was achieved using an initial 1: 20,000 dilution of GH antiserum for GH assay. T3 and T4 were purchased from Sigma Chemical Company and Chemicon Co, respectively. T3 was used for iodination. The rabbit anti T4 was prepared by Tabeshyarnoor Co., Tehran, Iran. A six-point standard curve ranging from 2.2 to 25 ng T4/mL was used. An average assay binding of 60% was achieved, using an initial 1: 1000 dilution of T4 antiserum for T4 assay. For T3 assay, T2 was purchased from Sigma Chemical Company. T2 was used for iodination. The rabbit anti T3 was prepared by Tabeshyarnoor Co., Tehran, Iran. A six-point standard curve ranging from 0.32 to 5.2 ng T3/mL was used. An average assay binding of 70% was achieved, using an initial 1:5000 dilution of T3 antiserum for T3 assays. Intra- and inter assay correlation coefficient of variations (CV) were 6.8 and 10.6% for GH, 8.1 and 7.3% for T3 and 8.8 and 8.3% for T4, respectively. All analyses were conducted using General Linear Model procedures (SAS, 1988).

Results

Table 1 Shows that mean BW and FCR of birds were affected significantly (P<0.05) using different energy and protein levels during three periods. Mean body weights were increased significantly (P<0.05) in broilers fed on high-energy diets compared with those fed low-energy diets during different periods. However, mean FCR were decreased significantly (P<0.05) in the three periods in birds fed low-energy diets compared with those fed high-energy diets.

Table 2 and Fig. 1-3 show that mean concentrations of plasma GH, T_3 and T_4 were affected significantly (P<0.05) using different energy and protein levels during different periods. Mean concentrations of plasma GH and T_3 were increased significantly (P<0.05) in broilers fed on low-energy and protein diets compared with those fed on high-energy and protein diets during three periods, whereas mean concentrations of plasma T_4 were decreased significantly (P<0.05). For example: in starter period mean concentrations of plasma GH and T_3 in birds fed on low-energy and protein diets (2800Kcal ME/Kg, 190gr/Kg) were 50 and 5.1 ng/mL respectively, whereas plasma T_4 remained at 6.4 ng/mL. However, in

Table 2: Mean concentrations of plasma T3, T4 and GH in the three periods in birds fed either 2800 or 3200 Kcal ME/Kg diets with five different protein levels

Period	Protein g/kg diet	2800 Kcal ME/Kg			3200 Kcal ME/Kg		
		GH ng/mL	T_3 ng/mL	T_4 ng/mL	GH ng/mL	T_3 ng/mL	T_4 ng/mL
Starter	230	36^{a}	3.2^{a}	8.5^{a}	28^{a}	2.4^{a}	9.3^{a}
	220	40^{ab}	3.7^{ab}	8.0^{ab}	32^{b}	2.9^{b}	8.8^{ab}
	210	43^{bc}	4.2^{bc}	7.5^{bc}	35^{bc}	3.4^{bc}	8.3^{bc}
	200	47^{cd}	4.6^{cd}	7.0^{c}	39^{c}	3.8^{c}	7.8^{cd}
	190	50^{d}	5.1^{d}	6.4^{d}	42^{d}	4.3^{d}	7.2^{d}
±s.e.		4	0.5	0.4	4	0.4	0.6
Grower	220	32^{a}	2.8^{a}	9.2^{a}	25^{a}	2.0^{a}	9.9^{a}
	210	36^{b}	3.3^{b}	8.6^{ab}	29^{ab}	2.5^{ab}	9.5^{ab}
	200	38^{bc}	3.6^{bc}	8.3^{d}	33^{bc}	2.8^{bc}	9.0^{b}
	190	41^{c}	4.0^{c}	8.0^{bc}	36^{cd}	3.2^{cd}	8.7^{bc}
	180	46^{d}	4.7^{d}	7.3^{c}	41^{d}	3.9^{d}	8.0^{c}
±s.e.		3	0.3	0.4	3	0.4	0.6
Finisher	190	26^{a}	2.3^{a}	10^{a}	18^{a}	1.7^{a}	10.8^{a}
	180	30^{ab}	2.8^{ab}	9.4^{ab}	22^{ab}	2.2^{ab}	10.2^{b}
	170	32^{bc}	3.1^{bc}	8.9^{b}	24^{c}	2.5^{b}	9.7^{bc}
	160	35^{c}	3.5^{c}	8.5^{bc}	27^{d}	2.9^{bc}	9.3^{cd}
	150	40^{cd}	4.2^{e}	8.0^{c}	32^{e}	3.6^{d}	8.8^{d}
±s.e.		4	0.4	0.4	4	0.4	0.5

[abcde] Mean with common superscripts within columns do not differ (p>0.05).

birds fed on high-energy and protein diets (3200Kcal ME/Kg, 230gr/Kg) mean concentrations of GH, T_3 and T_4 were 28, 2.4 and 9.3ng/mL respectively. These mean concentrations in birds fed on high-energy but low-protein diets were 42, 4.3 and 7.2ng/mL, respectively. In other words, at both energy groups, mean concentrations of plasma GH and T_3 were the least in chickens fed on the highest protein diets, whereas mean concentrations of plasma T_4 were the most. Also at both energy groups mean concentrations of plasma GH and T_3 were increased significantly (P<0.05) in broilers fed on low-protein diets compared with those fed on high-protein diets during each of the periods. Whereas, mean concentration of plasma T_4 were decreased significantly (P<0.05) in each of periods in birds fed on low-protein diets compared with those fed on high-protein diets.

Discussion

The objectives of the present study were to compare the effect of different energy and protein levels on mean plasma concentrations of GH, T_3 and T_4 in Lohmann male broilers. There are some reports regarding the relationship between dietary energy and protein and their subsequent effects on performance and intermediary metabolism (Burke and Marks, 1982; Darras *et al*., 2000; Harvey *et al*., 1983; Keagy *et al*., 1987; Lauterio and Scanes, 1987; March *et al*., 1964; Rosebrough *et al*., 1999; Vasilatos-Younken *et al*., 1999). The results of these studies about mean plasma concentrations of metabolic hormones and effect of different dietary energy and protein levels on them vary and there is no consensus about the direction and the amount. For example, plasma GH concentrations increased in broilers fed on low-protein diets compared with those fed on high-protein diets but also plasma T_3 and T_4 concentrations decreased in broilers fed on low-protein diets compared with those fed high-protein diets (Lauterio and Scanes, 1987). Whereas, other authors demonstrated that plasma GH and T_3 concentrations increased in birds fed on low-protein diet compared with those fed on high-protein diet, In addition, T_4 concentrations decreased in birds fed on low-protein diets (Keagy *et al*., 1987; Rosebrough and Mcmurtry, 1993; Rosebrough *et al*., 1999). However, in the current study the results of plasma concentrations of GH, T_3 and T_4 were vary. For example, plasma concentrations of GH reported (132-459, 0.75-1.36 ng/mL), although differences among strains and types of birds and assay methods may explicate the differences observed.

The present experiments demonstrated that feeding a diet containing 2800 KcalME/Kg resulted in the greatest plasma concentrations of GH compared with diet containing 3200 KcalME/Kg in all periods. Also other authors reported that plasma concentrations of GH were significantly increased (P<0.05) in birds that were fed on low-energy diets compared with those fed on high-energy diets (Gonzales *et al.,* 1998 and Vasilatos-Younken *et al.,* 1999). Other studies demonstrated that low GH levels were found in rapidly growing birds compared to those slowly growing birds (Burke and Marks, 1982; Rosebrough *et al*., 1999; Tanaka *et al*., 1983).

The results of the present study indicats that low GH levels were found in birds that are the heaviest compared with the lightest ones. The quantities of plasma concentrations of GH in the present experiments were not similar to those studies, but were approximately similar to the results reported by

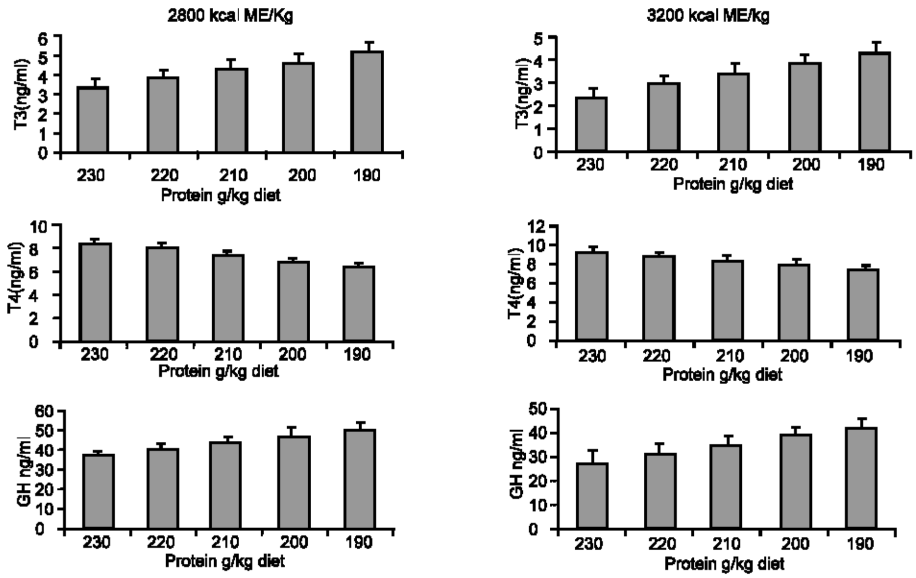

Fig. 1: Mean concentrations of plasma T3, T4 and GH in the starter period in birds fed either 2800 or 3200 Kcal ME/Kg diets with five different energy and protein levels.

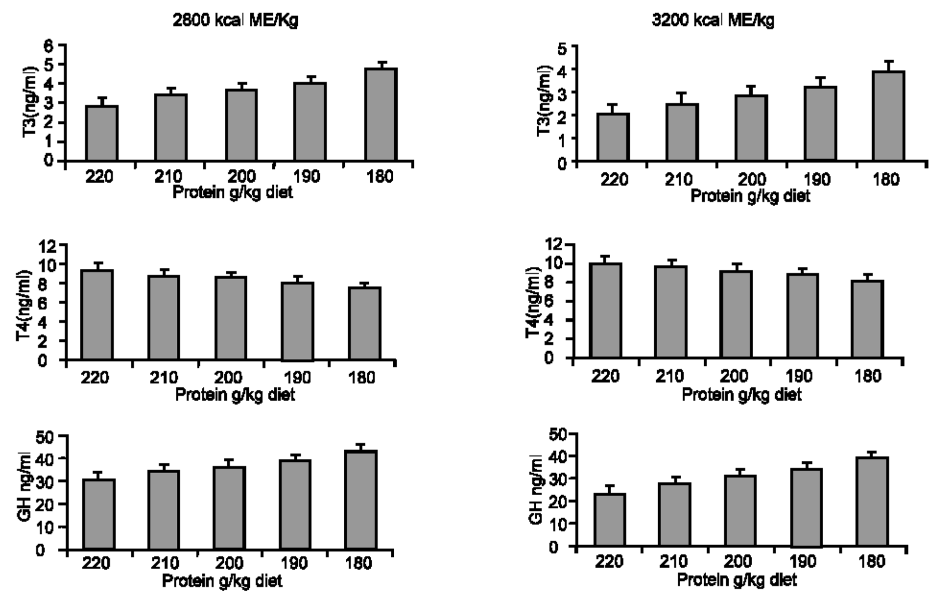

Fig. 2: Mean concentrations of plasma T3, T4 and GH in the grower period in birds fed either 2800 or 3200 Kcal ME/Kg diets with five different energy and protein levels.

Gonzales *et al.*, 1998. The results of the present study support those of other authors (Rosebrough and Mcmurtry, 1993; Tanake *et al.*, 1983) that showed that in the face of a constant energy level an increase in protein level would result in a decrease in plasma concentrations of GH. Other studies demonstrated that plasma concentrations of T_4 affected by different dietary energy and protein levels and found high T_4 levels in chicks that fed on high-energy and protein diets (Keagy *et al.*, 1987; Rosebrough and Mcmurtry, 1993; Williams and Njoya, 1998). Also, the present experiments demonstrated that plasma concentrations of T_4 increased significantly ($P<0.05$) in broilers that fed on high-energy and protein diets compared with those fed on low-energy and protein diets in each of periods. The amount of plasma concentrations of T_4 in the present study were approximately similar to the results that reported by others (Rosebrough and Mcmurtry, 1993). Generally, it has been reported that a reduction in plasma T_3 is accompanied by an increase in T_4 as a result of a reduction in peripheral monoiodination of T_4 (Klandorf *et al.,* 1981). Energy content of diet clearly

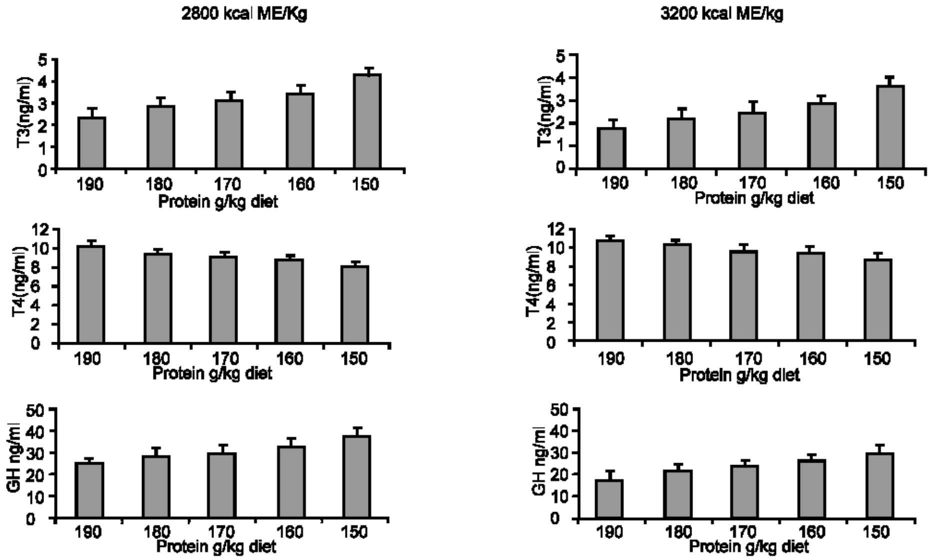

Fig. 3: Mean concentrations of plasma T3, T4 and GH in the finisher period in birds fed either 2800 or 3200 Kcal ME/Kg diets with five different energy and protein levels.

affects the plasma concentrations of T3 (Williams and Njoya, 1998) they demonstrated that low-energy diet resulted in higher plasma T_3 and lower plasma T_4 concentrations.

Also in the present study showed that plasma concentrations of T_3 were decreased significantly ($P<0.05$) in broilers fed on high-energy and protein diets compared with those fed on low-energy and protein diets during each of periods. Several factors could lead to high plasma T_3 levels in protein-deficient chicks. First, secretion rate and activity of the thyroid gland may be increased. However, others (March *et al.*, 1964) reported low thyroidal iodine uptake in protein-deficient chicks. Second, there could be slower clearance of T_3 from the blood. Usually, T_3 is cleared from chick plasma more quickly than T_4 because it is not bound tightly to plasma protein (Hutchins and Newcomer, 1966). On the other hand, plasma-binding characteristics for thyroid hormones might change in protein-deficient rats, in which case there is high total T_3 but low free T_3 in the plasma (Smallrige *et al.*, 1982). Finally, there might have been enhanced conversion of T_4 or T_3, an explanation that seems most probable, because the elevation of plasma T_3 was accompanied by a drop in plasma T_4 with no changes in plasma reverse T_3 (rT_3). If T_4 was not converted to T_3, then an increase in rT_3, the alternate, purportedly inactive metabolite of T_4 should have been seen (Keagy *et al.*, 1987). In addition, some studies suggested a change in the rate of conversion of T_4 to T_3 between starting, growing and finishing chickens (Williams and Njoya, 1998; Njoya, 1995). This is dependent either on dietary energy intake or on the amount of feed consumed and leads to the conclusion that the diet (or food consumption or energy intake) affects plasma thyroid hormone concentrations. Also, other authors suggested that GH could decrease hepatic type III deiodinase (D_3) that catalyses inner ring of a iodothyronine molecule (Geyten *et al.*, 1999; Vasilatos-Younken *et al*; 1999; Darras *et al.,* 2000). Outer ring deiodination (ORD) of T_4 is the only way to produce active T_3 and therefore ORD is important as an activating pathway. Inner ring deiodination (IRD) of T_4 or T_3 can only lead to inactive iodothyronines, namely reverse T_3 (rT_3).

Degradation of T3 by D3 is an important factor in the regulation of plasma T_3 levels and the cGH have inhibitory effect on D_3 (Darras *et al.*, 2000). It should also be mentioned that in many of the above studies, plasma concentrations of GH were increased by diets with low-energy and protein levels. D_3 activities were inhibited by GH and then were decreased degradation of T_3 and increased plasma concentrations of T_3. Analyses of our data also showed that in step with increase broilers age, mean concentrations of plasma GH and T_3 were decreased, whereas mean concentrations of plasma T_4 were increased in broilers fed on different energy and protein levels. It has been reported that food consumption or energy intake could affect plasma metabolic hormone concentrations Njoya, 1995). Our study demonstrated that food consumption or energy intake were the greatest and the least in finisher and starter periods, respectively. Also, in this study it was found that in almost all stages, food consumption or energy intake by broilers fed on diets with nearly similar protein levels were not changed ($P>0.05$) and that mean plasma concentrations of GH, T_3 and T_4 did not differ ($P>0.05$) in these broilers.

In summary, the hypothesis of the present study was that different dietary energy and protein levels and age in rearing periods in Lohmann male broilers could affect mean plasma concentrations of metabolic hormones, but diets with nearly similar protein levels did not influence food consumption and plasma concentrations of those hormones.

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