Global Veterinaria 6 (5): 459-467, 2011
ISSN 1992-6197

Relationship Between the Variations of Blood Thyroid Hormones Concentrations, Their Percentages and Some Biochemical Parameters During Different Ages of Layer Breeder

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Abstract: The thyroid is among the most important endocrine glands that its hormones activity has different effects on metabolism of various components in animal tissues. This study was carried out to determine the relationship between the changes of serum thyroid hormones (T_3, T_4, fT_3, fT_4) concentrations and their percentages, total protein, albumin, cholesterol, glucose concentrations and also albumin: globulin (A:G) T_3:T_4, fT_3:fT_4 ratios in different ages of layer breeders. A total of 49 layer breeder was randomly selected and then the birds were divided into seven age groups (22, 24, 26, 28, 30, 32 and 34 weeks). Blood samples were collected from the wing vein of the birds. The serum was analyzed to determine thyroxine (T_4), triiodothyronine (T_3), free thyroxine (fT_4), free triiodothyronine (fT_3) and total protein, globulin, albumin, glucose and cholesterol concentrations and also above mentioned ratios. The concentrations of thyroid hormones were determined by electrochemiluminescence method and other parameters were measured by photometric method. In the current study, there was a significant difference between age groups concerning the serum total protein ($P<0.001$), albumin ($P<0.01$), globulin ($P<0.001$), cholesterol ($P<0.01$) concentrations, T_3:T_4 ($P<0.05$) and fT_3: fT_4 ($P<0.001$) ratios and also concerning T_4 ($P<0.01$), T_3 ($P<0.001$), fT_3 ($P<0.01$) concentrations and their percentages ($P<0.05$). Our data from the layer breeder demonstrated that the higher concentration of total protein, globulin, T_3 and its percentage were observed in group VII and an increase in age results in significant increase in the plasma total protein ($P<0.001$, $r = 0.568$), globulin ($P<0.001$, $r =0.616$) and T_3 ($P<0.001$, $r = 0.534$) concentration also its percentage ($P<0.01$, $r = 0.388$), T_3:T_4 ($P<0.01$, $r = -0.426$) and fT_3:fT_4 ratios while results in significant decrease in T_4 percentage ($P<0.01$, $r = -0.379$). It was concluded that T_3 was metabolically more active compared to the other THs in layer breeder; also, age and related physiological changes had a significant effect on total protein, albumin, globulin and all the THs concentrations and also their percentages, except to fT_4. Furthermore, the determination of serum T_3:T_4 ratio was especially a good indication for analysis the data about the hormonal and blood biochemical metabolites changes and relationship between them compared to calculation of THs percentages in layer breeder.

Key words: Thyroid hormones · A: G ratio · Biochemical parameters · Plasma · Layer breeder

INTRODUCTION

Thyroid hormones (THs) have various effects on metabolism and development in poultry and mammals [1-3]. These hormones play important roles in the differentiation, development and maturation of some specific tissues in the vertebrate classes [1, 4-6]. For example, THs cause to development of chick brain by stimulating cell proliferation, migration and differentiation [6]. These hormones are involved in regulation of the energy metabolism and increase the basal metabolism rate of most tissues such as liver, kidney, heart and skeletal muscles [5, 7-9] and affect protein and adipose turnover rate [10, 11]. The effects of THs on protein metabolism may be anabolic or catabolic which is dependent on its levels. In low physiological levels they are anabolic while at high levels they are catabolic [5, 12]. Additionally, these hormones have an increasing effect on plasma glucose level; therefore, they are antagonistic to insulin [10, 12]. THs cause to enhance the rate of glycolysis, promote intestinal absorption of glucose and decrease of hepatic glycogen stores and lesser extend in skeletal muscles; at the same time they increase gluconeogenesis in liver [10, 12, 13]. The increasing effect of THs on energy

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metabolism is mainly manifested as heat production [7, 13]. THs, like steroids and vitamin D are hydrophobic compounds and have specific nuclear receptors which regulate the expression of targeted genes also include receptors for other small lipophilic hormones [14, 15]. They are bound to specific transport proteins such as thyroxin-binding globulin, prealbumin (Transthyretin) and albumin in plasma and carried throughout the circulating system to target tissues [10, 13, 15, 16]. In plasma these hormones are found in two forms: free and bound to specific proteins [12, 17]. Since chicken plasma does not contain a specific thyroxin-binding globulin, as does mammalian; therefore, THs cause to increase lipolysis in adipose tissue, degradation of cholesterol and its conversion to bile acid and exertion through the bile secretes [10, 12]. The level of plasma cholesterol is increased in hypothyroidism while is decreased in hyperthyroidism [12, 18]. Ths are most bound to albumin and prealbumin and equilibrium between the level of total protein and affinity of THs binding to them determine the level of free forms of THs within plasma [7, 10, 15, 17]. In chicken many factors affect on plasma THs concentrations that are including: food restriction, fasting and time of feeding, fattening, activity of deiodinases, diet composition, drugs, age, method of determination, environment temperature, sexual maturity, during of photoperiod and other hormonal and pathophysiological status [7, 19-21]. Similar to other mammalian, the greater parts of Ths are secreted as T_4, but T_3 is metabolically more active and involve in regulating oxygen consumption than T_4 in chicken. Overall, the effects of T_4 and T_3 are identical in birds, which are not in mammals [7]. There is a little information about the change of THs and their percentages during different ages and also about the relationship between THs and some plasma biochemical parameters in layer breeder. The objective of this experiment was focused on determination of relationship between the variation of serum THs concentrations and their percentages and some biochemical parameters including total protein, albumin, globulin, cholesterol and glucose concentrations and A:G, T_3:T_4 and fT_3: fT_4 ratios during different ages of layer breeder.

MATERIALS AND METHODS

The experiments were performed on 49 layer breeder poultry of the Ross 308 breed which reared at S. Developed Poultry Education Center, East Azarbaijan, Iran. The birds were nearly in the live body weight (1.9±0.12 Kg) and randomly divided into seven age groups that including: 22, 24, 26, 28, 30, 32 and 34 weeks. During the experiment, the broiler breeders fed the diets which their compositions are presented in table 1. Feed and water were supplied *ad-libitum* during the study. This experiment was performed in summer and all the birds were kept under the same hygienic, managerial and environmental conditions. Blood samples were collected by using disposable syringes from the wing vein of the

Table 1: Composition of the diets during different ages of broiler breeders

Ingredients (%)	22-24 weeks	25-26 weeks	27-30 weeks	31-34 weeks
Corn	57.5	62.5	52.5	49.2
Soy bean	20	22	22	22
Wheat	9.2	-	10	15
Phosphate	1.2	1	1	1
Oyster shell	2	-	4	4
Carbonate Calcium	1.2	8	4	4.5
Pollard	6	3	3	3
Premix [a]	0.6	0.6	0.6	0.6
Common salt	0.25	0.25	0.25	0.3
Methionine	0.1	0.1	0.1	0.08
Vit-D_3	0.1	0.1	0.1	0.1
Vit-E	0.2	0.3	0.3	0.2
Phytase [b]	0.05	0.015	0.015	0.02
Oil	1.5	2	2	-
Maxiplex	0.05	0.05	0.05	-
Amprolium	0.05	0.05	0.05	-
Total Calorie	2850	2800	2770	2640

a. Supplied per kilogram of diet: vitamin A, 9000 IU; Cholecalciferol, 3000 IU; vitamin E, 18 IU; vitamin K3, 2 mg; vitamin B12, 0.015 mg; thiamin, 1.8 mg; riboflavin, 6.6 mg; folicacid, 1 mg; biotin, 0.10; niacin, 35 mg; pyridoxine, 4 mg; choline chloride, 250 mg; ethoxyquine, 0.125. -Supplied per kilogram of diet: manganese sulphate, 100 mg; copper sulphate, 10 mg; selenium (sodium selenate), 0.2 mg; iodine (EEI), 1 mg; zinc sulfate, 100 mg; Fe, 50 mg.
b. Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 500 FTU microbial phytase per kilogram of diet.

birds directly into vacutainer tubes without an anticoagulant (Becton Dickinson, NJ, USA) at 7 – 9 A.M. The serum was separated by centrifugation at 750×g for 15 min and then frozen at –20°C until used. The serum was analyzed to determine thyroxine (T_4), triiodothyronine(T_3), free thyroxine (fT), free triiodothyronine (fT_3) concentrations using the Cobas electrochemiluminescence (ECLIA) kits (Roche Boeringer-Mannheim, USA) with the Hitachi analyzer at the Danesh medical laboratory, Tabriz, Iran and to determine of total protein, albumin, cholesterol and glucose concentration by spectrophotometric method using the commercial kits at the clinical pathology laboratory of Islamic Azad University, Shabestar branch, East Azarbaijan, Iran. Also, the level of globulin was determined using the method has been described previously by Stockham and Scott [17]. The validation for these hormones assays assessed the limits of detection, precision of standard curve following sample dilution and intra- and inter-assay coefficient of variation of the results. The data were analyzed by Duncan's multiple range tests for detection significant differences between the means and Pearson's correlation method using SPSS/ver.17 software. All values are shown as mean ± standard deviation (SD) and data with $P < 0.05$ was seen as statistically significant.

RESULTS

The mean ± standard deviations of all the studied parameters during different ages of layer breeder are presented in tables 2 and 3. The percentages of plasma thyroid hormones, T_3:T_4 and fT_3: fT_4 ratios during different ages of layer breeder are shown in table 4. According to the results, there was a significant difference among age groups concerning the serum total

Table 2: The values of albumin: globulin ratio and total protein, albumin, globulin, glucose and cholesterol concentrations during different ages of layer breeder

Group	N		TP (g/l)	Alb (g/l)	Glob (g/l)	A:G	Glu (mg/dl)	Chol (mg/dl)
I	7	Mean	42.28^{b}	24.60^{b}	17.67^{b}	1.42	196.76^{a,b}	151.35^{b}
		SD	2.06	1.82	2.17	0.25	24.86	24.32
II	7	Mean	53.59^{b}	32.47^{a}	21.12^{b}	1.57	185.95^{a,b}	243.63^{a}
		SD	7.26	7.28	2.91	0.43	9.37	40.54
III	7	Mean	48.25^{b}	19.32^{b}	28.93^{b}	0.71	166.67^{b}	173.36^{b}
		SD	10.72	4.57	9.07	0.25	12.07	66.02
VI	7	Mean	45.22^{b}	24.33^{b}	20.88^{b}	1.49	180.72^{a,b}	174.52^{b}
		SD	3.43	5.65	7.55	1.22	17.84	39.38
V	7	Mean	46.17^{b}	25.47^{b}	20.69^{b}	1.49	207.39^{a,b}	177.99^{b}
		SD	3.47	4.27	6.48	1.05	24.14	42.47
VI	7	Mean	80.34^{a}	24.04^{b}	56.29^{a}	0.45	175.86^{b}	198.84^{b}
		SD	6.38	4.32	9.61	0.14	17.12	36.68
VII	7	Mean	78.18^{a}	24.30^{b}	53.87^{a}	0.47	192.07^{a,b}	140.15^{b}
		SD	7.59	3.40	9.02	0.11	28.29	36.29

There is a significant difference among age groups concerning the studied parameters with different codes in a column (superscript letters a and b). Group I 22 weeks old; Group II 24 weeks old; Group III 26 weeks old; Group IV 28 weeks old; Group V 30 weeks old; Group VI 32 weeks old; Group VII 34 weeks old; SD standard deviation; TP total protein; Alb albumin; Glob globulin; A: G albumin: globulin ratio; Glu glucose; Chol cholesterol.

Table 3: The values of thyroid hormones during different ages of layer breeder

Group	N		T_4 (nmol/l)	T_3 (nmol/l)	fT_4 (pmol/l)	fT_3 (pmol/l)
I	7	Mean	25.21^{b}	0.97^{c}	11.32^{a,b}	6.16^{b}
		SD	11.36	0.42	2.07	5.62
II	7	Mean	39.27^{a}	1.82^{b,c}	10.05^{b}	3.86^{b}
		SD	5.77	0.48	1.99	1.17
III	7	Mean	26.87^{b}	1.28^{b,c}	10.89^{a,b}	15.61^{a}
		SD	11.35	0.59	3.38	6.35
VI	7	Mean	39.35^{a}	1.96^{b}	14.55^{a}	6.02^{b}
		SD	9.40	0.66	4.79	1.69
V	7	Mean	31.80^{a,b}	1.63^{b,c}	9.61^{b}	5.92^{b}
		SD	8.82	0.44	2.58	3.06
VI	7	Mean	38.15^{a}	2.16^{a,b}	10.64^{a,b}	4.71^{b}
		SD	8.58	0.82	3.47	2.18
VII	7	Mean	31.43^{a,b}	2.93^{a}	11.29^{a,b}	18.41^{a}
		SD	7.96	1.31	4.46	5.26

There is a significant difference among age groups concerning the studied parameters with different codes in a column (superscript letters a, b and c).Group I 22 weeks old; Group II 24 weeks old; Group III 26 weeks old; Group IV 28 weeks old; Group V 30 weeks old; Group VI 32 weeks old; Group VII 34 weeks old; SD standard deviation.

Table 4: The percentage of thyroid hormones during different ages of layer breeder

Group	N		T_4 (%)	T_3 (%)	fT_4 (%)	fT_3 (%)	T_3:T_4	fT_3:fT_4
I	7	Mean	95.50^{a}	4.43^{b}	0.049^{a,b}	0.022^{b}	0.040^{b}	0.473^{b}
		SD	3.46	3.44	0.024	0.013	0.022	0.416
II	7	Mean	95.54^{a}	4.42^{b}	0.025^{b}	0.009^{b}	0.047^{b}	0.393^{b}
		SD	0.93	0.93	0.006	0.004	0.011	0.139
III	7	Mean	94.81^{a}	5.08^{b}	0.044^{a,b}	0.068^{a}	0.046^{b}	0.587^{b}
		SD	2.93	2.94	0.025	0.063	0.023	0.327
VI	7	Mean	95.10^{a}	4.85^{b}	0.035^{a,b}	0.016^{b}	0.050^{b}	0.425^{b}
		SD	1.57	1.56	0.013	0.008	0.010	0.098
V	7	Mean	94.94^{a}	5.01^{b}	0.031^{a,b}	0.018^{b}	0.056^{b}	0.478^{b}
		SD	1.18	1.17	0.010	0.012	0.015	0.252
VI	7	Mean	94.57^{a}	5.39^{b}	0.027^{b}	0.011^{b}	0.056^{b}	0.388^{b}
		SD	1.80	1.80	0.005	0.007	0.020	0.117
VII	7	Mean	91.13^{b}	8.78^{a}	0.035^{a,b}	0.054^{a}	0.081^{a}	2.140^{a}
		SD	4.17	4.15	0.016	0.035	0.032	1.368

There is a significant difference among age groups concerning the studied parameters in a column with different codes (superscript letters a and b). Group I 22 weeks old; Group II 24 weeks old; Group III 26 weeks old; Group IV 28 weeks old; Group V 30 weeks old; Group VI 32 weeks old; Group VII 34 weeks old; SD standard deviation.

Table 5: Correlation coefficients of all the studied parameters in entire the layer breeders (n=49)

	T_4	T_3	fT_4	fT_3	T_3:T_4	fT_3:fT_4	T_4 (%)	T_3 (%)	fT_4 (%)	fT_3 (%)
Total protein	0.276	0.522**	-0.032	0.147	0.391**	0.433**	-0.271	0.001	-0.306*	-0.277
Albumin	0.280	0.180	0.028	-0.121	0.030	-0.024	-0.144	-0.175	0.007	-0.198
Globulin	0.172	0.456**	-0.040	0.184	0.362*	0.422**	-0.220	0.058	-0.303*	0.005
A: G	-0.200	-0.303**	0.112	0.141	0.206	-0.298	0.307*	0.305*	0.233	-0.197
Cholesterol	0.576**	0.002	-0.108	-0.352*	-0.351*	-0.322*	-0.415**	-0.395**	0.155	-0.086
Glucose	-0.215	-0.070	0.028	0.003	0.030	0.012	0.225	0.004	-0.166	0.221

* Correlation is significant at the 0.05 level (2-tailed). ** Correlation is significant at the 0.01 level (2-tailed). A: G albumin: globulin ratio

Table 6: The values of 95% CI of albumin: globulin ratio and total protein, albumin, globulin, glucose and cholesterol concentrations during different ages of layer breeder

The 95% confidence interval							
Group	N	TP (g/l)	Alb (g/l)	Glob (g/l)	A:G	Glu (mg/dl)	Chol (mg/dl)
I	7	40.37-44.18	30.87-34.15	15.66-19.67	1.18-1.65	173.76-219.75	128.85-173.84
II	7	46.87-60.37	25.73-39.20	18.42-23.81	1.17-1.96	177.28-194.61	206.13-281.12
III	7	38.33-58.16	15.09-23.54	20.54-37.31	0.47-0.97	155.50-177.83	112.30-234.41
IV	7	42.04-49.37	19.10-29.55	13.8927.86	0.36-2.61	164.22-197.21	138.09-210.94
V	7	42.96-49.37	21.52-29.41	14.69-26.68	0.51-2.46	185.06-229.71	138.71-217.26
VI	7	74.43-86.24	20.04-28.03	47.40-65.17	0.32-0.57	160.02-191.69	164.91-232.76
VII	7	71.16-85.19	21.55-27.44	45.52-62.21	0.36-0.57	165.90-218.23	106.58-173.71

Group I 22 weeks old; Group II 24 weeks old; Group III 26 weeks old; Group IV 28 weeks old; Group V 30 weeks old; Group VI 32 weeks old; Group VII 34 weeks old; TP total protein; Alb albumin; Glob globulin; A: G albumin: globulin ratio; Glu glucose; Chol cholesterol

Table 7: The values of 95% CI of T_3:T_4 and fT_3:fT_4 ratios, thyroid hormones and their percentages during different ages of layer breeder

The 95% confidence interval						
Group	N	T_4 (nmol/l)	T_3 (nmol/l)	fT_4 (pmol/l)	fT_3 (pmol/l)	T_3:T_4
I	7	14.70-35.71	0.58-1.35	9.40-13.23	0.96-11.35	0.019-0.060
II	7	33.93-44.60	1.37-2.26	8.20-11.89	2.77-4.94	0.036-0.057
III	7	16.37-37.36	0.73-1.82	7.76-14.01	9.73-21.48	0.024-0.067
IV	7	30.65-48.04	1.35-2.57	10.11-18.98	4.45-7.58	0.040-0.059
V	7	23.64-39.95	1.22-2.03	7.22-11.96	3.08-8.75	0.042-0.069
VI	7	30.21-46.08	1.40-2.91	7.43-13.84	2.69-6.72	0.037-0.074
VII	7	24.06-38.79	1.71-4.14	7.16-15.41	13.54-23.27	0.051-0.110
The 95% confidence interval						
Group	N	T_4 (%)	T_3 (%)	fT_4 (%)	fT_3 (%)	fT_3:fT_4
I	7	92.30-98.69	1.24-7.61	0.026-0.071	0.009-0.034	0.088-0.857
II	7	94.67-96.40	3.55-5.28	0.019-0.030	0.005-0.012	0.264-0.521
III	7	92.10-97.51	2.36-7.79	0.020-0.067	0.009-0.126	0.284-0.889
IV	7	93.64-96.55	3.13-6.02	0.022-0.047	0.008-0.023	0.334-0.515
V	7	93.84-96.03	3.92-6.09	0.021-0.040	0.007-0.029	0.244-0.711
VI	7	92.90-96.23	3.72-7.05	0.022-0.031	0.004-0.017	0.279-0.496
VII	7	87.27-94.98	4.94-12.61	0.020-0.049	0.021-0.086	0.874-3.405

Group I 22 weeks old; Group II 24 weeks old; Group III 26 weeks old; Group IV 28 weeks old; Group V 30 weeks old; Group VI 32 weeks old; Group VII 34 weeks old.

protein (P<0.001), albumin (P<0.01), globulin (P<0.001), cholesterol (P<0.01) concentrations, T_3:T_4 (P<0.05) and fT_3: fT_4 (P<0.001) ratios and also concerning T_4 (P<0.01), T_3 (P<0.001), fT_3 (P<0.01) concentrations and their percentages (P<0.05). Correlation coefficients and statistical analysis between some of the studied parameters in layer breeder are presented in table 5. Among all the studied parameters, with increase in age, we found a positive correlation with total protein, globulin, T_4, T_3, fT_3 concentrations and percentages of T_3 and fT_3. Although, with advancing in age of layer breeder, we showed a significant correlation with total protein (r = 0.568), globulin (r = 0.616), T_3 (r = 0.534) (P<0.001) and with percentages of T_4 (r = -0.379) and T_3 (r = 0.381), T_3: T_4 (r = 0.495) (P < 0.01) and fT_3: fT_4 (r = 0.487) (P < 0.01) ratios. As well as, the values of 95% confidence interval all of the studied parameters are shown in tables 6 and 7.

DISCUSSION

In this study, the concentration of serum total protein was higher than the values reported previously in layer hen [22, 23], broiler chicken [24, 25] and chicks [26], but was consistent or had a little difference with the values reported in hens [27] and turkeys [28, 29]. The results indicated that the concentration of total protein and globulin in blood serum of layer breeder gradually increased, especially after 32-34 weeks old. The differences detected with other reports are probably associated to age; diet composition, breed and period of egg laying [22-28] and among groups are due to physiological changes which are normal during different ages [16, 23]. The rise of total protein and globulin concentrations between the beginning and end of the experiment were highly significant (P<0.001). A considerable increase in total protein occur just prior and the peak egg laying. It could be attributed to an estrogen-induced increase in globulin concentrations [16, 28, 30], which was similar to the results of this experiment. The concentration of albumin was higher than the values previously reported in laying hen [22, 23], broiler chicken [24] and turkey [28]; although had a little difference with the values reported in chicken [31], turkey [29] and Japanese quail [32]. It was observed that age influenced on serum albumin concentration and its highest value was shown in group II. Concurrently with increase in age, the serum albumin concentration was non-significantly decreased which mainly attributed to the negative effect of egg laying and physiological changes during different ages on blood albumin concentration [16, 30]. The A:G ratio was higher than the other values reported in laying hen [22, 23], turkey [28] and was consistent with the values reported in chicken [24, 31]. The reasons of detected differences with the other values previously reported were similar to the total protein and albumin which above mentioned. In the current experiment, the concentration of glucose was consistent with the values reported in chicken [30, 31, 33, 34] and had a little difference with the other values reported in chicken [35], but its value was lower than the other reports in chicken [36, 37] and Japanese quail [32, 38]. Age had not significant effect on blood serum glucose concentration in layer breeder; therefore our result was consistent with the reports in Japanese quail [32]. Although, we observed a non-significant negative correlation between age and glucose concentration which was in agreement with the other reports suggested that the plasma glucose level decreased gradually after maturity through out the birds' life [29]. In birds, the change of blood glucose concentration during the growth and maturation, depending on the feed intake, production performance, body weight and relation to the change of the environment [39, 40]; these factors could be cause to differ our results with the other. The concentration of cholesterol was higher than the values reported in Japanese quail [32, 41], chicken and broiler [24, 35, 42], but was agree with the values reported in chicken [31]. Similar to glucose, age had not significant effect on serum cholesterol concentration in layer breeder which was consistent with previous report in chicken [40]. Development of reproductive stage may be caused to increase in serum cholesterol concentration where it reached to highest value at peak of production and before sexual maturity. This increase on serum cholesterol is attributed to simulation lipid metabolism by estradiol [16, 28, 30, 41]. The concentrations of plasma T_4 and T_3 were consistent with the values reported previously in chicken [7, 31], but was higher than the values reported in broiler chicken [43]. With regarding to the results, age had a positive effect on T_4 (non-significantly) and T_3 (significantly) concentration in layer breeder which their amounts were gradually increased with aging. In this experiment, the changes of these two hormones were not similar to the other animals [44, 45], but were agree with the other report in chicken [7]. Considering the metabolic role of these thyroid hormones in the body which are involvement in the growth and development of young organisms as well as economizing on energy in the chickens, when the organism need a great amount of

energy for intensive production period, our results could be expected. Moreover, this explains the rise of T_3 concentration during different ages in blood serum of layer breeder. It is well known that T_3 plays a more dominant role in bio-oxidation processes in cells than does T_4 [5, 7, 46]. The fT_4 and fT_3concentrations had a little difference with values reported in broiler breeder [21], but the fT_4 concentration was higher while the fT_3 concentration was lower than the other values reported previously in laying hen [47]. Overall, as aforementioned, many endogenous and exogenous factors such as diet, food restriction, fasting and time of feeding, fattening, activity of deiodinases, drugs, age, gender, method of determination, environment temperature, performance and sexual maturity, during of photoperiod affect the thyroid hormones concentration in poultry [7, 20, 21, 48-50] and most of them act as complex; therefore, the exact explanation effects of these factors on serum thyroid hormones is not possible in this experiment. Although, it seems that age and related physiological changes, diet and the rate of egg laying were the most important affecting factors on serum thyroid hormones concentration in the experiment. Age had a significant effect on all the thyroid hormones in layer breeder, except to fT_4. As well as, our results demonstrated that among all the studied thyroid hormones, with an increase in age, only the T_3 concentration was significantly increased in layer breeder which clear the predominant role of T_3 in birds' metabolism [46]. Similar to the T_4, T_3 and fT_3 concentrations, their percentages changed significantly during different ages of layer breeder. The changes of T_3 and its free form were against to the T_4 percentage, because their percentages were elevated while the T_4 percentage was decreased in older birds especially in 34 weeks-old layer breeders. As above mentioned, T_3 has a more predominant role compared to T_4 in birds' metabolism, but only the free form of hormone is responsible for its biological activity [17, 46]. Therefore, considering to the metabolically demands during the different ages of layer breeder, these variations of thyroid hormones and their percentages were expected. In this study, the value of T_3:T_4 ratio was lower than other values reported previously in broiler chicks [43] and was consistent reported in quail [51]. But, there was no data about the change of fT_3: fT_4 ratio in poultry. The T_3:T_4 and fT_3: fT_4 ratios affected significantly during different ages of layer breeder. Likely, it was mainly related to physiological and metabolically changes during different ages of birds. According to the results, among the thyroid hormones, only T_3 had a significant positive correlation with serum total protein and globulin concentrations which cleared that the T_3 is more metabolically active (especially in protein metabolism) than the T_4 in birds. As well as, our findings demonstrated the positive effect of THs on blood total protein concentration which had been reported by the other researchers, previously [10, 12, 18]. The significant positive correlation between the serum cholesterol and T_4 concentrations ($P<0.01$); probably, associated to biphasic effect of THs on lipid metabolism. The biphasic nature of THs does affect the cholesterol concentration, as does total protein [51]. Therefore, T_4 hormones had anabolic effect on serum cholesterol concentration within the obtained values during different ages of layer breeder. Concurrently, fT_3 had a significant negative correlation with the cholesterol concentration ($P<0.01$) and was in agreement with the previous reports [12, 18]. With regarding to our results, moreover to biphasic nature of THs, different forms of THs have various effects on lipid metabolism in layer breeder which needs to be investigated in other researches. Although, the determination of T_3:T_4 and fT_3:fT_4 ratios had more agreement with the other reports concerning to have a good correlation with the change of various metabolic substances (e.g. protein, lipid and carbohydrate) in layer breeders. But, its application need to be more demonstrated in poultry endocrinology and in clinical setting. As well as, among all the blood THs, T_4 was predominant compared to the other which agree with the previous reports in different animals and birds [13, 21, 46, 50].

CONCLUSION

Overall, the results demonstrated that T_3 was metabolically more active compared to the other THs in layer breeder and had significant positive correlation with the total protein and globulin concentration while T_4 had similar correlation with the cholesterol concentration. The fT_3 acted against to the T_4 and had a significant negative correlation with the cholesterol concentration. Age and related physiological changes and requirements had a significant effect on the results particularly on total protein, albumin, globulin and all the THs concentrations and also their percentages, except to fT_4. Furthermore, the determination of serum T_3:T_4 (especially) and fT_3:fT_4 ratios obtained a more acceptable results for analysis the data about the hormonal and metabolically changes and relationship between them and related biochemical parameters compared to calculation of THs percentages; therefore, they can be used as a good indication for evaluating the variation of THs in clinical setting of layer breeder.

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