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Relationship Between Plasma Free Thyroxine Levels and Some Biochemical Parameters in Two Strains of Broiler Chickens

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Abstract: The thyroid is an endocrine organ found in all vertebrates and the avian gland is similar in many respects to that of mammalian. In birds as in other vertebrates, product hormones are both thyroxine (tetra-iodothyronine or T4) and tri-iodothyronine (T3). Thyroid hormones influence all major metabolic pathways and are involved in the regulation of the basal metabolism of the majority of tissues and consequently in the metabolism of lipids, carbohydrates and proteins. In this study, the relationship between plasma free thyroxine, cholesterol, triglyceride, glucose and total protein in Isa 15 strain and Arbor Acres strain during the fattening period was investigated. At 28, 35 and 42 days of age, ten birds per strain were sacrificed by decapitation and blood for analysis was collected from the jugular vein. The concentration of free T4 was recorded by radio- immuno- assay method (RIA) and other parameters were measured using clinical chemistry tests on the ARCHITECTci 8200 system. Our data from this study indicates that in broiler chickens, plasma lipid profile and plasma glucose profile were not correlated to plasma free thyroxine. However, a significant correlation between plasma free thyroxine and plasma total protein ($r=0.42$, $p=0.01$) was described in Arbor Acres strain. A significant difference ($p<0.05$) for plasma total protein was observed between the two strains. No significant difference between the two groups was reported for the other measured parameters. The study suggested conducting more investigations on the dynamics of free thyroid hormone concentration changes in blood plasma and their correlations in broiler chickens.

Key words: Free thyroxine · Plasma · Biochemical parameters · Broiler chickens

INTRODUCTION

The thyroid is an endocrine organ found in all vertebrates and the avian gland is similar in many respects to that of mammalian. In birds as in other vertebrates, product hormones are both thyroxine (tetra-iodothyronine or T4) and tri-iodothyronine (T3). The mechanisms of hormone synthesis and release by avian thyroid gland are essentially equivalent to those in mammals [1].

Thyroid gland synthesizes and secretes a mixture of T3 and T4. Thyroxine is the predominant hormone of the circulating thyroid hormones in the domestic birds [2]. Most of them are bound to proteins in the blood (albumin, transthyretin, alpha- globulin) and a part of them is free [3]. Only free hormones are responsible for the biological activity of thyroid hormones and available to tissues [4].

Thyroid hormones are the most important humoral factors involved in setting and modulating the basal metabolic rate in target tissues, such as liver, heart, kidney and brain [5]. These hormones influence all major metabolic pathways. Their most obvious and well known action is an increase in basal energy expenditure obtained acting on protein, carbohydrates and lipid metabolism. With specific regard to lipid metabolism, thyroid hormones affect synthesis, mobilization and degradation of lipids, although degradation is influenced more than synthesis [6]. They favor lipolysis in adipose tissue resulting in a decrease in plasma cholesterol content and they may have an indirect effect on lipogenesis [5]. Thyroid hormones also stimulate protein synthesis [7] and enhance the rate of glucose oxidation and intestinal absorption. They increase glygenolysis and gluconeogenesis in the liver [5].

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Previous studies have examined the impact of various factors on T3 and T4 levels in birds, including species [8], age [9], energy intake and dietary composition [10,11], feeding regimen [12], photoperiod [13], geographic variation [14], ambient temperature [15], pathophysiologic status [16], methods of measurement [3], but were limited to the total form of hormones and to few broiler strains.

There is no previous studies investigating the relationship between the level of plasma thyroid hormones and cholesterol, triglycerides, glucose and total protein in broiler chickens including strains. Therefore, the present study was conducted:

- To compare the growth performance between Isa 15 strain and Arbor Acres strain.
- To determine the biochemical values including: plasma free thyroxine, cholesterol, triglyceride, glucose and total protein in Isa 15 strain and Arbor Acres strain.
- To compare blood profile of these two groups.
- To determine the relationship and correlation between all the cited parameters with plasma free thyroxine in the two strains of broiler chickens.

MATERIELS AND METHODS

Animal and Housing: The study was conducted in the poultry farm BENBOULAID (Constantine-Algeria). Experiments were carried out on 60 broilers of two strains: 30 heavy fast growing broilers Arbor Acres strain and 30 lighter- weight, fast growing broilers Isa 15 strain. The two groups of animals were housed in two different battery brooders but subject to equivalent conditions including feeding. Broilers Isa 15 strain were reared from 1 to 59 days of age and broilers Arbor Acres strain were reared from 1 to 57 days of age according to the technological recommendations for these breeds. Chickens were fed up during the first 11 days of age with a commercial starter diet and from day 12 until the end of the fattening period with a commercial grower diet. The content of crude protein (%) and metabolisable energy (kcal/kg of diet) was as follows: starter diet: 22.11- 2823.75 (Table 1), grower period: 20.32- 2908.53 (Table2).

The temperature was maintained between 22 and 24°C. The animals were exposed to a light:dark cycle of 16 hours light : 8 hours dark at 21 days of age. The lighting is then increased by 2 hours each week to 22 hours light at 42 days until slaughter. Water and feed were provided ad libitum for the two strains. In our experimentation, no clinical symptoms of disease or abnormal mortalities were observed.

Table 1: Composition of starter diet.

Starter feed composition	Metabolizable energy	% Proteins
-Maize : 50%	2823.75 Kcal/Kg of diet	22.11%
-Soya meals : 24%		
-Milling issues : 23%		
-Limestone: 1%		
-Phosphate : 1%		
-Mineral complex vitamins :1%		

Source ONAB

Table 2: Composition of grower diet.

Grower feed composition	Metabolizable energy	% Proteins
-Maize : 62%	2908.53 Kcal /Kg of diet	20.32%
-Soya meals : 26%		
-Milling issues : 8.50%		
-Limestone: 0.90%		
-Phosphate : 1.60%		
-Mineral complex vitamins :1%		

Source ONAB

Blood Samples: At 28, 35 and 42 days of age, ten birds per strain were sacrificed by decapitation. Blood for analysis was collected from the jugular vein and harvested into heparinized polystyrene tubes. After sampling, blood plasma was separated by centrifugation at 3,000 rpm for 10 minutes and the obtained plasma was stored in a freezer at -20°C for later analysis.

Biochemical Analysis: Plasma samples were assayed using clinical chemistry tests on the ARCHITECTci 8200 system. The blood plasma was analyzed for triglycerides by the enzymatic procedure of Fossati and Prencipe [17] and Mac Gowan *et al*. [18].

Cholesterol was analyzed by the enzymatic procedure of Allain *et al*. [19], modified by Roeschlau *et al*. [20].

Glucose was analyzed by the hexokinase method, based on a modification of Slein [21], using hexokinase and glucose-6-phosphate-dehydrogenase to catalyze the reaction.

Total protein was analyzed by the Biuret method [22].

Hormone Assays: The concentration of free T4 was recorded by the radio- immuno-assay method (RIA), using the principle of labeled antibody. Samples and standards are incubated with I125-labeled monoclonal antibody specific for T4, as tracer, in the presence of a biotinylated analog of thyroxine (ligand) in avidin-coated

tubes. There is a competition between the free thyroxin of the sample and the ligand for the binding to the labeled antibody. The fraction of antibody complexed with the biotinylated ligand binds to avidin-coated tubes. After incubation, the content of tubes is eliminated and bound radioactivity is measured. A calibration curve is established and unknown values are determined by interpolation from the curve. Specificity of the assay for the free T4 had been established by the supplier. Intra and interassay coefficient of variation were 8.3% and 7.5% respectively.

Statistical Analysis: The results were evaluated statistically by the Statview 1992-98 SAS Institute. Inc. Data were analyzed with one way analysis of variance (ANOVA). The student's t-test was used to evaluate strain differences. Z test correlation of Fisher was applied to assess the relation between plasma free thyroxine with cholesterol, triglycerides, glucose and total protein levels in the two strains. Comparisons were considered significant when p values were less than 0.05.

RESULTS

In Table 3, body weights of the two strains at 28, 35 and 42 days of age are presented. Chickens of Arbor Acres strain were heavier in this investigation than Isa 15 strain. Their body weights were higher at 28, 35 and 42 days of age. Difference in body weights between the two strains was statistically significant ($p<0.05$).

The mean±SE of the plasma free thyroxine, triglyceride, cholesterol, glucose and total protein concentrations at different ages in Isa 15 strain and Arbor acres strain are showed in Table 4. There was a significant difference ($p<0.05$) between the two strains only for the levels of plasma total protein, no significant differences ($p>0.05$) were observed for the other measured parameters in this investigation.

We observed no significant correlation between plasma free thyroxine and plasma cholesterol in Isa 15 strain ($r=0.16$, $p=0.40$) and in Arbor Acres strain ($r=-0.18$, $p=0.33$), no significant correlation was also showed between plasma free thyroxine and plasma triglyceride in Isa 15 strain ($r=0.03$, $p=0.83$) and in Arbor Acres strain ($r=-0.02$, $p=0.89$). The plasma concentrations of free thyroxine had no significant correlation with plasma glucose in Isa 15 strain ($r=0.14$, $p=0.46$) and in Arbor Acres strain ($r=-0.08$, $p=0.64$).

We noted a significant correlation between plasma free thyroxine and plasma total protein in Arbor Acres strain ($r=0.42$, $p=0.01$), no significant correlation was showed for Isa 15 strain ($r=-0.22$, $p=0.24$).

In this investigation, increasing age results in a significant decrease ($p<0.05$) in plasma free thyroxine between 28 days and 35 days in Isa 15 strain and a significant increase ($p<0.05$) in plasma free thyroxine was observed between 28 days and 42 days in Arbor Acres strain.

We also noted a significant decrease ($p<0.05$) in plasma glucose and plasma cholesterol between 28 days and 42 days in the two strains studied and a significant decrease ($p<0.05$) in plasma triglycerides between 28 days and 42 days was described only for Arbor Acres strain.

Plasma total protein levels demonstrated an increasing significant tendency ($p<0.05$) between 28 days and 35 days and between 35 days and 42 days in Arbor Acres strain.

Table 3: Body weights (mean±SEM) in Isa 15 strain and Arbor Acres strain (g).

	28days	35days	42days
Isa 15 strain	1211.10^{a} ±42.64	1693.40^{b} ±21.39	2128.50^{c} ±34.40
Arbor Acres strain	1408.50^{a}±25.68	1984.70^{b}±36.32	2625.10^{c} ±58.06

Table 4: Concentrations (mean±SEM) of plasma glucose, cholesterol, triglycerides and free thyroxin in Isa 15 strain and Arbor Acres strain.

			Parameter				
Strain	Age	n	Glucose (g/L)	Cholesterol (g/L)	Triglycerides (g/l)	Total protein (g/L)	Free thyroxin (Pmol/L)
Isa 15	28	10	2.32^{a}±0.13	1.00^{a}±0.16	0.67^{a}±0.17	28.50^{a}±2.35	20.76^{a}±3.19
	35	10	$2.19^{a,c}$±0.13	$0.90^{a,c}$±0.09	$0.70^{a,c}$±0.37	29.96^{a}±1.99	16.40^{b}±2.83
	42	10	1.12^{b}±0.59	0.41^{b}±0.22	0.43^{b}±0.14	29.42^{a}±1.04	$18.80^{a,b}$±2.17
Arbor	28	10	2.74^{a}±0.55	1.19^{a}±0.31	0.63^{a}±0.16	34.83^{a}±7.27	17.91^{a}±2.37
Acres	35	10	$1.72^{b,c}$±0.90	$0.78^{b,c}$±0.42	0.57^{a}±0.21	$27.28^{b,c}$±6.32	$17.02^{a,c}$±3.17
	42	10	1.31^{c}±0.56	0.65^{c}±0.39	0.57^{a}±0.39	36.34^{a}±5.77	21.09^{b}±1.44

^{a,b,c,} means in the rows with different letters differ significantly $P<0.05$.

DISCUSSION

Obtained data is in accordance with technological norms for Arbor Acres and Isa 15 strains. Arbor Acres chickens are heavy broilers selected on fast growth [23, 24] and Isa 15 chickens are lighter-weight fast-growing broilers (Table 3).

Selection for diverse productive parameters has also induced endocrine changes, more particularly at the levels of thyroid hormones which are closely related to avian metabolism.

The rise of free thyroxine concentrations among the beginning and the end of the experiment was statistically significant for Arbor Acres strain. However, in Isa 15 strain, this increase was not statistically significant, free thyroxine levels reduce statistically between 28 and 35 days of age and then increase between 35 and 42 days of age (Table 4). The increase of free thyroxine in Arbor Acres strain between 28 and 42 days of age coincided with the period in which the most rapid relative growth is observed. At this stage of development, chickens begin to accumulate enormous amounts of muscle.

The increased T4 levels are in agreement with previous results which showed that T4 increased consistently with age in broilers and White Leghorns [25]. Stojevic *et al.* [26], have reported the age- dependent (3-6weeks) increase in the concentration of T3 and T4 hormones. Moravej *et al*. [27], have showed that in step with increase broilers age, mean concentrations of plasma T4 were increased in broilers fed on different energy and protein levels. Luger *et al*. [28], have concluded that plasma T4 concentrations continuously increase with age in healthy broilers.

Considering the metabolic role of the thyroid hormones in the organism, these results should be expected. The changes in relative growth rate and free thyroxine concentrations support the classical observations that thyroid hormones are necessary for growth. It is evident that thyroid hormones are involved in wide range of metabolic activities influencing the growth and development of birds. The thyroid hormones are primarily involved in energy production by increasing the metabolic rate in turn heat production. Their importance is most visible in deficient animals that exhibit stunted growth and lower productivity [2]. Any reduction in physiological levels of thyroid hormones impairs the growth and development of embryos [29].

It is generally thought that T4 is the predominant thyroid hormone in circulation, but it has little inherent biological activity, the more metabolically active thyroid hormone is T3 [30]. Hayashi *et al*. [31], have showed that T4 is active and plays important metabolic roles especially in protein metabolism in chicken skeletal muscle cells. It is plausible that T4 plays major roles in the regulation of heat production and skeletal muscle protein metabolism in animals. Because normal plasma levels of T4 is about 6 times of that of T3, T4 is then more stable in the plasma than T3.

But free thyroxine is a better marker than total thyroxine for thyroid function and is more independent from the transport capacity of plasma proteins. As T4 is the principal hormone synthesized by thyroid gland, impairment in thyroid function would directly and early lead to fall serum free T4 concentrations. Because the fixation of T4 to proteins slowed down the liver hormone catabolism, bound T4 persisted in plasma for a long time and serum T4 concentrations would be less responsive to variations in thyroid biosynthesis [32]. This was the reason for monitoring free thyroxine hormone concentrations.

Thyroid hormones are involved in the regulation of the basal metabolism of the majority of tissues and consequently in the metabolism of lipids, carbohydrates and proteins [33]. In this investigation, there was no significant correlation between plasma free thyroxine, cholesterol and triglyceride in the two strains of broiler chickens.

Changes in plasma cholesterol showed a significant decrease ($p<0.05$) with age in the two groups, also plasma levels of triglyceride demonstrated a decreased tendency with age, that was significant ($p<0.05$) only for Isa 15 strain. Age- related changes, with a tendency of blood cholesterol and triglyceride to decrease significantly during the fattening period were also observed in chickens by Piotrowska *et al*. [34] and Peebles *et al*. [35].

A general correlation between blood levels of thyroid hormones (T3, T4, fT4) and lipid metabolism in general is well established in humans [36]. In domestic animals, studies on blood lipid profile showed the existence of variations between species and even within species [5]. There are contradictory findings regarding the relation between blood thyroid hormones, cholesterol and triglycerides. The blood cholesterol level generally varies inversly with thyroid activity [37]. In contrast, the concentrations of thyroid hormones were not correlated with cholesterol levels in camels [38], in goats [39] and in sheeps [5,40].

Thyroid hormones action has long been recognized as an important determinant of glucose homeostasis [41]. They stimulate all aspects of carbohydrate metabolism,

including enhancement of insulin-dependent entry of glucose into cells and increased gluconeogenesis and glycogenolysis to generate free glucose[42]..

According to our results, there was no significant correlation between plasma free thyroxine and plasma glucose levels in the two strains. No significant correlation was also reported in calves [43]. Changes in plasma glucose showed a significant decrease ($p<0.05$) with age in the two groups. Our findings are in agreement with previous studies on birds which also revealed an age-dependent gradual decline in plasma glucose concentrations [44].

There was a significant increase ($p<0.05$) in plasma concentrations of total protein between 28 days and 35 days and between 35 days and 42 days in Arbor Acres strain. Among numerous factors that can influence the level of plasma protein in broilers, age of the birds seems to be one of the most important factor, higher values are generally found in adult birds compared to young birds [45-50]. A significant difference was observed between Isa 15 strain and Arbor Acres strain for plasma total protein and a significant correlation between plasma free thyroxine and plasma total protein was observed in Arbor Acres strain. It has been demonstrated that in chickens, both T3 and T4 selectively stimulate the synthesis of proteins [51]. Eshkhatkhah *et al*. [43], have reported that the relation between plasma thyroid hormones and protein metabolism is dose dependent: when given in small and physiological doses, they favor protein metabolism, while in larger doses they cause protein catabolism.

It can be concluded that the increased secretion of thyroxine during this period of intensive meat production may be related to an increased metabolic rate especially for Arbor Acres strain. In broiler chickens, the plasma lipid profile and glucose profile were not related to plasma free thyroxine levels in the two strains studied. However, in broilers Acres Acres strain, we observed a significant correlation between the levels of free thyroxine and total protein. The study suggested conducting more investigations on the dynamics of free thyroid hormone concentration changes in blood plasma and their correlations, in view of their involvement in the growth and their great importance in poultry production.

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REFERENCES

1. Mc Nabb, F., 2000. Thyroids. In sturkie's avian physiology (fifth edition) pp: 461- 471.
2. Biswas, A., J. Mohan and K. Sastry, 2010. Age-dependant variation in hormonal concentration and biochemical constituents in blood plasma of Indian native fowl. Veterinary Medicine International. Vol 2010, article ID 737292, 5pages.
3. Thrall, M.A., G. Veisser, R. Allison and W. Campbell, 2012. Clinical chemistry of common domestic species. In: Veterinary Haematology and Clinical Chemistry.
4. Bobiniene, R., D. Gudavièiute and M. Miškiniene, 2010. The impact of iodine on biochemecal blood parameters in laying hens. Veterinaria Zootechnika. Vet. Med. Zoot. T., 51: 73.
5. Eshkhatkhah, B., M. Sadaghian, S. Eshkhatkhah, S. Pourabi and K. Najafian, 2010. Relationship between the blood thyroid hormones and lipid profile in Moghani sheep, influence of age and sex. Comp. Clin. Path., 19: 15-20.
6. Pucci, E., L. Chiovato. and A. Pinchera, 2000. Thyroid and lipid metabolism. Inter. J. Obes. Rel. Met. Dis., 24(2): 109-112.
7. Todini, L., 2007. Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. Animal., 1(7): 997-1008.
8. Gonzales, E., J. Buyse, J. Sartori, M. Loddi and E. Decuypere, 1999. Metabolic disturbances in male broilers of differents strains. 2. Relationship between the thyroid and somatotropic axes with growth rate and mortality. Poultry Science. 78: 516- 521.
9. Bobeck, S., M. Jastrzebski and M. Pietras, 1977. Age-related changes in oxygen consumption and plasma thyroid hormone concentrations in the young chickens. Gen. Comp. Endocrinology. 31: 169- 174.
10. Lauterio, T. and C. Scanes, 1987. Hormonal responses to protein restriction in two strains of chickens with different growth characteristics. J. Nutr., 117: 758- 763.
11. Swennen, Q., G. Janssens, S. Millet, G. Vansant, E. Decuypere and J. Buyse, 2005. Effects of substitution between fat and protein on feed intake and its regulatory mechanisms in broiler chickens: Endocrine functionning and intermediary metabolism. Poultry Science. 84: 1051- 1057.
12. De Beer, M., J. Mc Murty, D. Brocht and C. Coon, 2008. An examination of the role of feeding regimens in regulating metabolism during the broiler breeder groxer period. 2. Plasma hormones and metabolites. Poultry Science. 87: 264- 275.

13. Proudman, J. and T. Siopest, 2005. Thyroid hormone and prolactin profiles in male and female turkeys following photostimulation. Poultry Science. 84: 942- 946.
14. Burger, M. and R. Denver, 2002. Plasma thyroid hormone concentrations in a wintering passerine bird: their relationship to geographic variation, environmental factors, metabolic rate and body fat. Physiological and Biochemical Zoology. 75(2): 187- 199.
15. Tao, X., Z. Zhang, H. Dong and H. Zang and H. Xint, 2006. Responses of thyroid hormones of market- size broilers to thermoneutral constant and warm cyclic temperatures. Poultry Science. 85: 1520- 1528.
16. Lin, H., E. Decuypere and J. Buyse, 2008. Effect of thyroid hormones on the redox balance of broiler chickens. Asian- Australian Journal of Animal Science. 21(6): 794- 800.
17. Fossati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-2080.
18. Mc Gowan, M.W., J.D. Artiss and D.R. Strandberg, 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., 29: 538-542.
19. Allain, C.C., L.S. Poon and C.S. Chan, 1974. Enzymatic determination of total serum cholesterol. Clin. Chem., 20(4): 470-475.
20. Roeschlau, P., E. Bernt and W.A. Gruber, 1974. Enzymatic determination of total cholesterol in serum. Z. Klin. Chem. Klin. Biochem. 12: 226.
21. Slein, M.W., 1963. Methods of Enzymatic Analysis, (Bergmeyer, H.U. ed.) New York, Academic Press.
22. Andreasen, C., K. Latiner, I. Kircher and J. Brown, 1989. Determination of chicken and turkey plasma and serum protein concentrations by refractometry and the biuret method. Avian. Disease. 33: 93-96.
23. Uni, Z., Y. Noy and D. Sklan, 1996. Development of the small intestine in heavy and light strain chicks before and after hatching. Br. Poultry Science. 37(1): 63-71.
24. Sterling, K., G. Pesti and R. Bakalli, 2006. Performance of different broiler genotypes fed diets with varying levels of dietary crude protein and lysine. Poultry Science, 85: 1045- 1054.
25. Lu, J., J. Mc Murty and C. Coon, 2007. Developmental changes of plasma insulin, glucagon, insuline-like growth factors, thyroid hormones and glucose concentrations in chick embryos and hatched chicks. Poultry Science. 86: 673- 683.
26. Stojevic, Z., S. Milinkovic-Tur and K. Curcija, 2000. Changes in thyroid hormones concentrations in chickens' blood plasma during fattening. Veterinarski Arhiv., 70(1): 31- 37.
27. Moravej, H., H. Khazali, M. Shivazad and H. Mehrabani-Yeganeh, 2006. Plasma concentrations of thyroid hormones and growth hormone in Lohmann male broilers fed on different dietary energy and protein levels. International Journal of Poultry Science, 5(5): 457-462.
28. Luger, D., D. Shinder, V. Rzepakovsky, M. Rusal and S. Yahav, 2001. Association between weight gain, blood parameters and thyroid hormones and the development of ascites syndrome in broiler chickens. Poultry Science. 80: 965- 971.
29. Gregory, C., C. Dean and T. Porter, 1998. Expression of chicken thyroid stimulating hormone â subunit messenger ribonucleic acid during embryonic and neonatal development. Endocrinology, 139(2): 474-478.
30. Navidshad, B., M. Shivarzad, A. Zareshahneh and G. Rahimi, 2006. Effects of feed restriction and dieatry fat saturation on performance and serum thyroid hormones of broiler chickens. Int. J. of Poultry Science, 5(5): 436- 440.
31. Hayashi, K., H. Kuroki, T. Kamizono and A. Ohtsuka, 2009. Comparison of the effects of thyroxine and triiodothyronine on heat production and skeletal muscle protein breakdown in chicken. J. Poultry Science. 46: 212- 216.
32. Kurtdede, A., R. Asti, T. Sel, N. Kurtdede, H. Karagul, O. Otalay and M. Guzel, 2004. Effect of anti-inflammatory and immunosuppressives doses of prednisolone on serum triiodothyronine, thyroxine and free thyroxine concentrations and thyroxine morphology in the dog. Revue de Méd. Vét., 155(6): 324-330.
33. Dias, I.R., C.A. Viegas, A.M. Silva, H.F. Pereira, C.P. Sousa, P.P. Carvalho, A.S. Cabrita, P.J. Fontes, S.R. Silva and J.M.T. Azevedo, 2010. Haematological and biochemical parameters in churra-da-terra-quente ewes from the northeast of Portugal. Arq, Bras, Med. Vet. Zoot., 62(2) Belo Horizonte.
34. Piotrowska, A., K. Burlikowska and R. Szymeczko, 2011. Changes in blood chemistry in broiler chickens during the fattening period. Folia biologica (Krakow), 59(3-4): 183-187.
35. Peebles, E.D., J.D. Cheaney, J.D. Brake, C.R. Boyle and M.A. Latour, 1997a. Effects of added dietary lard on body weight and serum glucose and low density lipoprotein cholesterol in random bred broiler chickens. Poultry Science, 76: 29-36.

36. Maugeri, D., A. Santangelo, P. Parbagallo, M.R. Bonanno, M. Malaguarnera, I. Irizza, S. Speciale, M. Tomarchio, M.P. Curasi and P. Panebianco, 1999. Thyroid hormones and lipid metabolism in a group of patients over seventy. European review for medical and pharmacological Sciences. 3: 211-216.
37. Bartley, J.C., 1989. Lipid metabolism and its disease. In: Clinical biochelistry of domestic animals. 4th ed, edJ.J.Janeko. Academic press. Inc, New York. USA, pp: 106-141.
38. Wasfi, I.A., A.M.Hafez, F.M.A. El-Tayeb and A.Y. El-Taher, 1987. Thyroid hormones, cholesterol and triglycerides levels in the camel. Res. Vet. Sci., 42: 418.
39. Nazifi, S., H.R. Gheisari and F. Shaker, 2002. Serum lipids and lipoproteins and their correlations with thyroid hormones in clinically healthy goats. Veterinarski Arhiv., 72(5): 249-257.
40. Nazifi, S., M. Saeb, E. Rowghani, M. Hassankhani, F. Hassashahi and N. Ghafari, 2007. Studies on the physiological relationship between throid hormones, serum lipid profile and erythrocyte antioxidant enzymes in clinically healthy Iranian fat-tailed sheep. Bulgarian Journal of Vet. Med., 10(3): 161-167.
41. Farasat, T., A.M. Cheema and M.N. Khan, 2011. Relationship of thyroid hormones with serum fasting insulin and insulin resistance in euthyroid glycemic anomalies. Pak J. Zool., 43(2): 379-386.
42. Bowen, R., L. Austgen and M. Rouge, 2006. Mechanism of action and physiologic effects of thyroid hormones. In: Pathophysiology of the Endocrine System.
43. Eshkhatkhah, B., M.R. Nahand, S.P. Rasoul, S.M.R. Taj and H.J. Rad, 2012. Relationship between plasma thyroid hormones and some biochemical parameters in Iranian Sarabi calves. Comp. Clin Path., 21(2): 167-171.
44. Hernandez, M. and A. Margalida, 2010. Haematology and blood chemistry reference values and age-related changes in wild Bearded Vultures (Gy-paetus Barbatus). Journal of Wildelife Disease. 46(2): 390-340.
45. Rodgers, J.D. and G.H. Gass, 1983. The effect of age on serum protein in mice. Exp. Gerontol., 18: 39- 45.
46. Schmidt, E., A. Paulillo, R. Locatelli-Dittrich, E. Santin, P. Da Silva, O. Beltrame and E. De Oliveira, 2007. The effect of age on hematological and serum biochemical values on juvenile ring-necked pheasants (phasianus colchicus). Int. Jour of Poultry Science, 6(6): 459- 461.
47. Silva, P.R.L., O.C. Freitas Neto, A.C. Laurentiz, O.M. Junqueira and J.J. Fagliari, 2007. Blood serum components and serum protein test of hybro- PG broilers of different ages. Brazilian Journal of Poultry Science. 9: 229- 232.
48. Szabo, A., M. Mezes, P. Horn, Z. Suto, G.Y. Bazar and R. Romvari, 2005. Developmental dynamics of some blood biochemical parameters in the growing turkey (Meleagris Gallopavo). Acta Veterinaria Hungarica, 53(4): 397- 409.
49. Rajman, M., M. Jurani, D. Lamosova, M. Macajova, M. Sedlackova, L. Kostal, D. Jesova and P. Vyboh, 2006. The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comp. Bio-chem. Physiol. A., 145: 363-371.
50. Filipovic, N., S. Stojevic, S. Milinkovic-Tur, B. Ljubic and M. Zdelar-Tuk, 2007. Changes in concentration and fractions of blood serum proteins of chickens during fattening. Veterinarski arhiv. 77(4): 319-326.
51. Roman, Y., C. Bomsel-Demontoy, J. Levrier, D. Ordonneau, D.C. Duvernoy and M. Saint Jalme, 2009. Influence of molt on plasma protein electrophoretic patterns in Bar-headed geese (Anser Indicus). J. Wildlife Disease. 45(3): 661-667.