

## Determination of Plasma Thyroid Hormones by Chemiluminescence and Radioimmunoassay Methods in Calves

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**Abstract:** The various methods for determination of the plasma thyroid hormones concentrations have been studied in some species and selection of a proper method is necessary for accurate determination of these hormone levels. The aim of this study was to evaluate the plasma thyroid hormones concentrations by both chemiluminescence (CLIA) and Radioimmunoassay (RIA) methods in calves. Blood samples were collected from the jugular vein of 30 clinically healthy animals, aged 1-6 months and of different sexes during summer. The plasma was analyzed to determine thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ) concentrations. According to the results, there was a significant difference in the  $T_4$  ( $P < 0.0001$ ) and  $T_3$  ( $P < 0.05$ ) concentrations between two methods and that levels of these hormones were higher in RIA method. The linear regression analysis of both hormones showed that the RIA and CLIA results was significantly correlated ( $T_4$ :  $P < 0.01$ ,  $r = 0.677$ ;  $T_3$ :  $P < 0.05$ ,  $r = 0.538$ ). Additionally, we observed a significant difference between two sexes concerning the thyroid hormones in both methods, except to the  $T_3$  when using the CLIA method. In conclusion, determination of the  $T_4$  and  $T_3$  concentrations using the RIA method had an acceptable limit of sensitivity and precision; also it was more appropriate than the CLIA method for research application in calves.

**Key words:** Thyroid hormones • Chemiluminescence immunoassay • Radioimmunoassay • Plasma • Calve

### INTRODUCTION

With progress of technology, new methods and devices are created to determinations of plasma hormones; but most of these progresses are focused on the determination of plasma parameters in human. These new methods have some advantages and disadvantages compare with the old methods; but these effects are not cleared in domestic animals. There are various methods to determine of plasma thyroid hormones concentrations such as RIA [1], ELISA [2], chromatography [3], chemiluminescence immunoassay [4-7], electrochemiluminescence immunoassay [7, 8], HPLC [9], gas-liquid chromatography [10] and chromatography on polyacrylamide gel [3], different methods of fluorometry [11, 12], ultra filtration [13] and bioluminescent immunoassay [14] and equilibrium dialysis [15]. The RIA method is used commonly for

determination of the plasma thyroid hormones in most veterinary medicine [1, 16-21]. This method have some disadvantages (such as radioactive waste, expensive instrumentation and harmful to the operators in a long time) compare with the other new method [2], so new and safe methods such as CLIA and electrochemiluminescence (ECLIA) methods are replaced it now and they are used routinely in medical diagnostic laboratories. It was demonstrated that the CLIA method is relatively simple, inexpensive instrumentation and has wide dynamic range, high sensitivity and specificity in medical diagnostic tests. But, some of these advantages are not proved in veterinary diagnostic laboratories and there is no previous information about it. The CLIA method was used to measurement of plasma thyroid hormones concentrations in some species of ruminant [22, 23].

The aim of this study was to compare RIA and CLIA methods in determination of plasma thyroid hormones in calves.

## MATERIALS AND METHODS

This study was carried out on blood samples taken from thirty clinically healthy calves. These animals were, free from internal and external parasites, aged 1 - 6 months and two sexes at the Improvement Center of Sarabi cow, located in East Azarbaijan province, Iran. All the blood samples were collected before feeding by jugular venepuncture into vacutainers containing heparin as an anticoagulant in summer and the mean ambient temperature was 34°C. Plasma was separated by centrifugation at  $750 \times g$  for 15 min and stored at -20°C until used. The levels of plasma  $T_4$  and  $T_3$  were measured by DiaSorin CLIA kits (Strada per Crescentino-13040 Saluggia (Vercelli) - Italy) with the LIAISON analyzer and BIOSOURCE RIA kits (BioSource Europe SA 8, Rue de l'Industrie \_ 1400 - Nivelles \_ Belgium) with the Kontron analyzer (Kontron Co. Sweden). 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 independent sample *t* - test and Pearson's correlation method; also, the linear regression analysis and its curves were performed using SPSS/ver.17 software. All values are shown as mean  $\pm$  standard deviation (SD).

## RESULTS

The  $T_4$  and  $T_3$  concentrations of all the plasma samples from the calves and their concentrations according to sex are presented in Tables 1 and 2, respectively. A significant difference was found between two sexes concerning the thyroid hormones concentrations in both methods ( $P < 0.05$ ), except for the  $T_3$  when using the CLIA method. Additionally, the levels of these hormones are higher in female than male animals when used the both mentioned methods.

The linear regression analysis curve of the measured thyroid hormones concentrations by the RIA and CLIA methods are shown in Figs. 1 and 2.

The intra-assay coefficients of variations results for  $T_4$  and  $T_3$  were 4.6 and 8.8 % with the CLIA method; 4.2 and 8.1% with the RIA method, respectively. The inter-assay coefficients of variations results for  $T_4$  and  $T_3$  were 5.8 and 10.1 % with the CLIA method and 3.5 and 8.4% with the RIA method, respectively.

Table 1: The plasma concentrations of thyroid hormones of calves using RIA and CLIA methods.

Parameters	Group	Sex	N	Mean $\pm$ SD	P-value
$T_4$ (nmol/l)	RIA	Male	14	53.61 $\pm$ 19 <sup>b</sup>	<0.05
		Female	16	77.06 $\pm$ 13.4 <sup>a</sup>	
	CLIA	Male	14	21.79 $\pm$ 8.7 <sup>b</sup>	<0.05
		Female	16	37.9 $\pm$ 12.7 <sup>a</sup>	
$T_3$ (nmol/l)	RIA	Male	14	0.95 $\pm$ 0.69 <sup>b</sup>	<0.01
		Female	16	1.74 $\pm$ 0.54 <sup>a</sup>	
	CLIA	Male	14	0.87 $\pm$ 0.38	>0.1
		Female	16	1.15 $\pm$ 0.30	

There was a significant difference between groups with different letters (a, b) in each parameters.

Table 2: The plasma concentrations of thyroid hormones in different sexes of calves using RIA and CLIA methods.

Parameters	Group	N	Mean $\pm$ SD	P-value
$T_4$ (nmol/l)	RIA	30	67.40 $\pm$ 19.46 <sup>a</sup>	<0.0001
	CLIA	30	31.24 $\pm$ 13.65 <sup>b</sup>	
$T_3$ (nmol/l)	RIA	30	1.41 $\pm$ 0.74 <sup>a</sup>	<0.05
	CLIA	30	1.00 $\pm$ 0.38 <sup>b</sup>	

There was a significant difference between sexes within group with different letters (a, b) in each parameters.

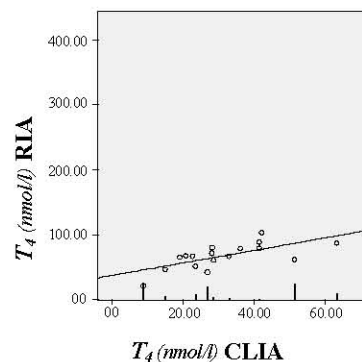


Fig. 1: Comparison of plasma  $T_4$  concentration of calves determined by the RIA and CLIA methods expressed by linear regression ( $y = 37.25 + 0.966x$ ,  $r = 0.677$ ,  $P < 0.01$ ).

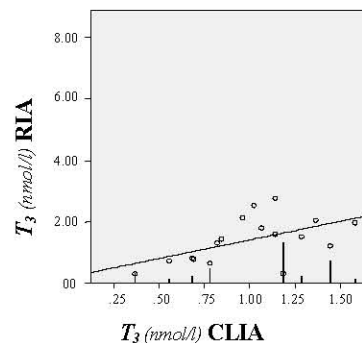


Fig. 2: Comparison of plasma  $T_3$  concentration of calves determined by the RIA and CLIA methods expressed by linear regression ( $y = 0.659 + 0.239x$ ,  $r = 0.538$ ,  $P < 0.05$ ).

## DISCUSSION

The thyroid hormones affect both development and functions of most tissue in animals [23, 24] and determination of these hormones is very important in the diagnosis of thyroid disorders. Many factors such as endogenous and exogenous factors effects on the plasma thyroid hormones concentrations in domestic animals [16, 17, 22-27]. The determination of these hormones concentrations using the routine methods has significant effects on their concentrations in some species [2, 7]. In this study, a significant difference was found between the RIA and CLIA methods concerning the  $T_4$  and  $T_3$  concentrations and their levels were lower using the CLIA method. This result is consistent with the other report in sheep [7]. The levels of  $T_4$  and  $T_3$  using the RIA method had a little difference with other report in calves [28] and was higher than the values reported in cattle [29].

It seems that the levels of plasma thyroid hormones are lower in CLIA than the other routine methods such as RIA and ECLIA in ruminant. The data from this study indicate that, there was a significant difference between two sexes in both methods, except for the  $T_3$  concentration when using the CLIA method. Also, the levels of these hormones were higher in female than male animals. This result is consistent with other reports in sheep and Sarabi calves [7, 22]. The comparison of these two methods revealed that the methods used to measuring plasma thyroid hormones concentrations had a significant impact on the values obtained.

It was concluded that the CLIA method is not proper method to determine of the plasma thyroid hormones concentrations in ruminant, especially in calves.

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