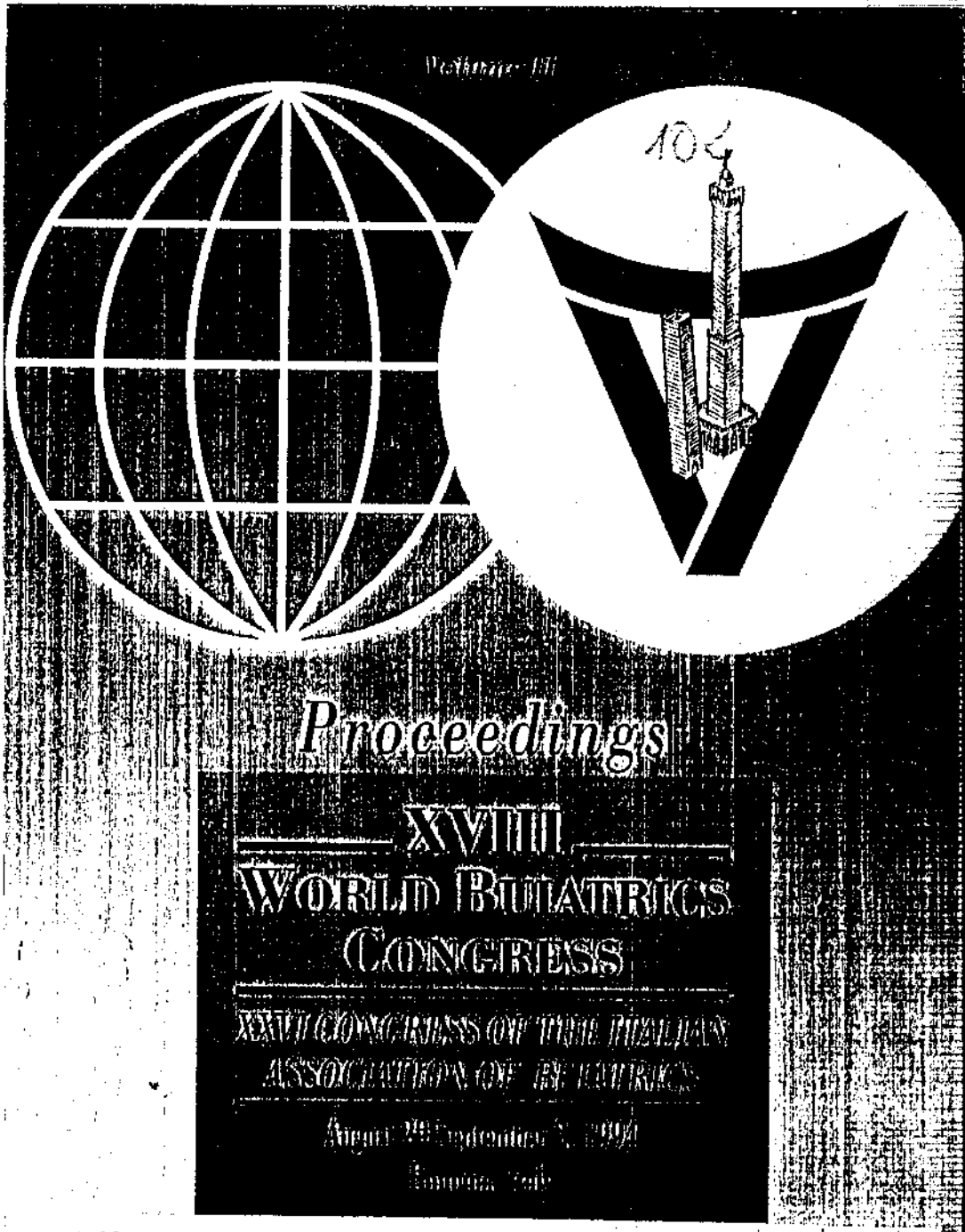

CIRCADIAN VARIATIONS OF THYROID HORMONE CONCENTRATIONS IN LACTATING AND DRY BROWN SWISS COWS.

Della Croce G.(1), Martino E.(2), Nannipieri S.(1), Bartalena L.(2), Grasso L.(2), Bagliacca M.(3), Valentini A.(4), Bogazzi A.(5), Biagi G.(1)

(1) Istituto Clinica Medica Veterinaria, Università di Pisa (ITALY)
(2) Istituto Endocrinologia, Università di Pisa (ITALY)
(3) Dipartimento Produzioni Animali, Università di Pisa (ITALY)
(4) Istituto Zootecnia, Università della Tuscia-Viterbo (ITALY)
(5) Libero Professionista

INTRODUCTION

The presence of circadian variations of thyroid hormone concentrations in the cow has not been fully determined yet and controversial results have been obtained (1, 5). This rhythm seems to be influenced by different physiological conditions, such as lactation (7) and pregnancy (3, 4, 6, 7), but no direct comparison of lactating and dry cows has been carried out.

The purpose of the present study was to ascertain whether concentration of thyroid hormones in lactating and dry Brown Swiss cows undergoes circadian variations and, in this case, to characterise it.

MATERIALS AND METHODS

Animals. Twelve Brown Swiss cows (6 lactating and 6 dry) were included in this study. The animals, purchased at ages ranging from 4 to 9 years, had no clinical problems.

Table 1 - Nutrition plan.

	Lactating	Dry
Hay		15-20 Kg
Soy bean meal	0.92 Kg	
Bran	2.15 Kg	
Corn gluten	2 Kg	
Corn germ	1.07 Kg	
Corn	1.53 Kg	
Grass silage	4.92 Kg	
Fresh beet pulps	8 Kg	
Wheat straw	1.7 Kg	
Dried alfalfa	5.7 Kg	
Stillage	0.6 Kg	
Vit./Min. integrator	0.030 Kg	
TOTAL	28.62 Kg	15-20 Kg

The animals were kept in fixed stables. The cows were fed according to the plan in Table 1.

The lactating cows were milked twice daily (at 6:00 a.m. and 6:00 p.m.); and every cow produced about 20 litters of milk daily.

Blood samples were collected from all cows by puncture of the jugular vein at hourly intervals for 24 hours and the first blood sample being collected at 7:00 a.m.

Assays. Sera were frozen until analysed. The assay was carried out in duplicate. All samples belonging to the same animal were run in the same assay to avoid inter assay variations. The inter assay coefficient of variations was < 5%. Serum thyroid hormone concen-trations were measured by commercial RIA kits (Total T4 and T3: Byk Gulden Italia; Free T4 and T3: Kodak Diagnostici S.p.A.).

To study the circadian rhythms the data have been analysed by least squares means using the following non-linear model: Hormone = $a + b * \sin(2\pi / 24 * \text{hour}) + c * \text{hour}$.

To study the eventual differences between lactating and dry cows the data have been analysed by a multivariate general linear model: Hormone = constant + status, where status is the physiological moment (lactating or dry) of cows.

RESULTS

The results of the statistical analysis are reported in Tables 2-4.

In Table 2 the least square estimated means of the thyroid hormone concentrations are reported at hourly intervals during the 24 hours according to the different physiological moment. Significant differences were found in total T_4 and T_3 concentrations in lactating and dry cows since the former had higher serum total T_4 and T_3, however serum free T_4 and T_3 levels, which more accurately define thyroid status (2), were similar, indicating that the two groups of animals did not differ as to their metabolic status.

Table 2 - Estimated means and standard deviations of thyroid hormone concentrations (small different letters in the same row indicate significant differences: $p < 0.05$; capital different letters: $p < 0.01$).

	TT4 (mg/dl)		TT3 (ng/dl)		FT4 (pg/ml)		FT3 (pg/ml)	
	Lactating n = 6	Dry n = 6	Lactating n = 6	Dry n = 6	Lactating n = 6	Dry n = 6	Lactating n = 6	Dry n = 6
7:00 a.m.	3.8±0.95	2.4±1.34	125±14.0	83± 65.9	12.2±1.43	11.9±1.63	3.9±.67	3.5±0.67
8:00 a.m.	4.4±1.35	3.1±0.83	130±35.1	80± 84.0	11.7±1.69	12.8±0.64	3.9±.62	3.3±0.71
9:00 a.m.	4.3±1.64	3.2±0.75	157±23.0	116±102.9	12.1±1.45	13.0±1.19	3.9±.64	3.6±0.43
10:00 a.m.	4.4±1.87	3.7±1.20	190±35.6a	155±115.0b	12.1±1.49	13.6±2.55	4.8±.88	3.9±0.79
11:00 a.m.	4.4±1.35	3.6±1.30	172±17.9	161±111.5	12.9±1.89	15.2±2.27	4.6±.81	4.1±0.98
12:00a.m.	5.2±1.15a	3.5±1.00b	190±37.8	122± 82.4	13.8±1.24	15.0±1.55	4.8±.84	3.9±0.70
1:00 p.m.	5.8±2.62a	3.4±0.70b	185±28.4A	91± 55.4B	13.7±2.09	14.5±1.91	4.7±.78	3.8±0.89
2:00 p.m.	4.5±1.54	3.5±0.64	179±13.0A	104± 57.7B	15.1±2.41	14.7±1.65	4.7±.66	3.9±0.74
3:00 p.m.	5.1±1.31	4.2±1.29	181±19.8A	99± 56.2B	15.6±2.38	14.3±2.12	4.7±.75	3.9±0.86
4:00 p.m.	5.1±1.37	4.0±1.34	181±16.2A	101± 53.5B	15.1±1.93	15.4±1.22	4.7±.75	4.0±0.79
5:00 p.m.	5.5±1.69	3.9±1.10	189±22.4A	85± 53.9B	15.8±1.37	15.7±1.61	5.0±.82	3.9±0.93
6:00 p.m.	5.9±1.81a	3.7±1.11b	176±18.9A	83± 44.5B	15.1±1.83	15.0±3.12	4.6±.88	3.8±1.03
7:00 p.m.	5.3±0.90	3.9±1.51	220±50.0A	85± 68.5B	14.2±2.16	15.7±2.67	4.7±.87	3.8±0.55
8:00 p.m.	6.0±1.44A	3.3±1.04B	201±36.8A	97± 66.9B	14.9±1.49	15.3±1.65	4.7±.85	4.0±0.86
9:00 p.m.	5.7±1.24a	3.9±1.39b	216±30.0a	103± 94.6b	15.0±1.53	15.5±2.04	4.8±.87a	3.7±0.99b
10:00 p.m.	5.6±0.97a	3.9±1.16b	219±31.1A	97± 48.1B	15.5±2.54	15.6±1.79	4.8±.89a	3.8±0.63b
11:00 p.m.	6.3±1.98a	3.7±1.09b	193±38.4A	87± 40.7B	17.1±3.93	15.7±2.37	4.7±.57a	3.9±0.70b
12:00 p.m.	6.0±1.35A	3.6±0.82B	215±32.6A	87± 35.5B	15.2±2.58	15.1±1.51	4.7±.53a	3.6±0.80b
1:00 a.m.	5.5±1.65	3.9±1.24	216±13.7A	100± 68.6B	15.3±2.43	15.7±1.53	4.6±.86	4.2±1.34
2:00 a.m.	5.5±1.45a	3.6±0.94b	212±23.5A	91± 51.2B	14.9±2.33	15.4±3.97	4.8±.88	3.7±0.89
3:00 a.m.	6.0±2.51a	3.3±1.26b	188±14.0A	82± 57.2B	14.5±1.58	14.0±2.80	4.3±.80	3.7±0.82
4:00 a.m.	5.1±0.84a	3.7±1.21b	185±20,2A	64± 32.4B	14.5±1.97	14.3±1.92	4.3±.56a	3.5±0.53b
5:00 a.m.	5.8±2.14	3.9±1.40	186±47.5A	71± 38.2B	15.2±2.08	14.2±1.93	4.2±.69	3.6±0.77
6:00 a.m.	5.1±1.21a	3.5±1.05b	182±40.6A	71± 29.2B	14.6±2.07	13.8±2.23	4.1±.65	3.7±1.16

It might therefore postulated that the above differences in serum total thyroid hormone concentrations be ascribed to differences in thyroid hormone transport protein level, and in particular to an increase in thyroid hormone binding capacity in lactating cows. As to circadian variations, although, as illustrated in Table 2, some differences in serum total T_4 and T_3 concentrations, were found sometimes during the day, no clear-cut circadian changes could be found.

Figure 1 - Estimated means and standard deviations of total T_4 and T_3 concentrations.

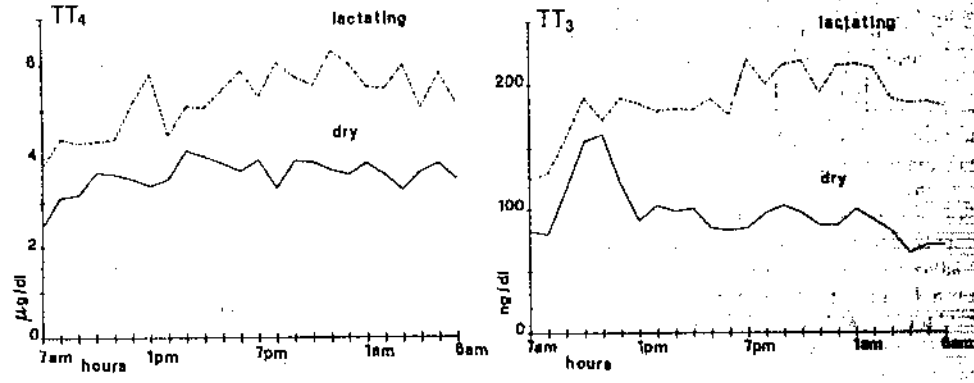

Table 2 - R-squared values (* = p<0.01).

	TT4	TT3	FT4	FT3
Lactating	0.993*	0.990*	0.995*	0.996*
Dry	0.992*	0.964*	0.996*	0.998*
Total	0.995*	0.986*	0.996*	0.997*

The non-linear model used explained most of the variance of the samples as the R^2 values were all statistically significant ($p < 0.01$) (Table 3). In Table 4 the estimated values of a, b and c parameters for total T_4 and T_3, free T_4 and T_3 concentrations and the respective asymptotic standard errors (A.S.E.) are reported.

The A.S.E. values of the b parameters, are very high and we argue that there is not any significant circadian rhythm for all the hormones considered (Table 4). This finding might be due in part to the small number of animals, but it is worth noting that the lack of significant hormone circadian variation was even more evident when serum free T_4 and T_3, which more reliably reflect the metabolic status, were taken into consideration (Table 2).

Table 3 - Estimated values of a, b and c parameters of TT_4, TT_3, FT_4 and FT_3 concentrations and the respective asymptotic standard error (A.S.E.).

		Lactating		Dry		Total	
	Par.	Mean	A.S.E.	Mean	A.S.E.	Mean	A.S.E.
TT4	a	4.7	0.303	3.2	0.211	3.9	0.210
	b	-0.272	0.224	0.153	0.156	-0.059	0.155
	c	0.043	0.023	0.033	0.016	0.038	0.016
TT3	a	168.9	12.37	113.5	12.42	141.2	11.04
	b	-8.815	9.146	5.648	9.184	-1.584	8.160
	c	1.430	0.935	-1.369	0.938	0.030	0.834
FT4	a	13.06	0.659	14.18	0.612	13.62	0.581
	b	-0.235	0.487	-0.283	0.452	-0.259	0.430
	c	0.109	0.050	0.036	0.046	0.073	0.044
FT3	a	4.47	0.198	3.72	0.115	4.09	0.148
	b	-0.015	0.146	0.085	0.085	0.035	0.109
	c	.0.006	0.015	0.005	0.009	0.005	0.011

In lactating cows total T_4 and T_3 concentrations were lower in the morning and higher in the afternoon (Table 2). For what concerns the total T_3 concentrations, it can be noted from Table 2 that the values are highly variable both in dry and lactating cows. Mainly in dry Brown Swiss cows there is a high individual variability, particularly from 9:00 to 11:00 a.m., with standard deviation over 100.

CONCLUSIONS

These results of our study are in agreement with previous reports (1,3,4,5,6,7,8) and underscore the lack of significant differences in the circadian rhythm of thyroid hormone variations in two different conditions, lactation and pregnancy.

Serum total T_3 concentrations are lower in dry than lactating. This behaviour can be explained by the different diet supplied to the two groups (Table 1). On the other hand it must also be considered the different metabolic burden sustained by the lactating cows, which are at the same time pregnant and might, therefore, show a greater increase in thyroid hormone binding capacity. This twofold burden causes a considerable metabolic stress, which influences the endocrine system and especially the thyroid, which plays a fundamental role in the development and functional maintenance of the mammary epithelium. Therefore it can be supposed that at the time of the cow drying, also the thyroid functionality slows in anticipation of the further coming of the calving and following lactation.

REFERENCES

1 - Anderson R.R., Nixon D.A., Akasha M.A.: "Total and free thyroxine and triiodothyronine in blood serum of mammals" Comp. Biochem. Physiol., 1988, 89A, 401.

2 - Bartalena L., Robbins J.: "Variations in thyroid hormone transport proteins and their clinical implications" Thyroid, 1992, 2, 237.

3 - Bertoni G., Lombardelli R.: " Variazioni ematiche di alcuni ormoni al termine della gravidanza ed inizio della lattazione in bovine da latte" Atti SIBCA, 1985, 1, 213.

4 - Bitman J., Tao H., Akers R.M.: "Triiodothyronine and thyroxine during gestation in dairy cattle selected for high and low milk production" J. Dairy Sci., 1984, 67, 2614.

5 - Curtis R.J., Abrams J.T.: "Circadian rhythms in the concentration of thyroid hormone in the plasma of normal calves" Br. Vet. J., 1977, 133, 134.
6 - Ferlazzo A., Fazio E., D'Aura G., Panzera M.: "Variazioni diurne dei parametri della funziona-lità tiroidea nella bovina gravida" Acta Med. Vet., 1991, 37, 125.
7 - Ferlazzo A., D'Aura G., Panzera M.: "Funzionalità tiroidea nella bovina gravida" Atti SISVet., 1988, 42, 277.
8 - Refsal K.R.S., Hasnau J., Nachreiner R.F., Convey E.M.: "Diurnal variation in serum triiodothyronine and thyroxine in lactating dairy cows" J. Anim. Sci., 1980, 51, Abs. 517, 320.

SUMMARY
Blood sample were collected at hourly interval for period of 24 h from twelve Brown Swiss cows (6 lactating and 6 dry, at the last mounth of pregnany), at ages ranging from 4-9 years. The first blood sample was collected at 7:00 a.m. The total T_4,and T_3, free T_4 and T_3 concentrations were determined by commercial RIA kits. To study the circadian rythms the data have been analyzed by least squares means using the following non-linear model: Hormone = a + b * sin (2π / 24 * hour) + c * hour and to study the eventual differences between lactating and dry cows the data have been analyzed by a multivariate general linear model: Hormone = constant + status. Any cyclic daily rhythm was evidenced in the thyroid hormone concentrations; statistical significative differences were found between the total T_4 and T_3 and free T_3 levels in lactating and dry Broun Swiss cows. The higher concentrations were measured in lactating cows.

RIASSUNTO
Sono stati effettuati prelievi di sangue a 12 bovine da latte di razza Bruna Alpina (6 in lattazione e 6 in asciutta, ad un mese circa dal parto), di età compresa tra 4 e 9 anni. I campioni sono stati raccolti dalla jugulare ogni ora nell'arco dell'intera giornata a partire dalle ore 7:00 del mattino. Le concentrazioni della TT_4, della TT_3, della FT_4 e della FT_3 sono state determinate con il metodo radioimmunologico. I dati sono stati sottoposti ad analisi statistica mediante il metodo dei minimi quadrati impiegando un modello non lineare a periodo di 24 ore (Ormone = a + b * sen (2π / 24 * ora) + c * ora) per evidenziare l'eventuale ritmo circadiano, mentre per valutare le differenze dovute al momento fisiologico, lattazione ed asciutta, è stato usato il modello: Ormone = costante + status. Non è stato evidenziato alcun ritmo ciclico giornaliero per nessuno degli ormoni considerati, mentre sono state individuate differenze significative fra le concentrazioni ormonali delle bovine in lattazione e quelle in asciutta, con i valori più alti negli animali in lattazione.

RESUMEN
Se ha efectuado un muestreo de sangre en 12 vacas de raza Pardo Suiza (6 en lactancia y 6 en descanso, un mes antes del parto), entre 4 y 9 años de edad. Las muestras se obtuvieron de la vena yugular cada ora por todo el día a partir de las 7 de la mañana. Las concentraciónes de TT_4, de TT_3, de FT_4 y de FT_3, fueron determinadas através del método radioimmunológico. Los resultados fueron sometidos a análisis estadística con el método de los mínimos cuadrados, utilizando un modelo no linear con periodo de 24 horas (Hormona = a + b * sen (2π / 24 * hora) + c * hora) para evidenciar un eventual ritmo cíclico jornalero, mientras para evaluar las differencias debidas al momento fisiológico, lactancia o descanso, se ha utilizado el siguiente modelo: Hormona = constante + status. A través de estas metódicas no se ha revelado algún ritmo cíclico diario en ninguna de las hormonas consideradas, mientras se ha individuado diferencias significativas entre las concentraciones hormonales de las vacas en lactancia y en descanso, con valores más altos en los animales en lactación.

Report supported by MPI 40%
The Authors contributed in equal parts.