Circulating Thyroid Hormones and Indices of Energy and Lipid Metabolism in Normal and Hormonally Induced Oestrus Cows

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Abstract: In a field study, circulating thyroid hormones, their free forms and indices of energy and lipid metabolism were measured in blood samples of 16 dairy cows expressing detectable oestrus signs. The cows were divided into two equal groups according to their days in milk (DIM=53-90 and DIM=100-150). In each group, 4 cows expressed the oestrus signs normally and the others were induced by hormone injection. Serum thyroxin (T4), free thyroxin (fT4), triiodothyronine (T3), free triiodothyronine (fT3), glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglyceride (TAG), cholesterol, very low density lipoproteins (VLDL-cholesterol), low density lipoproteins (LDL-cholesterol) and high density lipoproteins (HDL-cholesterol) were investigated. Comparison of all oestrus cows (normal or induced) between DIM groups (n=8 each) revealed lower levels of T4 (P=0.027) and T3 (P=0.022), but higher concentrations of fT4 (P=0.031) and fT3 (P=0.006) in the cows with lower DIM. Higher concentrations of TAG and VLDL (P=0.021) and cholesterol (P=0.046) as well as a tendency (P=0.074) for lower levels of BHB were other remarkable findings in cows with lower DIM. In cows with DIM=53-90, the normal oestrus cows had higher levels of T3 (P=0.044) as well as tendencies (P=0.083) for higher T4 and lower fT4 compared with induced cows. In cows with DIM=100-150, however, no significant difference was observed between the normal and induced oestrus cows. In conclusion, the cows that express oestrus signs normally may have better metabolic and thyroid hormone conditions compared to those that express heat by hormone injection. With progress in DIM, however, such differences may become less evident.

Keywords: Thyroid hormones, Oestrus, Dairy cows, Metabolic status.

INTRODUCTION

In dairy cows, postpartum decrease in thyroid functions has been noticed among the coordinated changes in hormones and metabolites to cope with negative energy balance (NEB) that recovery from them is essential for returning to normal functions [1-4]. Thyroid hormones govern the control of metabolism in nearly all somatic tissues (5) and their abnormal levels may lead to reduced reproductive functions [6-8]. Although the duration of decreased concentrations of thyroid hormones may be as short as one month [9], it may take for a long period of time from three [3] to five months or even more [2]. This means that reduced thyroid function may not terminate in early lactation and may extend to mid- and even late- lactation.

While the duration of decreased thyroid functions may extend beyond mid- lactation [2], cows are usually inseminated during early lactation and/or mid lactation (e.g. from day 45 to day 150 of lactation) if they express oestrus normally or by hormone injection. Oestrus cows normally have higher circulating thyroid hormones [10, 11]. Thus, in both normal and hormonally induced oestrus cows, recovery from the effects of NEB and similar metabolic conditions with regard to thyroid hormones and other metabolites is anticipated in spite of days in milk (DIM). In other words, it is logic to expect similar metabolic conditions in normal and induced heat cows for satisfactory reproductive responses.

This article presents the results of a field study that compared the levels of circulating thyroid hormones and indices of energy and lipid metabolism in dairy cows with normal or induced oestrus signs before and after 100 DIM. Serum thyroxin (T4), free thyroxin (fT4), triiodothyronine (T3), free triiodothyronine (fT3), glucose, beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglyceride (TAG), cholesterol, very low density lipoproteins (VLDL-cholesterol), low density lipoproteins (LDL-cholesterol) and high density lipoproteins (HDL-cholesterol) were investigated.

MATERIALS AND METHODS

Animals and Samplings

The study was done in two dairy farms with 120 milking cows each and controlled and constant and almost similar nutritional management, located 70 km north of Shiraz, Fars province, Iran in close vicinity to each other. Blood samples of 8 cows in each farm expressing detectable oestrus signs (standing heat and

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other simultaneous behavioural changes) were taken from jugular vein into tubes without anticoagulant. The samples were taken in the morning (1000 to 1200 Hrs) during July and August, when the temperature-humidity index reached to 84 for several hours in days. The cows were divided into two equal groups according to their days in milk (DIM=53-90 and DIM=100-150). In each group, there were 4 cows that exhibited oestrus signs normally and 4 cows that were induced by hormone injection (two injections of $PGF_{2\alpha}$; 11 days apart). The reason for hormonal treatment was that the oestrus signs were not detected in these cows following the voluntary waiting period and/or thereafter. No other abnormality was reported for these cows. All cows were planned to be inseminated (or were inseminated) within the same oestrus time. The cows were adult Holsteins with standard production (305, 2X, ME) of ≥8000 kg and without any previous report of disease or abnormal condition in the same lactation (veterinary inspections and/or examinations were carried out bimonthly). According to their DIM (<150), the cows were grouped together (as high producers) and were fed based on NRC [12]. The last milk record of the cows (within one week before or after sampling) and BCS of the cows at the day of sampling were recorded.

Measurements

The samples were kept on ice for about two hours before centrifugation and decanting. Sera were stored at –20ºC for further analysis. Serum T_4, T_3, fT_3 and fT_4 were measured by radioimmunoassay (RIA) method (kits from Immunotech Company, Immunotech-Radiova-Prague-Czech Republic). Glucose was measured by glucose oxidase method. BHB was measured by Williamson-Mellanby spectrophotometery method and NEFA were determined by spectrophotometery method defined by Burtis and Ashwood [13]. Cholesterol was determined by modified Abell-kendall/Levey-Brodie (A-K) method [13]. triglyceride were determined by the enzyme procedure of McGowan *et al.* [14] and VLDL-cholesterol was estimated as one-fifth of triglyceride [15]. HDL-cholesterol was measured by a precipitation method. The precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the sera to aggregate non-HDL lipoproteins, which were then sedimented by centrifugation (10,000 *g* for 5 minutes). The residual cholesterol was measured using enzymatic method [13]. Cholesterol was quantified in the serum precipitate and in the HDL-cholesterol supernatant using the same enzymatic method. LDL-cholesterol was calculated as the difference between cholesterol measured in the precipitate and in the HDL-cholesterol fraction.

Statistics

The cows with DIM=53-90 were compared to those with DIM=100-150 using Mann-Whitney U non-parametric test. The induced cows of each DIM group were compared with non-induced oestrus ones of the same range of DIM (n=4 for each group) using the same test. The SPSS statistical software, version 20, IBM Inc, USA was used for the study.

RESULTS

The results for thyroid hormones and the measured serum metabolites (mean ± SD) are depicted in Table **1**. Comparison of all oestrus cows (normal or induced) between DIM groups (n=8 each) revealed lower levels of T4 (P=0.027) and T3 (P=0.022), but higher concentrations of fT4 (P=0.031) and fT3 (P=0.006) in the cows with lower DIM. Higher concentrations of triglyceride and VLDL-cholesterol (P=0.021) as well as a tendency (P=0.074) for lower levels of BHB were other remarkable findings in cows with lower DIM. Examining normal and induced oestrus cows in separate DIM groups revealed higher levels of T3 (P=0.044) as well as tendencies (P=0.083) for higher T4, lower fT4 and higher NEFA in the normal oestrus cows with DIM=53-90 than in induced cows with the same range of DIM. No significant difference was observed between normal and induced oestrus cows in DIM=100-150 except a tendency (P=0.053) for higher concentrations of LDL-cholesterol in normal cows.

DISCUSSION

Higher levels of T4 and T3 in cows with DIM more than 100, coincided with lower triglyceride and VLDL-cholesterol, could be a sign of relief from the adverse effects of NEB. At the same time, since only the free forms of thyroid hormones can be utilized in cellular metabolism, lower levels of fT4 and fT3 could indicate to lower tissue demands due to relief from NEB. Lower levels of triglyceride and VLDL-cholesterol in cows above 100 days in milk could be explained as a response to improved energy status and liver functions. Cows with T3 levels below 1.4nmol/L may have diminished oestrus signs [8]. Thus, it could be concluded that the cows with DIM below 100 may probably not be as ready as the cows above 100 days in milk to re-establish normal reproductive functions.

Table 1: Thyroid Hormones and other Measured Parameters (mean±SD) in Oestrus Cows Below and Above 100 Days in Milk with Normal and Induced Oestrus. Bold Lines Show Significant Differences

	All cows		**DIM=53-90**		**DIM=100-150**	
	DIM=53-90 (*n*=8)	**DIM=100-150 (*n*=8)**	**Normal (*n*=4)**	**Induced (*n*=4)**	**Normal (*n*=4)**	**Induced (*n*=4)**
T4 (nmol/L)	**50.38±14.87**	**80.38±30.36**	60.00±14.54	40.75±7.59	70.00±26.78	90.75±33.87
	P=0.027		P=0.083			
T3 (nmol/L)	**0.90±0.22**	**1.70±0.46**	**1.03±0.25**	**0.78±0.10**	1.63±0.57	1.78±0.39
	P=0.002		**P=0.044**			
fT4 (pmol/L)	**48.58±17.88**	**30.75±8.60**	37.90±14.41	59.25±15.31	26.75±5.32	34.75±10.08
	P=0.031		P=0.083			
fT3 (pmol/L)	**1.56±0.26**	**1.06±0.25**	1.62±0.32	1.50±0.22	1.10±0.27	1.03±0.26
	P=0.006					
T4:T3	56.1±11.4	47.3±11.8	59.03±10.47	53.33±13.09	44.66±15.27	49.84±8.70
	56:1	47:1	58:1	52:1	43:1	51:1
BCS	**2.75±0.41**	**2.81±0.39**	3.00±0.35	2.63±0.43	3.06±0.24	2.56±0.38
	P=0.033					
Production	33.56±8.61	33.51±4.22	33.00±12.87	36.05±4.38	34.12±2.59	30.98±2.27
Glucose	54.88±22.71	52.00±23.76	52.75±21.33	57.00±27.14	50.50±30.77	53.50±19.09
BHB	565.72±299.07	926.35±369.02	679.84±303.49	451±286.13	971.80±511.80	880.91±224.28
	P=0.074					
NEFA	0.21±0.09	0.29±0.16	0.27±0.10	0.15±0.05	0.34±0.21	0.24±0.07
			P=0.083			
TAG	**0.23±0.05**	**0.15±0.04**	0.23±0.07	0.22±0.02	0.15±0.01	0.15±0.06
	P=0.021					
VLDL (mmol/l)	**0.06±0.01**	**0.03±0.01**	0.05±0.01	0.04±0.00	0.03±0.00	0.03±0.01
	P=0.021					
Cholesterol	**6.49±2.06**	**4.93±1.20**	7.27±2.69	5.71±1.02	5.23±1.37	4.64±1.13
	P=0.046					
HDL (mmol/L)	2.98±1.37	2.57±0.87	3.52±1.31	2.43±1.36	2.13±0.88	3.01±0.68
LDL (mmol/L)	3.09±1.77	2.29±1.47	3.00±2.05	3.17±1.76	3.03±1.41	1.55±1.25
					P=0.083	

Lower concentration of cholesterol in cows above 100 days in milk could be a result of its higher utilization in steroidogenesis influenced by higher levels of thyroid hormones. Involvement of thyroid hormones in regulating steroidogenesis has been demonstrated [16, 17]. A negative correlation between serum T_3 and cholesterol has been reported with pooled data of dairy cows at various stages of lactation cycle from early lactation to dry period [2]. Blaszczyk *et al.* [16] showed negative correlations in bovine follicular fluid between fT3 and cholesterol concentrations and between fT4 with cholesterol and HDL-cholesterol. In humans and dogs, cholesterol varies inversely with thyroid activity [18]. In human, decrease in thyroid hormones during NEB results in increased cholesterol and LDL-cholesterol [19, 20]. Thus, increased ovarian demands during oestrus and better steroidogenesis in cows with DIM above100 could have a contribution in lower levels of fT4 and fT3. Another noticeable finding in these cows was a tendency (P=0.074) for higher levels of BHB, a ketone body which can be utilized in most cells as a fuel for energy. During NEB it can be substituted for glucose to prevent its decline in blood [21]. In the cows above 100 days in milk higher concentrations of BHB together with a glucose level close to that of cows below 100 days in milk could indicate to provision of the animal with more fuel in energy metabolism. In other words, in cows below 100 days in milk, while the concentration of glucose was similar to that of cows above 100 days in milk, the lower level of BHB could render the animals to energy deficit at the critical time of oestrus.

Comparison between normal and induced oestrus cows revealed some differences in circulating thyroid hormones in cows with lower DIM (53-90). In this range of DIM, normal cows showed higher levels of T3 as well as tendencies for higher levels of T4 and fT4. Other parameters were not different between normal and induced cows. These findings could indicate to a better and less challenging metabolic condition in cows that exhibit oestrus signs normally. In cows above 100 days in milk the normal and induced cows were not different. Thyroid activity varies during the oestrus cycle, with maximal activity during oestrus phase [10, 11]. It could be concluded that, in the cows with lower DIM, hormonal induction of oestrus may not be coincided with elevated thyroid hormones because the animals have not completed a normal cycle or they may not be metabolically ready for swift and desirable response to hormonal induction. Cows above 100 days in milk may have less metabolic limitations for balancing the secretion of thyroid hormones in response to hormonal induction of oestrus.

In conclusion, hormonal induction of oestrus may be accompanied with low levels of circulating thyroid hormones as well as unstable metabolic conditions which may potentially affect reproduction. The expectation for similar metabolic conditions in normal and induced heat cows for satisfactory reproductive responses could be achieved at higher days in milk.

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ABBREVIATIONS

BHB	=	beta-hydroxybutyrate
fT3	=	free triiodothyronin
fT4	=	free thyroxin
HDL-cholesterol	=	high density lipoprotein
LDL-cholesterol	=	low density lipoprotein
NEB	=	negative energy balance
NEFA	=	non-esterified fatty acids
T4	=	thyroxin
T3	=	triiodothyronin
TAG	=	triglyceride
VLDL-cholesterol	=	very low density lipoprotein

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