



Serum thyroid hormone evaluation during transition periods in dairy cows

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Abstract. Thirty-five multiparous Holstein dairy cows were selected from a high-producing dairy farm in north-eastern Italy: 16 in second lactation (L2), 10 in third lactation (L3) and 9 in fourth lactation (L4). Blood sampling was carried out 7 ± 5 days before calving (Pre/C) and 7 ± 5 days after calving (Post/C). Serum triiodothyronine (T3), thyroxine (T4) and a thyroid-stimulating hormone (TSH) were assessed. Two-way analysis of variance (ANOVA) showed statistically significant effects of class (L2, L3, L4) and of period (Pre/C, Post/C) on the parameters studied (T3, T4, TSH). In particular, Bonferroni's multiple comparison test showed lower values in post-calving than in the pre-calving in L2 and L3 for TSH; lower values in post-calving than in the pre-calving in L2 for T3; and lower values in post-calving than in the pre-calving in L2, L3 and L4 for T4. Our results improve the knowledge of endocrine and metabolic changes occurring in dairy cows during transition periods and may be useful to supply a new strategy for the improvement of dairy cow farm management and reproductive performance.

1 Introduction

The time from late pregnancy to early lactation is known as a transition period, and it is recognized as the period between 3 weeks before and 3 weeks after parturition (Contreras and Scordillo, 2011; Morgante et al., 2012). During this period, physical, behavioral, metabolic and hormonal changes are likely to occur in dairy cows, which could lead to a decline in productive and reproductive performance (Morgante et al., 2012). Adaptation of the endocrine system during the transition period, primarily the thyroid gland, is the key factor in maintaining metabolic balance. It is known that immediately before and after parturition as well as during the first stage of lactation, increased mammary gland activity results in energy deficiency, increased lipomobilization from body reserves and intensive ketogenesis and lipogenesis in the liver (Šamanc et al., 2010).

Excessive fat mobilization can induce an imbalance in hepatic carbohydrate and fat metabolism, which may re-

sult in metabolic disorders such as ketosis (Goff and Horst, 1997). In this regard, thyroid hormones are considered to play an important role in the etiopathogenesis of ketosis due to their very low levels in the blood of transition cows that bring about a decrease in energy metabolism, mobilization of body fat reserves and their partitioning toward high milk production (Djokvic et al., 2014; Gueorguiev, 1999). Thyroxine (T4) is the predominant thyroid hormone in the circulation; it has little inherent biological activity and it is commonly viewed as a prohormone. The metabolically active thyroid hormone, triiodothyronine (T3), is produced by enzymatic 5'-deiodination of T4 within the thyroid and in extrathyroidal tissues (Leonard and Visser, 1986). Conversely, 5'-deiodination of T4 generates the inactive thyroid hormone metabolite, reverse-T3 (rT). The extrathyroidal activity of thyroxine 5'-deiodinase (5'D) is an important control point for regulating the thyroid status of animal tissues in various physiological and pathological situations (Chopra et al.,

Table 1. Analytical composition of diets administered during pre-calving (Pre/C) and post-calving (Post/C) period.

Chemical composition (%)	Pre-calving	Post-calving
Crude protein	13.37	16.59
Ethereal extract	4.30	6.01
NDF	40.77	30.17
ADF	24.82	20.37
NFC	34.17	38.81
Starch	14.74	28.46

NDF: Neutral Detergent Fiber; ADF: Acid Detergent Fiber; NFC: Non Fiber Carbohydrates.

1978; Kohrle, 1994; Larsen, 1982; Leonard and Visser, 1986; Wartofsky and Burman, 1982). All three thyroid hormones are present in the circulation; however, inherent physiological effects are attributed almost only to T3 (Leonard and Visser, 1986; Dickson, 1990; Flier et al., 2000). Thyroid hormones have circadian and ultradian rhythmicity also in the plasma of lactating dairy cows (Bitman et al., 1994), and concentrations of T4 and T3 in cattle are influenced by a variety of environmental factors, such as the ambient temperature (Pratt and Wettemann, 1986; McGuire et al., 1991) and dietary components and intake (Awadeh et al., 1998; Richards et al., 1995; Tiirats, 1997). Cows in postpartum negative energy balance (NEB) respond to decrease the concentrations of T3 and T4 and increase the concentration of rT3 (Petes et al., 1985; Ronge et al., 1988; McGuire et al., 1991; Yambayamba et al., 1996.) The positive correlation between circulating thyroid hormone concentrations and energy balance is well known in many species including cattle (Kunz and Blum, 1985; Janan et al., 1995; Leyva-Ocariz et al., 1997; Nikolic et al., 1997; Capuco et al., 2001; Cassar-Malek et al., 2001).

In view of such consideration, the aim of this study was to evaluate the modifications of the levels of thyroid hormones (T3, T4, TSH) during transition periods in dairy cows.

2 Materials and methods

2.1 Farm and animals

Thirty-five multiparous Holstein cows were selected from a high-producing dairy farm in northeastern Italy (45°24' N, 12°52' E, 12 m a.s.l.): 16 in second lactation (L2), 10 in third lactation (L3) and 9 in fourth lactation (L4). Farms undergo a dry period of 60 days and a period of steaming-up of 15 days before calving. A farm was selected with a milk production (about 10 000 kg for year); milk yield quality was not different for all cows: an average of 3.7 % of milk fat and 3.4 % of milk protein. All the animals were clinically healthy and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart rate, respiratory

profile, appetite, fecal consistency and hematologic profile. The ratio and the chemical composition of diets used during steaming-up and subsequent early lactation is reported in Table 1.

2.2 Blood sampling and hormonal analysis

The data were collected from June to October 2013. Blood sampling was carried out 7 ± 5 days before calving (Pre/C) and 7 ± 5 days after calving (Post/C). Blood samples were collected into serum vacuum tubes (BD Vacutainer Systems, Preanalytical Solutions, Plymouth, UK).

The tubes were centrifuged (Labofuge 400, Heraeus) at 1750 g for 10 min and the obtained sera were stored at -18° , within 1 h after blood collection. Serum hormonal profile analyses included triiodothyronine (T3), thyroxine (T4) and thyroid-stimulating hormone (TSH). T3, T4 and TSH concentrations in each sample were quantified with a commercial IMMULITE® 2000 kit (Immulate 2000 Total T3/L2KT36; Immulate 2000 Total T4/L2KT46; Immulate 2000 Third Generation TSH/L2KTS6; Siemens, Italy).

All treatments, housing and animal care reported above were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments.

2.3 Statistical analysis

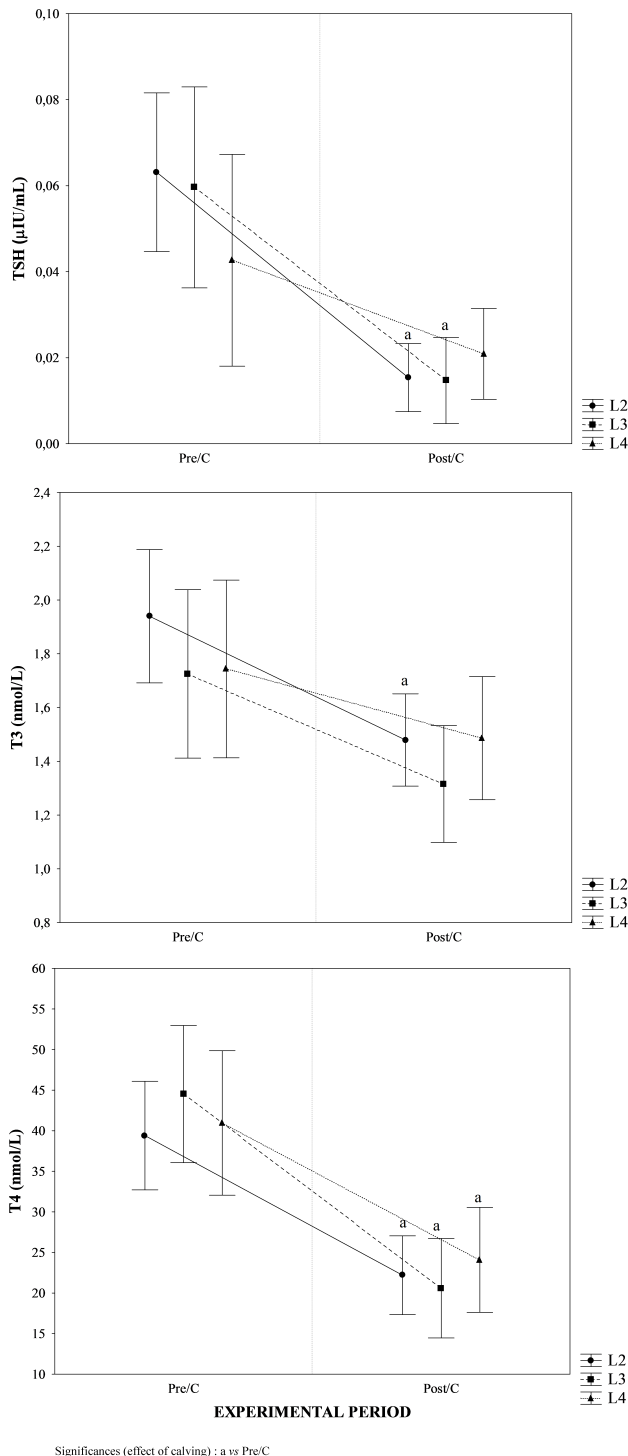
Two-way analysis of variance (ANOVA) was used to determine statistically significant effects of class (L2, L3, L4) and of period (Pre/C, Post/C) on the parameters studied (T3, T4, TSH). A P value of <0.05 was considered statistically significant. Bonferroni's multiple comparison test was applied for post hoc comparison. Obtained data have been analyzed using the software STATISTICA 7 (Stat Soft Inc.).

3 Results

Two-way repeated measures ANOVA showed a significant effect of two periods (Pre/C and Post/C) on TSH, T3 and T4 (Fig. 1). In particular, Bonferroni's multiple comparison test showed lower values in Post/C than in Pre/C in L2 ($P < 0.001$) and L3 ($P < 0.05$) for TSH; lower values in Post/C than in Pre/C in L2 ($P < 0.05$) for T3; and lower values in Post/C than in Pre/C in L2, L3 ($P < 0.0001$) and L4 ($P < 0.01$) for T4.

4 Discussion

Previous studies have reported differences in hematological and hematochemical reference ranges for dairy cows, associated with age, gender, breed and production (Lumsden et al., 1980; Dias et al., 2006). The hormonal activity of thyroid gland has an important role in the transitional period for determining the cell metabolism intensity, metabolism of



Significances (effect of calving) : a vs Pre/C

Figure 1. Statistically significant effects of period (pre-calving, post-calving) on TSH, T3 and T4 recorded during the transition period in Holstein dairy cows. Vertical bars report 95 % confidence intervals.

lipids and carbohydrates and lactation course itself by its thyroid hormones (Nikolic et al., 1997). In dairy cows, low T3

and T4 have been observed in the first trimester of lactation (Pethes et al., 1985).

Also our results confirm that the act of parturition in cows is accompanied by marked changes in circulating thyroid hormone profiles. The reduction of serum T3 and T4 we found in post-calving is probably a reflection of the decreased thyroid hormone secretion rate due to the energy deficiency state as well as to the large demand for these hormones by the mammary gland.

At the beginning of galactopoiesis, in fact, there is an increase in the number of T3 receptors in the mammary gland secretory cells during lactation (Wilson and Gorewit, 1980), and there is a greater activity of the organ-specific type-2 deiodinase enzyme, which generates T3 intracellularly from T4, and also of the type-3 deiodinase enzyme, which in turn deactivates the thyroid hormones (Pezzi et al., 2003); there is an actual secretion of T4 through the milk, which may represent between 4 and 7 % of the total required for the maintenance of metabolic functions (Akasha and Anderson, 1984). Alterations in plasma T4 levels associated with the energy balance and metabolism reflect both the changes in TSH-regulated thyroid secretion rate (central regulation) (Riis and Madsen, 1985) and the balance of extrathyroidal enzymatic T4 activation and inactivation (peripheral autoregulation) (Pethes et al., 1985; Capuco et al., 2001; Cassar-Malek et al., 2001). In addition, the decrease in T4 concentrations could yet be caused by the additional mammary gland secretion of maternal iodine during lactation.

Our results are consistent with previous reports in the literature relative to the effects of time during the periparturient period on thyroid hormone concentrations in cows, and they underline the importance of monitoring the hormonal status during transition periods in order to understand when adjustments of regulatory mechanisms break through physiological limits predisposing the cow to metabolic problems.

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