Circulating metabolic hormones in different metabolic states of high producing Holstein dairy cows

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Abstract:

BACKGROUND: Information regarding the metabolic hormones in different metabolic states of high producing dairy cows can aid high producing herds to manage and resolve the metabolic and production problems. Furthermore, it can be considered as a tool to evaluate metabolic status of dairy cows. Herd managers can also evaluate the energy input-output relationships by assessing the metabolic profile to prevent and control negative energy balance, metabolic disorders and nutritional insufficiencies. **OBJECTIVES:** The present study was performed to clarify the metabolic hormone profile in each metabolic state of high producing Holstein dairy cows. The results of this research can reveal the normal metabolic state of these animals. **METHODS:** 25 multiparous Holstein dairy cows were divided to 5 equal groups containing early, mid and late lactations, far-off and close-up dry periods. Blood samples were collected from all cows and sera were analyzed for concentrations of triiodothyronine (T3), thyroxine (T4), free T3 (fT3), free T4 (fT4), cortisol and insulin like growth factor-1 (IGF-1). **RESULTS:** Serum T3 concentration in early lactation group was significantly higher than other ones ($p<0.05$). T4 levels in early and mid lactation dairy cows were lower than other studied groups, significantly. The cows in early lactation and close-up dry periods had the highest and the lowest serum fT3 concentrations, respectively ($p<0.05$). fT4 in far-off dry cows was significantly higher than others. The highest and the lowest circulating levels of cortisol were detected in mid and late lactation periods, respectively ($p<0.05$). The lowest concentrations of IGF1 were detected in early lactation period and its highest levels were seen in mid and late lactation and far-off dry cows ($p<0.05$). **CONCLUSIONS:** Metabolic hormones change in different metabolic states of high producing Holstein dairy cows. The presented metabolic hormone profile can be considered as criteria to monitor the metabolic status of dairy cows at different metabolic states.

Introduction

Dairy cows pass different physiological states during their producing life. Metabolic characteristics in each state are different from others. Transitional period is one of these states which includes 3 weeks before and 3 weeks after calving when metabolic processes are adapted to providing energy for parturition and lactogenesis (Overton and Waldron, 2004). Subsequently, in early lactation, as a state causes negative energy balance, a high mobilization of lipids from body fat reserves as well as hypoglycemia occur (Reist et al., 2002). The negative energy balance is continued to mid lactation period and in this period the cow has the highest amount of milk production. At the end of lactation period, the dry matter intake is parallel to milk synthesis; hence in late lactation the negative energy balance is resolved (Van Knegsel et al., 2007)

Negative energy balance is defined as disproportion between input-output relationships which can cause metabolic diseases. Metabolic diseases may be created due to an imbalance between the rates of input of dietary nutrients and output of production (Quiroz-Rocha et al., 2009). When the imbalance is continued, it may lead to change in the amount of body reserves of certain metabolites (Radostits et al., 2007). Excess negative energy balance, fat mobilization and subsequent elevations in ketone body concentrations play a contributing role in the expression of metabolic diseases such as fatty liver syndrome and clinical ketosis (Goff and Horst, 1997). A severe negative energy balance during some metabolic states of high producing dairy cows may also increase the risk of retained placenta, metritis, and mastitis through impaired immune function (Kim et al., 2005).

The hormonal activities in each metabolic state are different from the other ones. Metabolic hormones such as thyroid hormones, insulin like growth factors and cortisol have important roles in each metabolic period for determining cell metabolism intensity, metabolism of lipids and carbohydrates and the lactation courses (Nikolic et al., 1997). Under the conditions of negative energy balance and high lipid mobilization, the circulatory concentrations of metabolic hormones can change in dairy cows (Pezzi et al., 2003; Reist et al., 2002).

Determining metabolic hormones can be a reliable tool for early diagnosis of nutritional deficiency or metabolic disease that would be a major forward step in attempting to optimize flock production and obtain maximum yields at minimum costs (Radostits et al., 2007). Therefore, information regarding circulating metabolic hormones is considered as a useful diagnostic aid to uncover the problems in difficult herd situations (Găvan et al., 2010). Furthermore, the genetically superior cows may be selected via evaluating their circulating metabolic hormones (Rowlands et al., 1973).

There are several literatures on metabolic hormones in transition and pre parturition periods in dairy cows (Nikolic et al., 1997; Roberts et al., 1997; Djoković et al., 2007) but based on the author's knowledge, comprehensive information about circulating metabolic hormones in different metabolic states of high producing Holstein dairy cows is lacking. Determining the normal base line values of circulating metabolic hormones in early, mid and late lactation and far-off and close-up dry cows and comparing them together in a single comprehensive study were the aims of the present research.

Materials and Methods

Animals: The present study was carried out in winter 2014 on 25 multiparous Holstein dairy cows from a high producing industrial dairy farm around Shiraz, southwestern Iran. These cows were housed in open-shed barns with free access to water and shade. The total mixed rations were formulated and prepared for all animals according to National Research Council (NRC) requirements. At this farm, a dry period of 60 days has been considered for late pregnant cattle. Milk production was about 10,000 kg for one year, an average of 3.6 of milk fat %, and 3.3 of milk protein %. All the animals were clinically healthy, had no history of debilitating disease, and were free from internal and external parasites due to routine antiparasitic programs at this farm. Body condition score (BCS) of these animals was estimated based on 0 to 5 system (Alapati et al., 2010). Cattle were divided to 5 equal groups containing early (30.2±5.7 days after calving, with 3.25±0.25 BCS), mid (108.1±8.4 days after calving, with 3.25±0.25 BCS) and late lactations (184.5±5.7 days after calving, with 3.5±0.25 BCS), far-off (281.9±5.4 days after calving, 228.4±8.6 days of pregnancy, with 3.5±0.25 BCS) and close-up dry periods (312.1±8.3 days after calving, 255.6±6.3 days of pregnancy, with 3.5±0.25 BCS).

Blood sampling and hormonal assays: Blood samples were collected from all cows through jugular venipuncture in plain tubes. Immediately after blood collections, sera were separated by centrifugation for 10 minutes at 3,000 g and stored at -22°C until assayed. Serum triiodothyronine (T3) concentrations were determined using a competitive enzyme immunoassay kit (Padtan Elm Co., Tehran, Iran). The intra- and inter-assay CVs of the assays were 12.6% and 13.2%, respectively. The sensitivity of the test was 0.2 ng/mL. Serum thyroxine (T4) concentrations were measured using a competitive enzyme immunoassay kit (Monobind Inc., CA, USA). The intra- and inter-assay CVs of the assays were 3.0% and 3.7%, respectively. The sensitivity of the test was 0.4 mg/dl. Serum free T3 (fT3) and free T4 (fT4) concentrations were determined by the fT3 and the fT4 ELISA kits (DiaPlus Inc., San Francisco, CA, USA). The intra- and inter-assay CVs of the fT3 assays were 4.1% and 5.2%, respectively. The sensitivity of the test was 0.05 pg/mL. The intra- and inter-assay CVs of the fT4 assays were 4.5% and 3.7%, respectively. The sensitivity of the test was 0.05 ng/dL. Serum cortisol concentrations were determined by Enzyme Immunoassay Colormetric method (AccuBind® ELISA kit; Monobind Inc., CA, USA). The sensitivity of the test was 0.25 µg/dl. Serum levels of insulin like growth factor-1 (IGF-1) were evaluated by ELISA kit (ImmunoDiagnosticSystem®) with the sensitivity equal to 3.1 µg/l.

Statistical analyses: All data are presented as mean ± standard deviation (SD). Differences among the averages of concentrations of serological factors in the different groups were analyzed by one-way ANOVA and the least significant difference (LSD) test was used to find differences using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). The level of significance was set at $p<0.05$.

Results

The levels of circulating metabolic hormones (mean±SD) in different metabolic states of high producing Holstein dairy cows are presented in Table I. Serum T3 concentration in early lactation group was significantly higher than other ones ($p<0.05$). T4 levels in early and mid lactation dairy cows

Table 1. Circulating metabolic hormones (mean±SD) in different metabolic states of high producing Holstein dairy cows (n=5). Different letters indicate significant differences in each row ($p<0.05$).

Hormones	Early lactation	Mid lactation	Late lactation	Far-off dry	Close-up dry
T3 (ng/dl)	143.00±22.15[a]	122.60±8.14[b]	141.25±9.50[b]	128.00±20.79[b]	126.20±17.75[b]
T4 (µg/dl)	3.61±0.68[a]	3.64±0.80[a]	4.11±0.27[b]	4.17±0.78[b]	4.09±1.18[b]
fT3 (ng/l)	3.29±0.67[a]	2.82±0.13[b]	2.90±0.26[b]	2.83±0.20[b]	2.52±0.31[c]
fT4 (ng/l)	4.50±0.79[a]	4.91±1.29[a]	5.31±0.90[a]	6.34±1.61[b]	4.99±2.64[a]
IGF1 (µg/l)	53.40±12.21[a]	84.20±18.00[b]	84.50±36.78[b]	86.20±27.73[b]	67.80±43.60[c]
Cortisol (ng/ml)	15.02±7.51[a]	21.58±6.26[b]	6.32±4.34[c]	16.76±3.65[a]	11.68±6.12[a]

were lower than other studied groups, significantly. The cows in early lactation and close-up dry periods had the highest and the lowest serum fT3 concentrations, respectively ($p<0.05$). fT4 in far-off dry cows was significantly higher than others. The highest and the lowest circulating levels of cortisol were detected in mid and late lactation periods, respectively ($p<0.05$). The lowest concentrations of IGF1 were detected in early lactation period and its highest levels were seen in mid and late lactation and far-off dry cows ($p<0.05$).

Discussion

Physiological systems of dairy cows work together to challenge with energy intake and output in order to maintain adipose tissue. Furthermore, adipose tissue secretes leptin and cytokines, which induces satiety and has been linked to hormones such as thyroid hormones, cortisol and IGF-1. Thus, adipose tissue is acted upon by a number of physiological stimuli, including hormones, and simultaneously, is an active component in the regulation of its own lipid content (Van Knegsel et al., 2007). All of the hormones mentioned above are associated with each other in changing metabolic states. Hence, the present study was performed to clarify the levels of thyroid hormones, cortisol and IGF-1 as metabolic biomarkers in different metabolic states of high producing Holstein dairy cows.

Thyroid gland produces the thyroid hormones containing T3 and its prohormone T4, which are tyrosine-based hormones. Thyrotropes of the anterior pituitary gland secrete thyroid stimulating hormone and this hormone regulates the production of T3 and T4 by follicular cells of the thyroid gland. T4 is the major circulating thyroid hormone which has a longer half-life than T3. T4 is changed to the active T3 and T3 is more potent than T4. Circulating fT3 and fT4 represent the amount of T3 and T4 that are not bound to proteins. Evaluating the fT3 and fT4 can be used to assess and manage disorders of the thyroid gland (Yen, 2001).

Thyroid hormones are primarily responsible for metabolism regulation. They increase the metabolic rate, change protein synthesis, regulate osteoblasts and nervous system maturation and increase the sensitivity to catecholamines. The proper circulating levels of thyroid hormones are necessary for developing and differentiation of all cells. These hormones also are responsible for regulation of protein, fat, carbohydrate and vitamin metabolism. Numerous physiological, pathological and pharmacological stimuli influence thyroid hormone metabolism (Tan et al., 1998; Taylor et al., 2004).

The results of the present study showed that circulating levels of thyroid hormones in each metabolic state were different from others (Table 1). The characteristics of each metabolic state of dairy cows, with emphasis on negative energy balance, can interfere with the thyroid hormone levels and activities. The intensity of oxidation in mitochondria of cells is closely linked with the functional state of the thyroid gland, so it is justifiably considered that the conditions of negative energy balance and the increased lipid mobilization from body fat reserves result in lipid infiltration of liver cells (Djoković et al., 2007). The reason is the decreased capacity of mitochondria to oxidize fatty acids in the conditions of low concentrations of thyroid hormones in blood (Johannsen et al., 1993).

Djoković et al. (2007)suggested that a hypothyroidal status was established in ketotic cows and that the blood concentrations of free fatty acids, triacylglycerols, total cholesterol and glucose served as major biochemical indicators in determining liver steatosis in the dairy cows in transitional period. Thyroid hormones are important for maintenance of pregnancy and for a normal ensuing lactation cycle. It is found that thyroid hormone is a necessary hormone in the development of mouse mammary tissue (Vonderhaar and Greco, 1979).

Based on our research, T3 and T4 levels were decreased near to parturition. In the dairy cow, plasmaT4 levels gradually decrease around the time of parturition. There is a gradual rise following calving with prepartum levels of T4significantly higher compared to postpartum concentrations (Goff and Horst, 1997). Levels of T3 and T4 were measured in Estonian cows during different stages of lactation. During early lactation, plasma thyroid hormone concentrations were lower and progressively increased as lactation continued (Tiirats, 1996).

Cortisol is the major stress hormone produced by the adrenal glands in the ruminant (Hunter et al., 1970). Based on our finding the highest levels of cortisol were seen in mid lactation period when the cows had the high milk yield. It may be suggested that the negative energy balance in this period can be considered as a stress factor to induce the elevation of circulating cortisol. Hunter et al. (1970) mentioned that during the transition period, cortisol is elevated prior to, during and following parturition, signifying an increased release of the adrenocorticotropin (ACTH). A normal response to stressful conditions is activation of the hypothalamic-pituitary-adrenal axis (Christison and Johnson, 1972). Physiological stressors cause the release of the hypothalamic corticotropin releasing factor which increases ACTH (Collins and Weiner, 1968). During the periparturient period, stressors are above what the cow normally experiences, eliciting an increased release of ACTH (Hunter et al., 1970).

Several studies mentioned that at the time of parturition, cortisol peaks, followed by a sharp drop in concentrations in early lactation (Hunter et al., 1970; Goff and Horst, 1997). The current study showed that after parturition, serum cortisol concentrations were elevated in early lactation period. Beerda et al. (2004) have shown that high yielding dairy cows have lower cortisol response to ACTH administration than low yielding cows. One possible reason could be difficulties in synthesizing cortisol when their energy demands increase in peak lactation.

Growth hormone is in control of metabolism in the dairy cow in all stages of lactation (Sjaastad et al., 2010). The effect of growth hormone on lactation is discussed to be only partly direct and instead mediated by IGF-1 (Svennersten-Sjaunja and Olsson, 2005). Receptors for IGF-1 can be found in all mammary cells and release of IGF-1 is stimulated by binding of growth hormone to hepatocytes in the liver (Tucker, 2000), which is the main source of circulating IGF-1 (Akers, 2002).

The concentration of IGF-1 can be seen as an indicator of the metabolic state of the dairy cow (Taylor et al., 2004). The results of the current study showed that the lowest circulating levels of IGF-1 were in early lactation period. Abribat et al. (1993) showed that the levels of IGF-1 are lower at the start of lactation and increase during the whole lactation, inversely related to milk yield.

Based on Roberts et al.'s study (1997), determining the circulating concentrations of growth hormone and IGF-1 may provide a clinical approach for evaluating the nutritional status of individual cows within herd basis. Under adequate dietary availability, circulating concentrations of IGF-1 are under positive regulation by growth hormone (Vicini et al., 1991).

The concentrations of IGF-1 were decreased near to parturition in close up dry cows (Table 1). As previously demonstrated, circulating concentrations of IGF-1 decline at parturition and gradually increase over time, whereas concentrations of growth hormone increase at parturition and then decline over time (Vicini et al., 1991; Roberts et al., 1997). The magnitude in decline and duration of time required for IGF-1 levels to return to prepartum levels are greater in animals subjected to dietary restriction (Hunter et al. 1970) and may interact with genetic potential for milk production (Roberts et al., 1997).

Finally, the present research revealed the circulating baseline levels of some metabolic hormones in dairy cows.

In conclusion, it could be stated that metabolic hormones change in different metabolic states of high producing Holstein dairy cows. These changes are induced commonly by metabolic alterations of cow's body systems such as lactogenesis, pregnancy and parturition.

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هورمون‌های متابولیک در حالات مختلف متابولیکی گاوهای شیری هلشتاین پر تولید

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چکیده

زمینه مطالعه: آگاهی از هورمون های متابولیک در حالات مختلف متابولیکی گاوهای شیری پرتولید می‌تواند گله‌های شیری پرتولید را در مدیریت و برطرف ساختن مسائل متابولیک و تولید یاری کند. همچنین، می‌تواند به عنوان ابزاری برای ارزیابی وضعیت متابولیک گاوهای شیری در نظر گرفته شود. مدیران گله‌ها نیز می‌توانند نسبت ورود و خروج انرژی به واسطه ارزیابی پروفایل متابولیک گلـه آزموده و از توازن منفی انرژی، اختلالات متابولیک و نارسـایی‌های تغذیـه‌ای جلوگیری کنند. **هدف:** مطالعه حاضر به منظور مشخص ساختن پروفایل هورمون‌های متابولیک در هر دوره متابولیکی گاوهای شیری هلشتاین پرتولید انجام شد. نتایج این پژوهش می‌تواند وضعیت متابولیک طبیعی این حیوانات را بازگو کند. **روش کار:** تعداد ۲۵ رأس گاو شیری هلشتاین چند شکم زاییده به ۵ گروه مساوی شامل ابتدا، میانه و انتهای شیردهی و ابتدا و انتهای دوره خشکی تقسیم شدند. نمونه‌های خون از تمام گاوها اخذ شد و سرم‌ها به منظور ارزیابی غلظت‌تری یودوتیرونین (T۳)، تیروکسـین (T۴)، T۳ آزاد (fT۳)، T۴ آزاد (fT۴)، کوزتیزول و هورمون رشـد مشابه انسولینی ۱ (IGF-۱) مورد ارزیابی واقع شدند. **نتایج:** غلظت سرمی T۳ در گروه ابتدای شیردهی به طور معنی‌داری بیش از سایر گروه‌ها بود (p<۰/۰۵). میزان T۴ در ابتدا و میانه شیردهی به طور معنی‌داری کمتر از سایر گروه‌های مورد مطالعه بود. گاوهای ابتدای شیردهی و انتهای خشکی به ترتیب بیشترین و کمترین میزان سرمی fT۳ را دارا بودند (p<۰/۰۵). میزان fT۴ در گاوهای ابتدای خشکی به طور معنی‌داری بیش از سایرین بود. بیشترین و کمترین میزان کورتیزول به ترتیب در دوره‌های میانی و انتهایی شیردهی مشاهده شد (p<۰/۰۵). کمترین غلظت IGF-۱ در دوره ابتدای شیردهی و بیشترین میزان آن در میانه و انتهای شیردهی و همچنین در ابتدای خشکی مشاهده شد (p<۰/۰۵). **نتیجه‌گیری نهایی:** هورمون‌های متابولیک در دوره‌های مختلف متابولیکی گاوهای شیری هلشتاین پرتولید تغییر می‌کنند. پروفایل هورمون‌های متابولیک ارائه شده می‌تواند به عنوان شاخصی به منظور ارزیابی وضعیت متابولیک گاوهای شیری در دوره‌های مختلف متابولیک در نظر گرفته شود.

واژه‌های کلیدی: گاو شیری، مدیریت گله، هورمون های متابولیک، میزان طبیعی، دوره های فیزیولوژیک

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