Study on serum glucose, insulin, NEFA, BHBA and lipid profile in different productive status of high producing Holstein dairy cows

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Abstract:

BACKGROUND: Metabolic profiles have been used in efforts to predict pre parturient problems and fertility, to diagnose metabolic diseases, and to assess nutritional status in dairy cows. These profiles may help to confirm the diagnosis of sub clinical diseases, to be aware of possible causes of infertility in the herds, or to monitor improvement in herd animals.

OBJECTIVES: This study investigated changes in the metabolic profile of high producing Holstein dairy cows from early lactation to close-up dry periods. The results of the current research can provide useful guidelines for management strategies during different physiological phases of high producing Holstein dairy cows.

METHODS: Twenty-five multiparous high producing Holstein dairy cows were selected from a high producing industrial dairy farm. Cattle were divided into 5 equal groups of early, mid and late lactation and far-off and close-up dry cows. Blood samples were collected from all cows and sera were separated to evaluate glucose, insulin, β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA), cholesterol, triglyceride (TG), high, low and very low density lipoproteins (HDL-cholesterol, LDL-cholesterol and VLDL-cholesterol).

RESULTS: The highest levels of insulin were detected in mid lactation and close-up dry periods. The changing patterns of BHBA and NEFA were significant and the highest levels of these biomarkers were detected in the early lactation group. The decreasing pattern of BHBA and NEFA were seen from early lactation to far-off dry cows. Significant elevations were seen in these biomarkers from far-off to close-up dry cows.

CONCLUSIONS: The results of the present study show that metabolic biomarkers change in high producing Holstein dairy cows, under different physiological states. These changes are induced commonly by negative energy balance, lactogenesis and fetal growth in each state.

Key words: changing pattern, lactogenesis, fetal growth, Holstein dairy cows, metabolic profile

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Introduction

Different physiological states such as pregnancy and lactation are considered to change metabolism in animals (Tanritanir et al., 2009). Each physiological state can have influence
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on the health and subsequent performance of dairy cows, since cows develop serious metabolic and physiological changes during these periods (Tanaka et al., 2011). During pregnancy and lactation, all metabolic pathways are involved in sustaining fetal growth and lactogenesis, respectively (Bell et al., 2000). The periods of pregnancy and lactation induce a huge metabolic challenge to the high-yielding dairy cow and the metabolic profiles can be used as important criteria to evaluate the health status of these animals (Hawagane et al., 2009).

High rates of body condition score losses take place due to negative energy balance after calving which is indicated by alterations in blood metabolic and hormone profiles (Wathes et al., 2009). Providing glucose as the primary metabolic fuel for maintenance, fetal growth and milk production, is the necessary part of protection against negative energy balance in dairy cows (De Koster and Opsomer, 2013). In dairy cows, the massive energy demand is partly provided through gluconeogenesis and glucose has a central role in metabolism and homeostatic control (Herdt, 2000). Insulin has a direct control on mobilization and utilization of glucose. Changing the insulin dynamic in different physiological states of dairy cows takes place and insulin resistance as a metabolic dysfunction induces metabolic diseases and interferes with energy balance in dairy cows (De Koster and Opsomer, 2013).

Negative energy balance in dairy cows induces lipolysis and lipid mobilization. Evaluating the concentrations of non-esterified fatty acids (NEFA) and β-hydroxybutyric acid (BHBA) is indicative of lipid mobilization and fatty acid oxidation (Wathes et al., 2009). NEFA reflects the magnitude of mobilization of fat from storage and BHBA indicates the completeness of oxidation of fat in the liver (De Koster and Opsomer, 2013).

Evaluating the metabolic profile based on the laboratory measurement of certain components of the blood can reflect the productive performance, nutritional and health status of dairy cows, with or without presence of clinical abnormalities. Information regarding changing the metabolic profile can assist veterinarians to evaluate the metabolic situation and alteration of dairy cows at herd levels.

There are several literatures on metabolic profile in lactation, pregnancy and transition periods of dairy cows (Ghanem et al., 2012; Piccione et al., 2012; Fiore et al., 2014), but based on the author’s knowledge, the literature is lacking comprehensive studies on the changing patterns of metabolic parameters, at different physiological states of dairy cows. Hence, the present study was undertaken to give a clear metabolic profile in high producing Holstein dairy cows from early lactation to close-up dry periods. The results of the current research can provide useful guidelines for management strategies during different physiological phases of high producing Holstein dairy cows.

Materials and Methods

Animals: The present study was carried out during winter the period of 2014 on 25 multiparous Holstein dairy cows from a high producing industrial dairy farm around Shiraz, Southwest 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 the National Research Council (NRC) requirements. A dry period of 60 days was considered in the farm. Milk production was about 10,000 kg for a year, an average of 3.6% of milk fat, and 3.3% of milk protein. All animals were clinically healthy, had not history of debilitating disease, and free from internal and external parasites due to routine antiparasitic programs at the farm. Body condition score (BCS) of these animals were estimated based on 0 to 5 system. Cattle were divided into 5...
equal groups of 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 serological 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 min at 3,000 g and stored at -22°C until assayed. Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method (ZistChem®, Tehran, Iran). Insulin was measured by bovine insulin ELISA kit (Cusabio®, China, specificity 100%, and precision: intra-assay and inter-assay CV < 8% and 10%, respectively). BHBA and NEFA were assayed by colorimetric method (Ranbut®, Ireland). The sera were analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Abbel et al., 1952; Burtis and Ashwood, 1994), triglyceride (TG) by the enzymatic procedure of McGowan et al. (1983). Lipoproteins were isolated using a combination of precipitation and ultra centrifugation. High density lipoprotein (HDL-cholesterol) was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins which were separated by centrifugation (10,000×g for 5 min). The residual cholesterol was then measured by enzymatic method (Burtis and Ashwood, 1994). Low density lipoprotein (LDL-cholesterol) was calculated as the difference between the total cholesterol measured in the precipitate and HDL fraction minus 0.2×triglyceride (LDL=total cholesterol–HDL cholesterol–0.2×TG). Very low density lipoprotein (VLDL-cholesterol) was estimated as one-fifth of the concentration of triglycerides (Friedewald et al., 1972).

**Statistical analyses:** All data are presented as mean ± standard deviation (SD). Differences between the average concentrations of different serological factors in the different groups were analyzed by one-way ANOVA and the least significant difference (LSD) test was used to find differences. Repeated measures of ANOVA was used to evaluate the changing patterns of different studied parameters during physiological states of dairy cows using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). The level of significance was set at p<0.05.

**Results**
Normal levels (Mean±SD) of metabolic biomarkers in different physiological states of high producing Holstein dairy cows are shown in Table 1. The dynamics of the metabolic profile during the different physiological states are shown in Figures 1 to 4. There were no significant changing patterns in glucose and lipid profile (p>0.05). The significant changes were seen in insulin dynamics during the physiological periods. The highest levels of insulin were detected in mid lactation and close-up dry periods (Fig. 1). The dynamics of BHBA and NEFA were significant and the highest levels of these biomarkers were detected in early lactation group. The decreasing pattern of BHBA and NEFA were seen from early lactation to far-off dry cows. The significant elevations are seen in these biomarkers from far-off to close-up dry cows.

**Discussion**
Glucose metabolism changes during lactation and pregnancy in cows (Bickerstaffe et al., 1974), goats (Debras et al., 1989) and sheep (Wilson et al., 1983). This might be related to the uptake of insulin by the lactating mam-
mary gland (Faulkner and Pollock, 1990) and perhaps due to uptake by the pregnant uterus. Another possibility is that insulin uptake by the liver changes during lactation and pregnancy. Insulin is a protein hormone secreted by the β-cells of the pancreas which stimulates translocation of glucose transporters, resulting in glucose uptake by tissues. In ruminants, volatile fatty acids from the gastrointestinal tract are the major energy source rather than direct sources of glucose. Thus, insulin plays a slightly different role in ruminants vs non-ruminants (Kahn, 1978). Elevating volatile fatty acid concentrations during lactation can interfere with glucose-induced insulin secretion (Bossaert et al., 2008). Therefore, it has been reported that elevated circulating volatile fatty acid levels is one of the factors that may account for the impaired hepatic insulin extraction in non-ruminants (Lewis et al., 2002).

The results of the present study show that insulin levels in early lactating dairy cows are significantly lower than close-up dry cows (Table 1; Fig. 1; p<0.05). Other researchers also mentioned that both basal concentration of insulin and insulin response to endogenous glucose can be lower in lactating than in dry dairy cows (Sartin et al., 1985) and lower in
high-yielding lactating cows compared to low yielders (Sartin et al., 1988). Faulkner and Pollock (1990) also suggested that the sensitivity of glucose utilization was increased in lactating sheep compared to non-lactating sheep. However, Debras et al. (1989) reported that the insulin-stimulated glucose utilization above basal levels was greatly impaired during early lactation compared with the dry period in goats; they suggested that a decrease in insulin sensitivity in some insulin-sensitive tissues might occur.

Fatty acids released from adipose tissue circulate as NEFA, which are a major source of energy to the cow during this period. The concentration of NEFA in blood reflects the degree of adipose tissue mobilization (Pullen et al., 1989). Therefore, as negative energy balance increases, more NEFA are released from body fat and the concentration of NEFA in the blood increases. Based on the findings of this study, the highest levels of NEFA were seen in early lactation groups which were in negative energy balance, physiologically. Serum concentrations of NEFA were decreased to the lowest levels in the far-off dry period.

Adipose tissue depots in the cow were oriented toward mobilization of NEFA at this time, rather than lipid deposition (McNamara, 1991). Lipogenesis (fat synthesis) was essentially shut down, and sensitivity to lipolytic signals (epinephrine and norepinephrine) was greatly enhanced.

As the concentration of NEFA in blood increases around calving or in early lactation, more NEFA are taken up by the liver (Emery et al., 1992). Once taken up by the liver, NEFA can be completely oxidized to carbon dioxide to provide energy for the liver, partially oxidized to produce ketone bodies that are released into the blood and serve as fuels for other tissues, or reconverted to storage fat. If NEFA uptake by the liver becomes excessive, fatty liver may develop. Negative energy balance and carbohydrate insufficiency in the liver after calving leads to increased production of ketone bodies such as BHBA, which can result in ketosis. According to Grummer et al. (2004), the increase in plasma NEFA concentration led to increase in ketogenesis by hepatocytes. The metabolic adaptations that support the onset of lactation include increased mobilization of fatty acids from adipose tissue and increased hepatic gluconeogenesis (Bell, 1995).

Overfeeding during the dry period results in increased esterification rates in adipose tissue prepartum and greater lipolytic rates (and thus higher NEFA) post-partum (Rukkwamsuk et al., 1999). During late pregnancy, metabolic demands shift to fetal growth and the tissues of the dam become increasingly insulin resistant, in order to supply substrates for the fetus while maternal tissues increase reliance on NEFA and BHBA (Bell, 1995). These changes in metabolic priorities might help to explain why far-off diets seem to have a more lasting effect on energy balance and metabolic health in early lactation.

The highest levels of BHBA were seen in early lactation cows and these concentrations decreased to the lowest levels at the far-off dry period. Excessive elevation of NEFA and BHBA during the close-up dry period and early lactation caused adipose tissues to be more insulin resistant, further drive down dry matter intake, and perpetuate a cycle of metabolic disorders in these cows (Pires et al., 2007).

Ruminants have an inherently low capacity for synthesis and secretion of VLDL to export TG from the liver (Pullen et al., 1989), and a similar capacity to reconvert NEFA back to TG (Graulet et al., 1998). Moreover, the rate of production of TGs in the liver increased at the time of calving (Grum et al., 1996). Consequently, cows fed typical diets during the dry period and transition period had an increased concentration of TG in the liver, a day after calving (Grum et al., 1996). Total cholesterol in the mild lactation cows was significantly
higher than other groups (p<0.05; Table 1). Probably because, during the puerperal period, there was an increase in the demands for regulatory mechanism, responsible for all the processes involved with milking (Krajnicakova et al., 2003). At this purpose, characteristic changes in lipid metabolism were found during pregnancy and lactation in most mammals (Roche et al., 2009). Endocrine profiles changed and lipolysis as well as lipogenesis were regulated to increase lipid reserve during pregnancy, and, subsequently, these reserves were utilized following parturition and the initiation of lactation (Roche et al., 2009). Similar results, however, were found by other researchers, showing that concentrations of total lipid increased at parturition, despite the kind of feed administered (Douglas et al., 2004).

The results of the present study showed that metabolic biomarkers change in different physiological states of high producing Holstein dairy cows. These changes are commonly induced by negative energy balance, lactogenesis and fetal growth in each state. The presented metabolic profile can be considered as a tool, to assess the energy balance in dairy cows at different physiological states. It can be used to evaluate the metabolic situations of herd and manage the metabolic and production disorders. Information regarding the dynamics of metabolic profile can aid veterinarians to evaluate and follow the metabolic status of dairy cows to carry out diagnosis, prognosis, treatment and control of metabolic disorders.

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References

12. Fiore, E., Gianesella, M., Arfuso, F., Giudice, E., Piccione, G., Lora, M., Stefani, A., Mor-


پروفایل گلوکوز، انسولین، اسیدهای چرب غیر استریفیه، بتا-هیدروکسی بوتیریک اسید و پروفوایل چربی سرم در دوره‌های مختلف تولیدی گاوها هنگام شیرگیری پرتولید

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چکیده

پروفایل متابولیک به منظور به‌پیشنهاد گروه گاوهای شیری مورد استفاده قرار گرفته است. این پروفایل ممکن است در تشخیص بیماری‌های تحت بالینی، اگرچه از عوامل محتملی نابایری در گلماها یا بهبود نظارت بر حیوانات گلما یا پای رسان باشد. هدف مطالعه حاضر به‌منظور دستیابی به تصویری از تغییرات پروفایل متابولیک در جریان گردش خون گاوها هنگام شیرگیری پرتولید از ابتدای شیرگیری تا انتهای دوره‌های مختلف تولیدی در حالی که واردهای انتها خشکی و ابتدای شیرگیری در بیمارستان مشاهده شدند. گروه‌های گاوها به جریان انسولین، اسیدهای چرب غیر استریفیه، بتا-هیدروکسی بوتیریک اسید (BHBA)، نیتروژن قلی، سولفات (NEFA) و لیپوپروتئین‌ها (LDL، HDL، VLDL) طبق باور در آزمایش‌های قبلی با کاهش تولید شیر و افزایش پنتونید در ابتدای شیرگیری پرتولید، احتمالاً صورت می‌گیرد.

نتایج:

نتایج مطالعه حاضر نشان داد که افزایش این بیومارکرهای گلوکوز در گروه‌های ابتدای شیرگیری و انتهای دوره‌های مختلف تولیدی معنی‌دار بود. نتایج نشان داد که نزدیکی بیومارکرهای مختلف در دوره‌های مختلف تولیدی به نظر می‌رسد و این می‌تواند الگویی مفید در اتخاذ استراتژی‌های مدیریتی در دوره‌های مختلف گاو‌ها باشد.

واژه‌های کلیدی: الگوی تغییرات، شیرگیری، رشد جنین، گاو‌های شیری هلشتنای، پروفایل متابولیک

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