Circulating Metabolic Profile of High Producing Holstein Dairy Cows

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Abstract

Assessing the metabolic profile based on the concept that the laboratory measurement of certain circulating components is a tool to evaluate metabolic status of dairy cows. Veterinarian also can evaluate the energy input-output relationships by assessing the metabolic profile to prevent and control of negative energy balance, metabolic disorders and nutritional insufficiencies. In the present study, 25 multiparous Holstein dairy cows were divided to 5 equal groups containing early, mid and late lactation, and far-off and close-up dry. Blood samples were collected from all cows through jugular venipuncture and sera were evaluated for glucose, insulin, β-hydroxybutyric acid (BHBA), non-esterified fatty acid (NEFA), cholesterol, triglyceride (TG), high, low and very low density lipoproteins (HDL, LDL and VLDL). Insulin levels in mid lactation and close-up dry cows were significantly higher than other groups (P<0.05) and the lowest insulin concentration was detected in far-off dry group. Serum concentrations of NEFA and BHBA in early and mid-lactation and close-up dry cows were significantly higher than late lactation and far-off dry animals (P<0.05). Baseline levels of cholesterol in mid and late lactation were significantly higher than other groups. The level of LDL in mid lactation cows was higher than others significantly, and its value in far-off dry cows was significantly lower than other group (P<0.05). It may be concluded that the detected changes among different groups induce commonly 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 physiologic states. It can be used to evaluate the metabolic situations of herd and manage the metabolic and production disorders.

Özet

Yüksek Verimli Holçayın İneklerin Metabolik Profil Dönüşü

Dolaşmındaki belirli bileşenlerin laboratuar ölçümleri ile metabolik profilin değerlendirilmesi, süt sığırının metabolik durumunu belirlemekle bir arada. Negatif dengeyi, metabolik hastalıkları ve besleme yetersizliklerini engellemek ve kontrol etmek için veteriner hekimler enerji giriş-çıkış ilişkisini metabolik profilin değerlendirilmesi ile yapabilirler. Bu çalışmada çoklu doğum sahibi 25 sütü Holçayın inekineği laktasyon başlangıç, ortası ve yakın kuru koyun olmak üzere eşit 5 gruba ayrılmıştır. Kan örnekleri tüm sütçü laktasyon ile alınmış ve sütçü sütülerin ve lezyonlara kanamışdır ve serumdan glukoz, insülin, β-hidroksiübüratik asit (BHBA), esetlerleme mümüssü yağ asitleri (NEFA), kolesterol, triglicerit (TG), yüksek, düşük ve daha düşük yoğunlukta lipoproteinler (HDL, LDL ve VLDL). Laktasyonun ortasındaki ve yakın kuru döngemeksi nektelemler diğer gruplardan çok daha yüksek (P<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. Laktasyon başlangıcında ve ortasındaki ve yakın kuru sıcaklıkta ineklerde NEFA ve BHBA serum konsantrasyonlar daha yüksek (p<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. Orta laktasyonun ortasındaki ve yakın kuru sıcaklıkta ineklerde yüksek (p<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. Orta laktasyonun ortasındaki ve yakın kuru sıcaklıkta ineklerde NEFA ve BHBA serum konsantrasyonları daha yüksek (p<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. Orta laktasyonun ortasındaki ve yakın kuru sıcaklıkta ineklerde yüksek (p<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. Orta laktasyonun ortasındaki ve yakın kuru sıcaklıkta ineklerde NEFA ve BHBA serum konsantrasyonları daha yüksek (p<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. 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Introduction

From several decades ago, the metabolic profile has been used as a tool for assessing metabolic status of dairy cows (Payne et al., 1970). The metabolic profile is based on the concept that the laboratory measurement of certain circulating components which reflect the nutritional status of the animal, with or without presence of clinical abnormalities. Evaluating metabolic profile is considered as a reliable tool for veterinarian to assess the input-output (nutrient-productivity) relationships (Ghanem et al., 2012). Negative energy balance is defined as disproportion between input-output relationships which can cause metabolic diseases. Metabolic diseases are created due to an imbalance between the rates of input of dietary nutrients and output of production (Ghanem et al., 2012). When the imbalance is continued, it may lead to change in the amount of body reserves of certain metabolites (Radostitis 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, clinical ketosis, and abomasal displacement (Goff and Horst, 1997). A severe negative energy balance during some physiological 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).

Metabolic profile can be also a reliable test to 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 (Radostitis et al., 2007). Therefore, information regarding circulating metabolic profile is considered as a useful diagnostic aid to uncover the problems in difficult herd situations (Gávan et al., 2010). Furthermore, metabolically superior cows can be identified via evaluating their circulating metabolic profile (Rowlands et al., 1973).

There are several literatures on metabolic profile in transition and preparturition periods in dairy cows (Ghanem et al., 2012; Fiore et al., 2014; Piccione et al., 2012) but based on the author’s knowledge, information about circulating metabolic profile in different physiological states of high producing Holstein dairy cows is lacking. Determining the normal base line values of circulating metabolic profile 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 at winter 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 National Research Council (NRC) requirements. At this farm, a dry period of 60 days has been considered. Milk production was about 10,000 kg for year, an average of 3.6 of milk fat %, and 3.3 of milk protein %. All the animals were clinically healthy, had not history of debilitating disease, and free from internal and external parasites due to routine antiparasitic programs at this farm. Body condition score (BCS) of these animals were estimated based on 0 to 5 system. 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), and 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 minutes 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). β-hydroxybutyric acid (BHB), non-esterified fatty acid (NEFA) were assayed by colorimetric method (Ranbut®, Ireland). The sera were analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Abell 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 ultracentrifugation. 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 sedimented by centrifugation (10,000×g for 5 min). The residual cholesterol was then measured by the 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 in the 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 among the averages of concentrations of serological factors in different groups were analyzed by one-way ANOVA and the least significant difference 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**

Normal levels (Mean±SD) of circulating metabolic biomarkers in different physiological states of high producing Holstein dairy cows are presented in Table 1. Insulin levels in mid lactation and close-up dry cows were significantly higher than other groups (P<0.05) and the lowest insulin concentration was detected in far-off dry group. Serum concentrations of NEFA and BHBA in early and mid-lactation and close-up dry cows were significantly higher than late lactation and far-off dry animals (P<0.05). But, there were no significant difference between late lactation and far-off dry groups. Baseline levels of cholesterol in mid and late lactation were significantly higher than other groups. The level of LDL in mid lactation cows was higher than others significantly, and its value in far-off dry cows was significantly lower than other group (P<0.05). The baseline values of glucose, TG, HDL and VLDL were not significantly differed among all studied animals (P>0.05; Table 1).

**Table 1.** Normal levels (Mean±SD) of circulating metabolic biomarkers in different physiological states of high producing Holstein dairy cows (n=5 in each group).

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Early lactation</th>
<th>Mid lactation</th>
<th>Late lactation</th>
<th>Far-off dry</th>
<th>Close-up dry</th>
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<tr>
<td>Glucose (mg/dL)</td>
<td>88.75±19.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.15±11.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.79±50.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.45±50.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.47±34.60&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Insulin (µU/mL)</td>
<td>29.07±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.80±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.33±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.05±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29.86±0.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.34±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.31±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.24±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.29±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>BHBA (µmol/L)</td>
<td>922.80±37.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>830.40±49.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>754.40±44.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>668.50±44.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>862.40±31.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>153.26±32.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>194.97±25.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>190.26±39.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>155.43±29.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.26±24.27&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TG (mg/dL)</td>
<td>123.40±21.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.60±8.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.80±10.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>115.50±5.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113.40±21.24&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>HDL (mg/dL)</td>
<td>100.96±22.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>106.80±18.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112.20±15.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>116.75±14.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>98.00±18.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>LDL (mg/dL)</td>
<td>57.50±17.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.40±19.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.20±19.77&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.50±17.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>50.00±14.67&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>VLDL (mg/dL)</td>
<td>24.68±4.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.32±1.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.80±2.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.10±1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.68±4.24&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a,b,c</sup>Different letters indicate significant differences in each row (P<0.05).

**Discussion**

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, the regulation of insulin secretion differs from that in non-ruminants wherein short-chain fatty acids at supraphysiological concentrations are more potent stimulators of insulin secretion than is glucose (Brockman, 1995). It was suggested that elevated free fatty acids concentration during lactation might interfere with glucose-induced insulin secretion (Bossaert et al., 2008). Therefore, it has been reported that elevated circulating free fatty acids levels is one of the factors that may account for the impaired hepatic insulin extraction in non-ruminants (Lewis et al., 2002). Thus, it is likely that negative energy balance of particularly high free fatty acids value observed during the periparturient period may be a key factor triggering low glucose tolerance in dairy cows (Terao et al., 2010).

The results of the present study showed that the highest levels of insulin were seen in mid lactation and close-up dry cows in comparison to other groups. Other researchers also detected this difference between lactating pregnant and non-lactating pregnant dairy cows (Sartin et al., 1985). They mentioned that the amount of insulin secreted during infusion of glucose or propionate...
was higher in pregnant cows than in the lactating and non-pregnant non-lactating cows (Sartin et al., 1985).

In early lactation group in this study, the low levels of insulin were detected. Other literature reported that both basal concentration of insulin and insulin response to secretagogues lower in lactating than in dry dairy cows (Sartin et al., 1985) and lower in high-yielding lactating cows than in low yielders (Sartin et al., 1988).

From late pregnancy to early lactation, plasma insulin levels decreases and adipocytes also become insulin resistant (Bell and Bauman, 1997). The end results are increased transcription of lipolytic enzymes (lipoprotein lipase, acetyl CoA carboxylase) and stimulation of glucose transport which is ultimately observed as onset of milk production (Vernon and Pond, 1997).

The highest levels of NEFA and BHBA were detected in early lactation group and their lowest amount was seen in far-off dry cows (P<0.05; Table 1). Fiore et al. (2014) mentioned that low insulin levels during postpartum in early lactation period may be due to a decreased responsiveness of pancreatic β-cells, caused by factors which inhibit the release of insulin, such as the increase of NEFA. NEFA value has an opposite trend respect to insulin. It may be due to the activation of lipid mobilization that represents another metabolic mechanism of adaptation to early lactation period (Piccione et al., 2012). In fact, it is well known that low insulin concentration and reduced insulin sensitivity of the tissues around parturition increase lipid mobilization and induces further raises in plasma NEFA concentrations (Hayirli, 2006).

The BHBA profile followed the same pattern of NEFA concentration. According to Grummer et al. (2004), the increase of plasma NEFA concentration led to the increase of ketogenesis by hepatocytes. Fiore et al. (2014) reported the high levels of BHBA in postpartum dairy cows. According to our results, the decrease of insulin and the increase of NEFA and BHBA may induce the difficulty of dairy cows to balance the input/output of energy demands after parturition.

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 (TGs). Ruminants have an inherently low capacity for synthesis and secretion of VLDL to export TG from the liver (Pullen et al., 1989), but a similar capacity to reconvert NEFA back to TG (Graulet et al., 1998). Moreover, the rate of production of TGs in the liver is increased at the time of calving (Grum et al., 1996). Consequently, cows fed typical diets during the dry period and transition period have an increased concentration of TG in the liver 1 day after calving (Grum et al., 1996). 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, which can result in ketosis (Radosits et al., 2007).

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 is 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 change and lipolysis and lipogenesis are regulated to increase lipid reserve during pregnancy, and, subsequently, these reserves are utilized following parturition and the initiation of lactation (Roche et al., 2009). Similar results, however, were found by other researchers, demonstrating that concentrations of total lipid increased at parturition, despite the kind of fed administered (Douglas et al., 2004).

**Conclusion**

The results of the present study demonstrated that metabolic biomarkers change in different physiologic states of high producing Holstein dairy cows. These changes are induced commonly 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 physiologic states. It can be used to evaluate the metabolic situations of herd and manage the metabolic and production disorders.

**Acknowledgment**

We would like to appreciate Mr. Javad Chalmeh for providing the cows to perform our study.

**REFERENCES**


Hayirli, A., 2006. The role of exogenous insulin in the complex of hepatic lipidosis and ketosis associated with insulin resistance phenomenon in postpartum dairy cattle. Veterinary Research Communications 30, 749-774.


