Araştırma Makalesi   Research Article

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 Holştayn İneklerin Metabolik Profil Döngüsü

Dolaşımdaki belirli bileşenlerin laboratuar ölçümleri ile metabolik profilin değerlendirilmesi, süt sığırlarının metabolik durumunu belirlemede kullanılan bir araçtır. 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 doğuma sahip 25 sütçü Holştayn ineği laktasyon başlangıcı, ortası ve sonu ile uzak ve yakın kuru olmak üzere eşit 5 gruba ayrılmıştır. Kan örnekleri tüm sığırlardan juguler venadan alınmış ve serumdan glukoz, insülin, β-hidroksibütirik asit (BHBA), eseterleşmemiş yağ asitleri (NEFA), kolesterol, trigliserit (TG), yüksek, düşük ve daha düşük yoğunluktaki lipoproteinler (HDL, LDL ve VLDL). Laktasyonun ortasındaki ve yakın kuru dönemdeki ineklerde insülin seviyeleri diğer gruplardan çok daha yüksek (P<0,05) iken en düşük insülin konsantrasyonu uzak kuru grubunda saptanmıştır. Laktasyon başlangıcında ve ortasındaki ile yakın kurudaki ineklerde NEFA ve BHBA serum konsantrasyonları geç laktasyondaki ve uzak kurudaki ineklere göre daha yüksektir (P<0,05). Orta ve geç laktasyonda taban kolesterol seviyeleri diğer gruplara göre önemli düzeyde yüksektir. Orta laktasyondaki ineklerde LDL seviyesi diğerlerine göre önemli düzeyde daha yüksektir ve uzak kurudaki ineklerde ise değeri önemli düzeyde daha düşüktür. Sonuç olarak farklı gruplar arasında saptanan değişikliklerin yaygın nedeni her durumdaki negatif enerji dengesi, laktogenezis ve fötal büyüme olabilir. Mevcut metabolik profil, farklı fizyolojik durumlardaki süt ineklerinde enerji dengesini belirlemek için bir araç olabilir. Sürünün metabolik durumunun değerlendirilmesi, metabolik ve üretim bozukluklarını yönetmede kullanılabilir.

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 (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, 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 (Radostits et al., 2007). Therefore, information regarding circulating metabolic profile is considered as a useful diagnostic aid to uncover the problems in difficult herd situations (Găvan 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), 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 (BHBA), 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).

Tablo 1. Yüksek verimli Holştayn ineklerin farklı fizyolojik dönemlerindeki metabolik döngü biyobelirteçlerin normal değerleri (ortalama±SD) (her grupta n=5).

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

[a,b,c] 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 secretogogues 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 (Radostits 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.

REFERENCES

Abell, L.L., Levy, B.B., Brodie, B.B., Kendall, F.E., 1952. A simplified method for the estimation of total cholesterol in serum and determination of its specificity. The Journal of Biological Chemistry 195, 357-366.

Bell, A.W., Bauman, D.E., 1997. Adaptations of glucose metabolism during pregnancy and lactation. Journal of Mammary Gland Biology and Neoplasia 2, 265-278.

Bossaert, P., Leroy, J.L.M.R., De Vlieger, S., Opsomer, G., 2008. Interrelations between glucose-induced insulin response, metabolic indicators and time of first

ovulation in high-yielding dairy cows. Journal of Dairy Science 91, 3363-3371.

Brockman, R.P., 1995. Pancreatic and Adrenal Hormonal Regulation of Metabolism. In: Control of Digestion and Metabolism in Ruminants. Milligan, L.P., Grovum, W.L., Dobson, A. (Eds), Banff Publishing, Canada, pp. 405-419.

Burtis, C.A., Ashwood, E.R., 1994. Tietz Textbook of Clinical Chemistry. 2nd ed., W. B. Saunders Com, Philadelphia, pp. 735-888.

Douglas, G.N., Overton, T.R., Bateman, H.G., Drackley, J.K., 2004. Peripartal metabolism and production of Holstein cows fed diets supplemented with fat during the dry period. Journal of Dairy Science 87, 4210-4220.

Emery, R.S., Liesman, J.S., Herdt, T.H., 1992. Metabolism of long chain fatty acids by ruminant liver. Journal of Nutrition 122, 832-837.

Fiore, E., Gianesella, M., Arfuso, F., Giudice, E., Piccione, G., Lora, M., Stefani, A., Morgante, M., 2014. Glucose infusion response on some metabolic parameters in dairy cows during transition period. Archiv Tierzucht 57, 1-9.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clinical Chemistry 18, 499-502.

Găvan, C., Retea, C., Motorga, V., 2010. Changes in the hematological profile of Holstein primiparous in peri-parturient period and in early to mid-lactation. Journal of Animal Science and Biotechnology 43, 244-246.

Ghanem, M.M., Mahmoud, M.E., Abd El-Raof, Y.M., El-Attar, H.M., 2012. Metabolic profile test for monitoring the clinical, haematological and biochemical alterations in cattle during peri-parturient period. Banha Veterinary Medical Journal 23, 13-23.

Goff, J.P., Horst, R.L., 1997. Physiological changes at parturition and their relationship to metabolic disorders. Journal of Dairy Science 80, 1260-1268.

Graulet, B., Gruffat, D., Durand, D., Bauchart, D., 1998. Fatty acid metabolism and very low density lipoprotein secretion in liver slices from rats and preruminant calves. The Journal of Biochemistry 124, 1212-1219.

Grum, D.E., Drackley, J.K., Younker, R.S., LaCount, D.W., Veenhuizen, J.J., 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. Journal of Dairy Science 79, 1850-1864.

Grummer, R.R., Mashek, D.G., Hayirli, A., 2004. Dry matter intake and energy balance in the transition period. Veterinary Clinics of North America, Food Animal Practice 20, 447-470.

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.

Kim, I.H., Na, K.J., Yang, M.P., 2005. Immune responses during the peripartum period in dairy cows with postpartum endometritis. Journal of Reproduction and Development 51, 757-764.

Krajnicakova, M., Kovac, G., Kostecky, M., Valocky, I., Maracek, I., Sutiakova, I., Lenhardt, L., 2003. Selected clinic-biochemical parameters in the puerperal period of goats. Bulletin of the Veterinary Institute in Pulawy 47, 177-182.

Lewis, G.F., Carpentier, A., Adeli, K., Giacca, A., 2002. Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocrine Reviews 23, 201-229.

McGowan, M.W., Artiss, J.D., Strandbergh, D.R., Zak, B., 1983. A peroxidase coupled method for the colorimetric determination of serum triglycerides. Clinical Chemistry 29, 538-542.

Payne, J.M., Dew, A.M., Manston, R., Faulks, M., 1970. The use of a metabolic profile test in dairy herds. Veterinary Record 87, 150-158.

Piccione, G., Messina, V., Marafioti, S., Casella, S., Giannetto, C., Fazio, F., 2012. Changes of some haematochemical parameters in dairy cows during late gestation, post partum, lactation and dry periods. Veterinary Medicine and Zootechnics 58, 59-64.

Pullen, D.L., Palmquist, D.L., Emery, R.S., 1989. Effect on days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. Journal of Dairy Science 72, 49-58.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D., 2007. Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs, and goats. 10th ed., Elsevier Saunders, Edinburgh, pp. 1661-1667.

Roche, J.R., Frggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J., Berry, D.P., 2009. Invited review: Body condition score and its association with dairy cow productivity, health and welfare. Journal of Dairy Science 92, 5769-5801.

Rowlands, G.J., Payne, J.M., Dew, S.M., Manston, R., 1973. A potential use of metabolic profiles in the selection of superior cattle. Veterinary Record 93, 48-49.

Sartin, J.L., Cummins, K.A., Kemppainen, R.J., Marple, D.N., Rahe, C.H., Williams, I.C., 1985. Glucagon, insulin, and growth hormone responses to glucose infusion in lactating dairy cows. American Journal of Physiology 248, 108-114.

Sartin, J.L., Kemppaben, R.J., Cllmminn, K.A., Williams, J.C., 1988. Plasma concentrations of metabolichormones in high and low producing dairy cows. Journal of Dairy Science 71, 650-657.

Terao, H., Fujita, M., Tsumagari, A., Sugino, T., Bungo, T., 2010. Insulin dynamics in transition dairy cows as revealed by intravenous glucose tolerance testing. Journal of Animal and Veterinary Advances 9, 2333-2337.

Vernon, R.G., Pond, C.M., 1997. Adaptations of maternal adipose tissue to lactation. Journal of Mammary Gland Biology and Neoplasia 2, 231-241.