

RELATIONSHIP BETWEEN ENDOCRINE PROFILE, ENERGY BALANCE AND MILK YIELD IN DAIRY COWS DURING LACTATION

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Abstract

The objective of the present study was to investigate relationship between endocrine profile with energy balance (EB) and milk yield (MY) in Simmental dairy cows during lactation. Fifteen late pregnant cows, 15 early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood samples were collected to measure growth hormone (GH), insulin, triiodothyronine (T3) and thyroxine (T4). Early lactation cows were found to have higher blood serum concentrations of GH ($p < 0.05$) and lower blood serum concentrations of insulin ($p > 0.05$), T3 ($p < 0.05$) and T4 ($p > 0.05$) compared to dry and mid lactation cows. Insulin and thyroid hormones were in positive correlation with EB ($p < 0.05$), and in negative correlation with MY (nonsignificant). GH was in positive correlation with MY ($p < 0.05$), but in negative correlation with EB (nonsignificant). Relationship between hormones showed significant positive correlation between insulin and thyroid hormones. These relations are not principally determined by EB or MY. Negative correlations between insulin or thyroid hormones with GH were observed. These relations are principally determined by EB or MY.

Key words: *cows, correlation, energy balance, hormones*

Introduction

Negative energy balance in dairy cows during transition period (21 days before and 21 days after calving) leads to many metabolic changes. These metabolic changes occur as a result of the entry of the mammary gland in the metabolic processes. Negative energy balance is the result of decreased food intake, higher consumption of glucose in the udder and decreased insulin sensitivity. These changes are in relation with many endocrine and metabolic adaptations. Characteristic endocrine adaptation is increased concentration of growth hormone (GH), decreased concentration of thyroid hormone (T3 and T4), decreased insulin and IGF-I concentration (Ingvarsten and Andersen, 2000).

Interdependent changes occur in the GH - insulin - IGF-I - glucose signalling pathway in early lactation (Lucy et al., 2001). GH concentration increases at this time; this increase is accompanied by an increase in IGF and IGF binding proteins in mammary secretions, suggesting a role for these factors in mammogenesis and lactogenesis (Tucker, 1994). When cows are in a negative EB, GH stimulates lipolysis; it alters the sensitivity of

adipose tissue to β -adrenergic agents (Bauman and Vernon, 1993). Similarly, plasma concentrations of insulin, another homeostatic hormone, would be changed by prepartum nutrition and this would affect nutrient supply to the udder. Insulin plays a role in the adaptation of organic matter metabolism in dairy cows during the transitional period and lactation, particularly in terms of nutrient redistribution and partitioning towards the mammary gland (insulin resistance) (Butler et al., 2003; Balogh et al., 2008). Thyroid hormones, primarily triiodothyronine (T3), are important in regulating the energy metabolism. Blood levels of thyroid hormones in periparturient cows decrease, particularly in early lactation, when body reserves are mobilized for high milk production (Bonczek et al., 1988; Tiirats, 1997; Huszenicza et al., 2002). Circulating thyroid hormone concentrations correlate positively with EB and negatively with daily MY in cattle (Blum et al., 1983; Tiirats, 1997; Eppinga et al., 1999; Capuco et al., 2001; Reist et al., 2002; Cassar-Malek et al., 2001).

The objective of the present study was to investigate the nutritional, metabolic and endocrine status in Simmental cows during transition period and mid lactation based on the relationships between blood metabolic hormones, EB and MY.

Material and methods

Fifteen late pregnant cows, 15 early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood was sampled from 25 to 1 (13 ± 9) days before partus, in the first month of lactation (16 ± 9 days), and in mid lactation cows between 3 to 5 months of lactation (115 ± 29 days). Blood samples were collected at 10 a.m. by puncture of the jugular vein into sterile disposable test tubes. Blood samples were collected to measure growth hormone (GH), insulin, triiodothyronine (T3) and thyroxine (T4). Serum concentrations of GH, insulin, T3 and T4 were determined by ELISA methods (Endocrine Technologies Inc. CA, USA) using Humareader Single plus (Human, Germany). Diet was suited to the energy requirements of late pregnancy, early and mid lactation cows. Weende methodology was used for the chemical analysis of the feed. Energy balance was calculated by NRC recommendation (2001). Actual energy balance was calculated as a difference between DMI and NEL of the ration offered minus DMI and NEL of the rest of the ration after feeding. Feeding space was provided to each individual cow in order to prevent mixing of their rations. MY was recorded every day by farm software.

Model and statistics: Difference between hormone concentration, EB and MY between three periods of lactation were calculated by ANOVA analysis and posthoc LSD test. Relationship between hormones, EB and MY was calculated by Pearson correlation coefficient. Finally, correlation and partial correlation between metabolic parameters were evaluated by Pearson correlation analysis. Partial correlation analysis is used to examine the correlation between endocrine and metabolic parameters with the effects of EB removed. Software used: Statgraphic Centurion, Statpoint Technologies Inc. Warrenton, Va, Virginia, USA.

Results and discussion

Early lactation cows were found to have higher blood serum concentrations of GH ($p < 0.05$) and lower blood serum concentrations of insulin ($p > 0.05$), T3 ($p < 0.05$) and T4 ($p > 0.05$) compared to dry and mid lactation cows.

Table 1. Blood hormones, energy balance and milk yield in late pregnant, early and mid lactation dairy cows (n=15 in each group). Results are expressed as mean \pm SD.

Parameter	Late pregnant cows	Early lactation cows	Mid lactation cows
GH (ng/ml)	11.4 \pm 8.67 ^a	17.13 \pm 3.87 ^b	11.45 \pm 4.42 ^a
Insulin (ng/ml)	0.55 \pm 0.44 ^a	0.39 \pm 0.21 ^b	0.65 \pm 0.47 ^c
T3 (ng/ml)	0.77 \pm 0.36 ^a	0.73 \pm 0.41 ^b	1.29 \pm 1.01 ^{bc}
T4 (ng/ml)	32.70 \pm 13.67 ^a	31.93 \pm 18.30 ^a	33.06 \pm 17.04 ^a
EB	10.15 \pm 9.5	3.56 \pm 10.19 ^b	12.15 \pm 8.4
MY	/	19.6 \pm 5.5	24.8 \pm 3.2

Values marked by letters (a, b, c) in one row describe significant differences at level $p < 0.05$ or higher

Insulin and thyroid hormones were in positive correlation with EB ($p < 0.05$), and in negative correlation with MY (nonsignificant). GH was in positive correlation with MY ($p < 0.05$), but in negative correlation with EB (nonsignificant).

Table 2. Relationship between hormones, EB and MY.

Parameter	GH	Insulin	T3	T4
EB	-0.26	0.32*	0.3*	0.31*
MY	0.51**	-0.29	-0.19	-0.17

* $p < 0.05$; ** $p < 0.01$

Relationship between hormones showed significant positive correlation between insulin and thyroid hormones. These relations are not principally determined by EB or MY. Negative correlations between insulin or thyroid hormones with GH were observed. These relations are principally determined by EB or MY.

Table 3. Relationship (correlation coefficients) between hormones in function of energy balance and milk yield.

Parameter	Insulin	GH
T3	0.35*	-0.21
	0.32*	-0.32*
	0.28	-0.34*
T4	0.37*	-0.26
	0.3*	-0.33*
	0.29	-0.31*
Insulin	/	-0.29
		-0.55**
		-0.33*

^a correlation between parameters after exclusion of EB or MY; ^b correlation between parameters controlled by energy balance; ^c correlation between parameters controlled by milk yield; * $p < 0.05$; ** $p < 0.01$

GH is a homeorhetic controller of metabolism, shifting the partitioning of nutrients between the various parts of the body during late pregnancy and lactation (Bonczek et al., 1988; Lucy et al., 2001). The transition and early lactation periods were considered as time periods that have the potential to enhance lactation performance. In the current study, early lactation cows had significantly higher GH levels than late pregnant and mid lactation

cows. GH dramatically increases lipid mobilization from the adipose tissue, and increases blood NEFA and BHB in early lactation cows (Tucker, 1994; Jindal and Ludri, 1994). In this study, GH was significantly positively correlated with MY and NEFA, but negatively with EB and DMI. These correlations have been reported by other authors (Jindal and Ludri, 1994; Balogh et al., 2008) and show that under NEB conditions, blood GH concentration increases what results in fat lipomobilisation, and stimulates MY in dairy cows during lactation. Therefore, lipolysis must be an important pathway to provide the precursors needed in the early postpartum period of cows especially to supply the energy required for milk production (Bonczek et al., 1988; Bauman and Vernon, 1993; Butler et al., 2003).

Insulin has an important homeostatic effect in regulating lipid metabolism. GH inhibits the ability of insulin to initiate lipogenesis in adipose tissue. Accordingly, GH reduces the action of insulin, restricts lipogenic enzyme activity, and reduces glucose utilization (Balogh et al., 2008). Blood insulin levels during the same period were non-significantly lower in early lactation cows than in late-pregnant and mid lactation cows. A significantly positive correlation coefficient between insulin and EB and DMI and a negative but non-significant coefficient with NEFA and BHB were obtained. These relationships were found in earlier studies (Jindal and Ludri, 1994; Xia et al., 2007) and are due to increased fat mobilization during insulin insufficiency. The decrease in blood insulin levels under NEB, reduced DMI and high blood GH values can cause an increase in blood NEFA and BHB levels, suggesting that the reduced anabolic effect of insulin on lipid metabolism leads to sudden uncontrolled mobilization of NEFA from body reserves, and ketogenesis in the liver. Similar results were reported elsewhere (Bonczek et al., 1988; Veenhuizen et al., 1991; Jindal and Ludri, 1994; Butler et al., 2003; Remppis et al., 2011).

Thyroid hormones, particularly T3 whose activity is 4 times greater than that of T4, are of importance in adapting the endocrine system during lactation, since their very low blood levels in transitional cows lead to a decrease in energy metabolism, mobilization of body fat reserves and their partitioning toward high milk production (Tiirats, 1997; Cassar-Malek et al., 2001; Huszenicza et al., 2002). This also involves disorders of metabolic balance and uncontrolled mobilization of lipids which, apart from being used for milk synthesis, very often remain within parenchymatous organs, liver, in particular. Blood levels of T3 and T4 in this experiment were lower in puerperal cows than in late pregnant and mid lactation cows, and exhibited a generally significantly positive correlation with EB and DMI, but a negative non-significant correlation with NEFA and BHB. These findings are consistent with those of other authors (Aceves et al., 1985; Jindal and Ludri, 1994; Tiirats, 1997; Eppinga et al., 1999; Capuco et al., 2001; Huszenicza et al., 2002; Đoković et al., 2007) suggesting that blood levels of thyroid hormones decrease in puerperal cows, particularly in those suffering from metabolic disorders, under marked NEB which involves increased mobilization of NEFA from body reserves.

Conclusion

Endocrine changes during lactation are in relation with energy balance and milk yield. Relationship between insulin and thyroid hormones was not controlled by milk yield and energy balance. Contrary to that, negative correlations between insulin or thyroid hormones with growth hormone are principally determined by EB or MY.

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References

1. Aceves C, Ruiz A, Romero C and Valverde C 1985. Homeorhesis during early lactation. Euthyroid sick-like syndrome in lactating cows. *Acta Endocrinol. (Copenh)* 110, 505-509.
2. Balogh O, Szepes O, Kovacs K, Kulcsar M, Reiczigel J, Alcazar JA, Keresztes M, Febel H, Bartyik J, Gy. Fekete S, Fesus L and Huszenicza Gy 2008. Interrelationships of growth hormone Alu I polymorphism, insulin resistance, milk production and reproductive performance in Holstein-Friesian cows. *Vet. Med.* 53, 604-616.
3. Bauman DE and Vernon RG 1993. Effects of bovine somatotropin on lactation. *Ann. Rev. Nutr.* 13, 437-461.
4. Blum JW, Kunz P, Leuenberger H, Gautschi K and Keller M 1983. Thyroid hormones, blood plasma metabolites and hematological parameters in relation to milk yield in dairy cows. *Anim. Prod. Sci.* 36, 93-104.
5. Bonczek RR, Young SW, Wheaton JE and Miller KP 1988. Responses of somatotropin, insulin, prolactin and thyroxine to secretion for milk yield in Holsteins. *J. Dairy Sci.* 71, 2470-2475.
6. Butler ST, Marr AL, Pelton SH, Radcliff RP, Lucy MC and Butler WR 2003. Insulin restores GH responsiveness during lactation-induced negative energy balance in dairy cattle: Effects on expression of IGF-I and GH receptor 1A. *J. Endocrinol.* 176, 205-217.
7. Capuco AV, Wood DL, Elsasser TH, Kahl S, Erdman RA, Van Tassell CP, Lefcourt A and Piperova LS 2001. Effect of somatotropin on thyroid hormones and cytokines in lactating dairy cows during ad libitum and restricted feed intake. *J. Dairy Sci.* 84, 2430-2439.
8. Cassar-Malek I, Kahl S, Jurie C and Picard C 2001. Influence of feeding level during postweaning growth on circulating concentrations of thyroid hormones and extrathyroidal 5'-deiodination in steers. *J. Anim. Sci.* 79, 2679-2687.
9. Djoković R, Šamanc H, Jovanović M and Nikolić Z 2007. Blood concentrations of thyroid hormones and lipids in the liver in dairy cows in transitional period. *Acta Vet. Brno* 76, 525-532.
10. Eppinga M, Suriyasathaporn W, Kulcsar M, Huszenicza Gy, Wensing T and Dieleman SJ 1999. Tyroxin and triiodothyronine in association with milk yield, β OH-butyrate, and non-esterified fatty acid during the peak of lactation. *J. Dairy Sci.* 82, 50-56.
11. Huszenicza Gy, Kulcsar M and Rudas P 2002. Clinical endocrinology of thyroid gland function in ruminants: A review of literature. *Vet. Med.* 47, 191-202.
12. Ingvarstsen KL and Andersen HR 2000. Integration of Metabolism and Intake Regulation: A Review Focusing on Periparturient Animals. *J. Dairy Sci.* 83, 1573-1597.
13. Jindal SK and Ludri RS 1994. Relationship between some hormones, metabolites and milk yield in lactating crossbred cows and buffaloes. *Asian-Aust J Anim Sci.* 7, 239-248.
14. Lucy MC, Hauser SD, Eppard PJ, Krivi GG, Clark JH, Bauman DE and Collier RJ 2001. Variants of somatotropin in cattle: gene frequencies in major dairy breeds and associated milk production. *Dom. Anim. Endocrinol.* 10, 325-333.
15. Reist M, Erdin D, von Euw D, Tschuemperlin K, Leuenberger H, Delavaud C, Chilliard Y, Hammon HM, Morel C, Philipona C, Zbinden Y, Kuenzi N and Blum JW 2002. Estimation

- of energy balance at the individual and herd level using blood and milk traits in high-yielding dairy cows. *J. Dairy Sci.* 85, 3314-3327.
16. Remppis S, Steingass H, Gruber L and Schenkel H 2011. Effects of energy intake on performance, mobilization and retention of body tissue, and metabolic parameters in dairy cows with special regard to effects of pre-partum nutrition on lactation. *Asian-Aust J. Anim. Sci.* 24, 540-572.
 17. Tiirats T 1997. Thyroxine, triiodothyronine and reverse-triiodothyronine concentrations in blood plasma in relation to lactational stage, milk yield, energy and dietary protein intake in Estonian dairy cows. *Acta Vet. Scand.* 38, 339-348.
 18. Tucker HA 1994. Lactation and its hormonal control. In *The Physiology of Reproduction* (ed. E. Knobil and J.D. Neill), pp. 2235–2263. Raven Press Ltd, New York.
 19. Veenhuizen JJ, Drackley JK, Richard MJ, Sanderson TP, Miller LD and Young JW 1991. Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. *J. Dairy Sci.* 74, 4238-4253.
 20. Xia C, Wang Z, Li YF, Niu S, Xu C, Zhang C and Zhang HY 2007. Effect of hypoglycemia on performances, metabolites, and hormones in periparturient dairy cows. *Agri Sci China* 6, 505-512.