BLOOD METABOLITES OF ESTONIAN HOLSTEIN COWS AND THEIR RELATION TO SOME FERTILITY PARAMETERS

K. Ling, H. Jaakson, J. Samarütel, A. Leesmäe

ABSTRACT. Blood metabolites of Estonian Holstein cows and their relation to some fertility parameters. *The objective of the study was to analyse relationships between some metabolites associated with cows energy and/or protein status and fertility parameters on two Estonian commercial farms. Farm or stage of gestation and/or lactation were the factors affecting urea, cholesterol, triglycerides, glucose, ketone bodies, NEFA and total lipids concentrations and AST activity. Service period was longer on farm B but there was no difference between the farms in days to first service. Correlations with fertility parameters were positive in the case of AST, GLDH, cholesterol and total lipids. Negative correlations had urea, glucose, ketone bodies, triglycerides and NEFA. The data from the two farms indicate that increased urea and ketone body levels may be potential risk factors of impaired fertility. Further investigations including progesterone profile analysis are needed to differentiate factors influencing intervals from calving to first ovulation and from first ovulation to actual conception.*

Keywords: *Estonian Holstein cow, fertility, metabolic status, energy balance, blood metabolites, aspartate aminotransferase – AST, glutamate dehydrogenase – GLDH, urea - UREA, glucose – GLC, ketone bodies – KB, triglycerides – TG, non-esterified fatty acids – NEFA, cholesterol – CHOL, total lipids – TL .*

Introduction

Several factors and interactions of the factors like nutrition, metabolic state, production, health and management affecting dairy cows fertility are reported in a number of investigations (Roxström *et al*., 2001; Clark *et al*., 2000; Mwaanga and Janowski, 2000; Mihm, 1999; Kruip *et al*., 1998). Already in dry period and still more in early lactation, changes take place in the endocrine system of cows, intensifying gluconeogenesis, lipolysis and ketogenesis. To cover the increased energy and glucose requirements, high-producing cows intensively utilise their fat deposits, negative energy balance being universal among them. Ketone bodies synthesised in liver in the course of incomplete oxidation of released fatty acids, particularly acetoacetic and β-hydroxybutyric acids, are essential energy sources in many tissues, and are saving glucose for lactose synthesis (Herdt, 2000). The detrimental effects of negative energy balance in early lactation appear to be manifested as reduced fertility during the breeding period.

The objective of our study was to found out the dynamics of some metabolites associated with energy and/or protein status as well as to analyse relationships between these metabolites and some fertility parameters on two high production level commercial farms in Estonia.

Materials and Methods

The study was carried out on two Estonian commercial farms (farm A about 250 and farm B about 1000 dairy cows) where cows were kept in tie-stall barns. On farm A data were collected from 1999 to 2001, on farm B from 1999 to 2000. Seasonal effects were minimized as on both farms most of the cows calved during a 90-day period from January till March. The cows were fed according to Estonian feeding standards, they were milked twice a day. The average milk yield on farm A was 6791, 7467 and 8665 kg energy corrected milk in 1999, 2000 and 2001 respectively. On farm B the average milk yield was 5916 kg energy corrected milk in 1999 and 6791 in 2000. On farm A 63 2–8 lactation (mean 3.46) cows and on farm B 62 1–8 lactation (mean 3.11, 6 first lactation heifers) cows were included in the final data analyses.

The cows of the farm A were fed silage, hay and straw in different combinations during the period of the study, 350–400 g concentrates + ensiled crushed grain or ray bran per kg of produced milk was added to the ration. All the cows were supplemented with mineral feed. The ration on farm B consisted of silage and 450 g concentrates per kg of milk.

Blood samples were taken from jugular vein (farm A in 1999) or coccygeal vein or artery during the following five stages of gestation or/and lactation: last 14 days of gestation (1–14 days before calving – DBC), first 14 days of lactation (1–14 days after calving – DAC); 28–42, 63–77 and 117–151 days after calving (DAC). Sera was separated as soon as possible and kept frozen at –20° C till analysing. The concentrations of metabolites and the activities of enzymes (aspartate aminotransferase – AST, glutamate dehydrogenase – GLDH, urea – UREA, glucose – GLC, ketone bodies – KB, triglycerides – TG, non-esterified fatty acids – NEFA,

cholesterol – CHOL, total lipids – TL) were measured spectrophotometrically. Glucose concentration in blood serum was determined according to Somogyi-Nelson (Lutskii *et al*., 1978), non-esterified fatty acids (NEFA) concentration according to a modified Liunggereni-Perason (Lutskii *et al*., 1978) and ketone bodies concentration according to Trubka (Trubka, 1974) method. AST and GLDH activity and UREA concentration were determined using an enzymatic-UV-kinetic method (Human Gesellschaft für Biochemica und Diagonostica GmbH test kits). Concentrations of triglycerides and total cholesterol were determined using enzymatic-colorimetric end-point method (Human Gesellschaft für Biochemica und Diagonostica GmbH test kits). Coefficients of variation of the methods were below 25%, the acceptable limit in clinical chemistry according to J. Lumsden (1998). The only exception was NEFA with 33.8%.

On both farms the management decision was to start insemination of cows not less than 50 days after calving.

Rough estimation of the energy balance (EB) of the cows was performed using intake data available from the farms. The intakes of the cows on farm A were estimated using everyday observations. Silage intake on farm B was estimated using an equation describing the correlation of intake (I) with silage dry matter (SDM) and organic matter content (SOM), intake of concentrates(CI), cow's body weight (BW) and energy corrected milk production (ECM), worked out at the department of feeding of the Institute of Animal Science, Estonian Agricultural University: $I = -14.2 + (0.00643 \times BW) + (0.149 \times SDM) + (0.319 \times SOM) + (0.112 \times ECM) + (0.291 \times CI)$ (Kärt, personal communication). In all feeds the content of dry matter, crude protein, crude fibre, crude fat, ether extract and major minerals were determined in the chemistry laboratory of Department of Animal Nutrition, Institute of Animal Science, Estonian Agricultural University. Contents of metabolizable energy (ME) and digestible protein (DP) in the feeds were calculated according to national feeding standards. Hygienic quality of ensiled feeds was evaluated. Cows on farm A received 8,31 MJ ME and 75.6 g of DP per kg of ECM milk, on farm B the respective figures were 5,88 and 73.5.

Control milking was performed twice a month on farm A and once a month on farm B. Data of milk recording from milk laboratory of Estonian Agricultural Register's and Information Centre were used to calculate energy corrected milk production for the of one or two preceding 14 days period for the farms A and B respectively. The above mentioned data were used to calculate the energy balance of the cows. In the context of our study energy balance is defined as energy intake minus energy requirements for a given yield and maintenance expressed per day for 14-day periods.

Analysis of variance, T-test and correlation analysis were used to evaluate the relationships between different parameters (SAS Systems and Excel statistical tools). To interpret results the following criteria of significance were used – significant ($P<0.05$), tendency (trend) to significance ($P<0.1$) and not significant ($P\geq0.1$). Natural logarithms were used if appropriate.

Results

Analysis of variance showed the significance ($P<0.001$) of the model with farm and stage of gestation and /or lactation as factors in the case of investigated blood metabolites and enzymes, except GLDH ($P<0.1$). Both factors individually as well as their interaction had significant influence ($P<0.05$) on CHOL, TG, GLC, KB and TL. Farm and stage of gestation and/or lactation separately influenced AST values ($P<0.01$). The influence of the stage of gestation and/or lactation was significant on NEFA ($P<0.001$) and the factors interaction on the UREA concentration ($P<0.001$).

Amongst investigated enzymes, especially AST activities throughout all periods expressed differences between farms (Figure 1). The values on farm B exceeded those of farm A. General trend of increase was evident during all the stages of gestation and/or lactation. GLDH expressed increase only on farm A. *Pre partum* activity on the farm tended to be lower, but exceeded farm B values 63–77 DAC.

UREA concentration changes outlined clearly in a different way on two farms. Before calving UREA concentration was higher on farm A. The following increase on farm B and decrease at least up to 28–42 DAC on farm A led to subsequent opposite difference between the farms.

The concentration curves of GLC and KB had opposite shapes. On both farms GLC nadir occurred during periods 1–14 DAC and 28–42 DAC with concurrent KB topmost level, that being significantly higher on farm B. GLC *periparturient* level was higher on farm B, although recovery from the nadir began earlier on farm A, 117–151 DAC GLC was again higher on farm B. NEFA and TG curves have similar opposite appearance as GLC and KB do. NEFA concentration had already risen *pre partum* and declined in the course of lactation. TG dynamics on farm A and B as well as differences between the farms and earlier recovery on farm A is very similar to GLC.

Increase in CHOL concentration up to 28–42 DAC was parallel on two farms. On farm A CHOL level continued to rise up to 63–77 DAC exceeding that of farm B by then. TL *pre partum* was lower on farm A but starting from 28–42 DAC exceeded that of farm B.

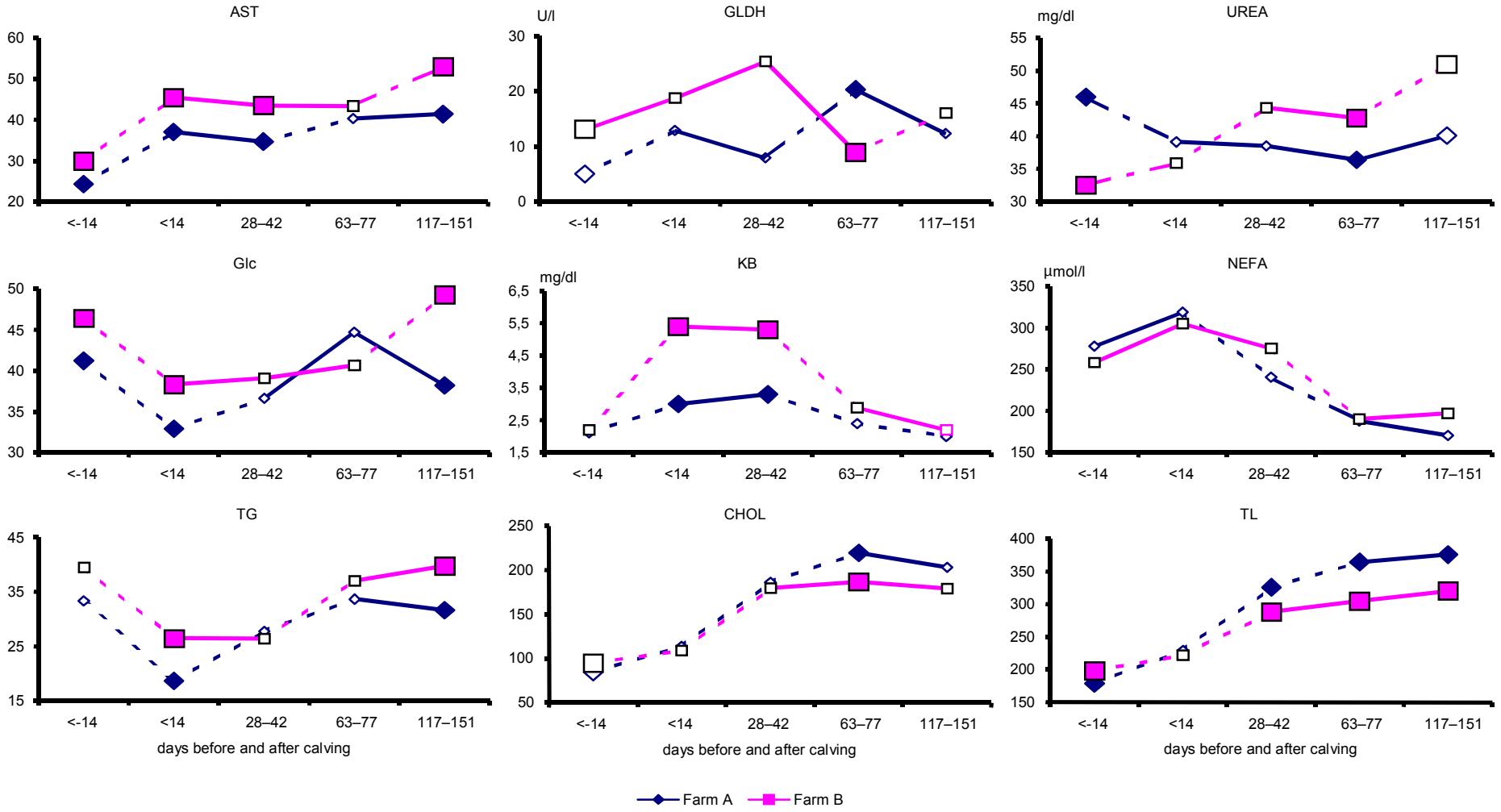

Figure 1. Dynamics of enzyme activities and metabolites concentrations on Farm A and B. Dotted lines indicate significant differences between stages and dashed-dotted lines tendency to differ. Big legend markers show differences between farms (filled: P<0.05, not-filled: P<0.1). Small legend markers indicate insignificant differences.
Joonis 1. *Ensüümiaktiivsuste ja metaboliitide kontsentratsioonide dünaamika farmides A ja B. Punktiirjoon näitab sigimistsükli staadiumite vahelist olulist erinevust (P<0,05), kriipspunktjoon suundumust erinevusele (P<0,1). Suured märgised osutavad farmidevahelisele erinevusele (täidetud: P<0,05, tühjad: P<0,1), väikesed märgised erinevuse puudumisele.*

Fertility parameters on farms are given in Table 1. Average interval CFS as well as SP was shorter on farm A, however, only service period difference being significant (P<0.05). Average interval between the first and last service was 29.4 days on farm A and 70.1 days on farm B the difference being significant (P<0.001). On farm A positive correlation (r=0.24; P<0.05) was observed between the interval from calving to first service and service period. Conception rate at first service was 52% on farm A, 28.6% on farm B. On farm A the number of services per conception was 2.0, on farm B 2.5.

Table 1. Fertility parameters on farms A and B
Tabel 1. *Sigimisnäitajad farmides A ja B*

Farm/*Farm*	A			B		
Parameter/*Näitaja*	$\bar{x}$ ± sd	n	Min-max	$\bar{x}$ ± sd	n	Min-max
Interval calving to first service (CFS) *Ajavahemik poegimisest esimese seemenduseni*	72.0±18.26	50	46–126	76.1±18.1	52	50–135
Service period (SP) / *Servisperiood*	100.8±44.63*	49	46–223	146.7±77.3*	48	56–458

* – significant difference between the farms (P<0.05)
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Correlations between fertility parameters and enzymes/metabolites as well as between individual enzymes/metabolites occurred in several cases, more clearly on Farm A (Tables 2 and 3). GLDH activity 1–14 DBC correlated with interval CFS. The same tendency was seen in GLDH activity 63–77 DAC. UREA concentration 28–42 DAC had a trend to correlate negatively with interval CFS. CHOL and TL correlated with interval CFS during stages 1–14 DBC and 28–42 DAC and between service period during 117–151 DAC.

Moreover, CHOL 63–77 DAC correlated with interval CFS. GLC concentration had a tendency to correlate negatively with both investigated fertility parameters during the stages 28–42 DAC and 63–77 DAC. There was a negative correlation between KB 1–14, and 28–42 DAC and CFS and between KB 1–14 DBC, 28–42 and 63–77 DAC and service period.

Table 2. Correlation coefficient between interval calving to first service and blood enzymes/metabolites of cows on farm A and B
Tabel 2. *Lehmade vere ensüümiaktiivsuste/metaboliitide kontsentratsioonide ja ajavahemiku poegimisest esimese seemenduseni vaheline korrelatsioonikordaja farmides A ja B*

	Farm A					Farm B				
	1–14 DBC	1–14 DAC	28–42 DAC	63–77 DAC	117–151 DAC	1–14 DBC	1–14 DAC	28–42 DAC	63–77 DAC	117–151 DAC
AST									0,23	
GLDH						0.71**			0,41*	
UREA			-0,24*					-0,24		
GLC									-0,25	
KB			-0,23				-0,28**			
TG								-0,15		
NEFA			-0,3*							
CHOL	0,35**		0,32**	0,49**	0,24					
TL	0,44**	0,2	0,37**			0,26*	0,23			

Empty cell – no correlation / *tühjad lahtrid – korrelatsiooni ei ilmnenud*;
xx – P≥0.1;
xx* – P<0.1;
xx** – P<0.05

Table 3. Correlation coefficient between service period and blood enzymes/metabolites of cows on farm A and B
Tabel 3. *Lehmade vere ensüümiaktiivsuste/metaboliitide kontsentratsioonide ja servisperioodi vahelised korrelatsiooni-kordajad farmides A ja B.*

	Farm A					Farm B				
	1–14 DBC	1–14 DAC	28–42 DAC	63–77 DAC	117–151 DAC	1–14 DBC	1–14 DAC	28–42 DAC	63–77 DAC	117–151 DAC
AST										
GLDH								0,37		
UREA							-0,23			
GLC			-0,26*						-0,23	
KB	-0,26		-0,24	-0,35**						
TG								-0,22		
NEFA				-0,32**						
CHOL										0,75**
TL					0,28*					0,64*

Empty cell – no correlation / *tühjad lahtrid – korrelatsiooni ei ilmnenud*;
xx – P≥0.1;
xx* – P<0.1;
xx** – P<0.05

During the first two weeks of lactation cows on both farms had nadir (–47 and –52 MJ on farm A and B respectively) of their energy balance (Figure 2). Quicker recovery from negative energy balance on farm A led to positive balance 6 weeks after calving while cows on farm B were still in negative energy balance 10 weeks *post partum.*

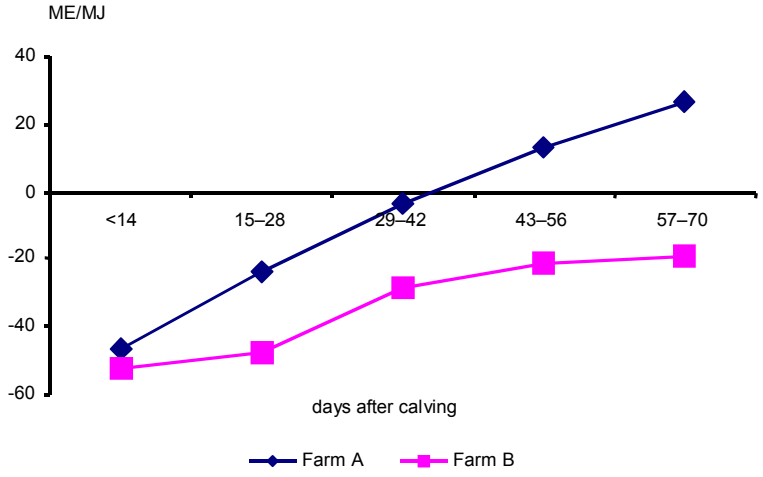

Figure 2. Estimated energy balance of cows on farm A and B
Joonis 2. *Lehmade hinnanguline energiabilanss farmides A ja B*

Discussion

AST and especially GLDH play a key role in protein metabolism. Their activity in blood may rise in case of insufficient or too abundant protein availability as well as energy deficiency. UREA concentration in blood or milk is often used as a criteria to evaluate protein/energy balance of the ration of cows (Moore, 1996; Carlsson, 1996). In our experiment AST activity and UREA concentration rose till 117–151 DAC on farm B. Noticeable difference in the curves of the UREA dynamics of the two farms was evidently a result of the differences in the protein/energy balance of the rations – 9.1 g digestible protein per 1 MJ ME on farm A compared to 12.5 on farm B. Several investigations report that increased UREA concentration can lead to impaired fertility of cows (Butler, 2000, review; Moore, 1996; Feddersen, 1994) as higher plasma urea concentrations may interfere with the normal inductive actions of progesterone on the microenvironment of the uterus and thereby cause

suboptimal conditions for support of embryo development (Butler, 2000, review). The results are consistent with the situation on farm B where SP was prolonged compared to farm A. The same relations between fertility and UREA concentration are reported by Clark *et al*. (2000). At the same time the authors also point to the negative correlation between post-partum UREA values and interval to first ovulation. The same trend was observed in our experiment (Table 2). Stable *post partum* UREA concentration on farm A compared to farm B can explain shorter SP on this farm as several investigations have related the decrease in reproduction capacity of cows to extremely low or extremely high urea content in body fluids (Pehrson, 1992; Feddersen, 1994).

In the dynamics of metabolites characterizing cows energy status also occurred some differences between farms. Reason for lower GLC level during all the investigated stages of gestation and/or lactation on farm A could be methodological – due to longer interval between blood collecting and analysing compared to farm B. At the same time rise from post-partum nadir began earlier on farm A than on farm B – from 1–14 DAC. Compared to farm A considerably risen level of KB with simultaneous GLC nadir during 1–14 DAC and 28–42 DAC was evident on farm B. TG like GLC on farm A started to rise from its 1–14 DAC nadir earlier than on farm B. The changes on farm A were accompanied by faster decline of NEFA from its post-partum topmost level. These relations may imply to quicker recovery from negative energy balance at the same time explaining shorter SP on farm A. Several investigations point out relations between EB and fertility (Mwaanga, Janowski, 2000; Mihm, 1999; Kruip *et al.*, 1998; Forshell *et al*., 1991). Positive correlation between low GLC level and negative EB has also been reported (Clark *et al*., 2000; Forshell *et al*., 1991).

Rukkwamsuk *et al*. (1998) have shown *pre partum* nutrition-associated relation between prolonged negative EB and NEFA staying on a high level for a long time *post partum*. Kruip *et al*. (1998) denote possible direct influence of high NEFA concentrations on ovary that leads to a lower progesterone production in *corpus luteum*. Clark *et al*. (2000) have referred to negative correlation between GLC level 11 days post-partum and post-partum anovulatory interval.

In our experiment there was a tendency to negative correlation between SP and GLC level 28–42 DAC, negative correlation between both fertility parameters and GLC level 63–77 DAC was not significant. The same relations appeared also in case of TG (Table 2 and 3). Negative correlations between investigated fertility parameters and KB as well as NEFA *post partum* (Table 2 and 3), described also by Clark *et al*. (2000), reveal the importance of lipid mobilisation during the period of negative energy balance.

CHOL pre partum level had tendency to be lower and 63–77 days or more after calving higher on farm A compared to farm B. There was a general trend for CHOL to rise on both farms. CHOL increase in the course of lactation together with intensive steroid synthesis has been reported in several investigations (Bösö *et al*., 2000; Pysera, Opalka, 2000). By Larson *et al*. (1997) and Villa-Godoy *et al.* (1988) impaired fertility may occur in cows with liver damages due to failed transport of CHOL to ovary whereby steroid synthesis is depressed and cyclicity delayed. As NEB on farm B was prolonged and rise in CHOL concentration modest relationships between these factors and longer SP compared to farm A may be supposed. However, Clark et al. (2000) refer to Rabiee's statement, that there is no correlation between ovarian CHOL uptake and progesterone output. Positive correlations found in our study between fertility parameters and CHOL level during investigated stages of gestation and/or lactation also show rather negative influence of high CHOL concentration on fertility (Tables 2 and 3).

TL curves reflect CHOL dynamics due to big portion of CHOL amongst TL, its correlation with fertility parameters are also similar to CHOL (Tables 2 and 3). We used several parameters to characterize dairy cow fertility. In our investigation CFS interval was similar on both farms but most of other parameters were different – first service conception rate was lower and number of services per conception higher on farm B leading to significantly longer service period. The decline in energy balance was more pronounced on farm B, the bottom of the energy balance curve was deeper and negative phase of the curve longer. On farm A the cows reached positive energy balance 6 weeks *post partum*. Their energy balance nadir is comparable to that of control cows within an investigation carried out in the Netherlands (Kruip *et al*., 1998) although the duration of the period overcomes that of the control cows by 2–3 weeks. On farm B the energy balance of the cows 10 weeks after calving was still negative. The situation was thus comparable to that of cows with induced deep and prolonged post-parturient negative energy balance accompanied by a long ovarian inactivity reported by Kruip *et al*. (1998). The detrimental effect of negative energy balance on fertility has also been demonstrated by De Vries and Veerkamp (2000) where low nadir of energy balance was related to delayed resumption of luteal activity. Butler (2000) has concluded that negative energy balance delays the time of first ovulation through inhibition of LH pulse frequency and low level of blood glucose, insulin and insulin-like growth factor-I (IGF-I) that collectively restrain oestrogen production by dominant follicles. Negative energy balance reduces serum progesterone concentrations and fertility. According to Reksen *et al*. (2001) dynamic changes in EB are even more important for subsequent reproductive performance than are mean EB or EB nadir, namely improvement of EB in late responders is much slower. The results favour farm A where the slope of EB increase was steeper than on farm B.

It seems to be that in our commercial dairy herds, whose production level is above average, cows tend to be in negative energy balance too long. Improving the situation would be one of the ways to amend cow fertility on the farms.

Conclusions

Our investigations indicate that increased UREA and KB levels may be potential risk factors of impaired fertility. Fertility situation on the two farms, similar in milk performance, was quite different. Interval CFS of the farms was slightly different but SP was considerably longer on farm B. Energy balance and metabolic status differences account partly for the dissimilarities. However, the comparable interval CFS indicates to similar potential in reproductive ability of the farms. Prolonged SP on farm B indicates to possible shortages in management resulting in impaired fertility.

Further investigations on cow health, nutrition and metabolic status as well as progesterone profile analysis are needed to differentiate factors influencing intervals from calving to first ovulation and from first ovulation to actual conception.

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Eesti Holsteini tõugu lehmade vere metaboliidid ja nende seos mõnede sigimisparameetritega

K. Ling, H. Jaakson, J. Samarütel, A. Leesmäe

Kokkuvõte

Laktatsiooni algusperioodil ei suuda suuretoodangulised lüpsilehmad suurenenud energia- ja glükoositarvet ainult sööda arvelt katta. Energiadefitsiidi kompenseerimiseks kasutavad nad intensiivselt organismi varulipiide ning nende energiabilanss on sel perioodil negatiivne. Pikenenud negatiivse energiabilansi perioodi kahjulikku mõju sigimisele on näidanud mitmed autorid. Käesoleva uurimuse eesmärgiks oli analüüsida võimalikke seoseid energia- ja valguainevahetust iseloomustavate metaboliitide ja kahe sigimisparameetri – ajavahemiku poegimisest esimese seemenduseni (interval calving to first service – CFS) ja servisperioodi (service period – SP) – vahel.

Katse viidi läbi kahes farmis (A ja B). Keskmine piimatoodang farmis A oli 1999. a 6542 kg, 2000. a 7467 kg ja 2001. a 8665 kg. Farmi B keskmine toodang oli 1999. a 5916 kg ja 2000. a 6791 kg. Vereproovid võeti kägiveenist (farmis A 1999. a) ning sabaveenist või -arterist sigimistsükli järgmistes staadiumites: 1–14 päeva enne poegimist (days before calving – DBC) ja 1–14, 28–42, 63–77 ning 117–151 päeva pärast poegimist (days after calving – DAC). Vereseerumist määrati spektrofotomeetriliselt järgmised ensüümiaktiivsused ja metaboliitide kontsentratsioonid: aspartaadi aminotransferaas (aspartate aminotransferase – AST), glutamaadi dehüdrogenaas (glutamate dehydrogenase – GLDH), karbamiid (urea – UREA), glükoos (glucose – GLC), üldketokehad (ketone bodies – KB), triglütseriidid (triglycerides – TG), esterifitseerimata rasvhapped (non-esterified fatty acids – NEFA), üldkolesterool (cholesterol – CHOL) ja üldlipiidid (total lipids – TL). Statistilises andmetöötluses kasutati dispersioonanalüüsi, t-testi ja korrelatsioonanalüüsi (SAS, Excel).

Farm ja/või sigimistsükli staadium kui faktor mõjutas UREA, GLC, KB, TG, NEFA, CHOL ja TL kontsentratsiooni ning AST aktiivsust ($P<0,05$). Metaboliitide, välja arvatud karbamiid, sisalduse üldine dünaamika oli mõlemas farmis sarnane (joonis 1). Samas ilmnes ka erinevusi. Nii algas GLC ja TG sisalduse poegimisjärgne tõus varem farmis A. Ühtlasi oli KB poegimisjärgne tase siin madalam ning NEFA sisalduse langus kiirem (joonis 1). Selline dünaamika viitab farmi A lehmade paremale energiaga varustatusele ja on kooskõlas leitud hinnangulise energiabilansiga (joonis 2). Nii AST kui ka UREA sisaldus farmis B tõusis, ületades farmi A keskmisi kõigi uuritud sigimistsükli staadiumite lõikes (AST) või alates 28–42 DAC (UREA). See võib iseloomustada ratsiooni tasakaalustamatust proteiini ja energia suhtes farmis B. CHOL sisaldus tõusis mõlemas farmis. Farmi A tase oli alates 63–77 DAC kõrgem. TL dünaamika oli sarnane CHOL omale, samas oli TL tase farmis A kõrgem juba 28–42 DAC (joonis 1).

Servisperiood oli pikem farmis B ($P<0,001$), kuid ajavahemik poegimisest esimese seemenduseni farmides ei erinenud (tabel 1). Ensüümiaktiivsuste ja metaboliitide kontsentratsioonide seosed uuritud sigimisparameetritega avaldusid sigimistsükli erinevate staadiumite lõikes järgmiselt: AST ja GLDH aktiivsused ning CHOL ja TL kontsentratsioonid korreleerusid uuritud sigimisparameetritega positiivselt, UREA, GLC, KB, TG ja NEFA kontsentratsioonid negatiivselt (tabelid 2 ja 3).

Kahe farmi võrdlemisel saadud tulemused näitavad, et suurenenud karbamiidi ja ketokehade sisaldus veres võib olla pikenenud servisperioodi potentsiaalne riskifaktor. Ajavahemik poegimisest esimese seemenduseni keskmiselt piimatoodangult sarnastes farmides ei erinenud, samas oli servisperiood farmis B pikem. Servisperioodi pikenemine viitab võimalikele söötmis- ja pidamistingimuste puudustele, mistõttu sigimispotentsiaal farmis B jäi osaliselt ära kasutamata.

Täiendavad lehmade tervise, söötmis- ja ainevahetusalased uuringud ning progesterooni profiilide analüüs on vajalikud selgitamaks faktoreid, mis mõjutavad ajavahemikku poegimisest esimese ovulatsioonini ja tiinestumiseni.

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