METABOLIC STATUS AND BODY CONDITION SCORE OF ESTONIAN HOLSTEIN COWS AND THEIR RELATION TO SOME FERTILITY PARAMETERS

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Summary. The objective of the study was to analyse relationships between some metabolites associated with cows energy and(or) protein status, body condition score (BCS) and fertility parameters on two Estonian commercial farms. Farm and(or) stage of gestation and(or) lactation were the factors affecting BCS and 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 between fertility parameters were positive in the case of AST, GLDH, cholesterol and total lipids. Negative correlations had urea, glucose, ketones, triglycerides and NEFA. Most of the correlations between BCS and metabolites occurred between 20 to 60 days in milk , that between BCS and AST, GLDH, glucose and ketone bodies being negative. Our investigations 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 physiological factors influencing intervals from calving to first ovulation and from first ovulation to actual conception.

Keywords: Estonian Holstein cow, fertility, metabolic status, body condition score, energy balance, blood metabolites.

ESTIJOS HOLŠTEINŲ VEISLĖS KARVIŲ MEDŽIAGŲ APYKAITA IR ORGANIZMO BŪKLĖS RYŠYS SU VAISINGUMU

Santrauka. Tyrimo tikslas – nustatyti santykį tarp kai kurių medžiagų, susijusių su karvių energija ir/arba baltymais, organizmo būkle (OBP) ir vaisingumo rodikliais dviejuose Estijos komerciniuose ūkiuose. Veiksniai, turintys įtakos OBP, šlapalui, cholesteroliui, trigliceridams, gliukozei, ketoniniams kūnams, NEFA ir bendrai lipidų koncentracijai bei AST aktyvumui, buvo ūkis ir/arba nėštumo stadija, ir/arba laktacija. Ilgesnis kergimo periodas buvo B ūkyje, tačiau pirmojo kergimo diena abiejuose ūkiuose sutapo. Vaisingumo parametrų ryšys buvo teigiamas AST, GLDH cholesteroliui ir bendram lipidų kiekiui. Neigiamas ryšys buvo nustatyta šlapalui, gliukozei, ketonams, trigliceridams ir NEFA. Didžiausias ryšys tarp OBP ir medžiagų apykaitos produktų nustatytas 20 – 60 pieno gamybos dieną, t. y. tarp OBP ir AST, GLDH, gliukozės ir ketoninių kūnų jis buvo neigiamas. Mūsų tyrimų rezultatai leidžia daryti išvadą, kad padidėjęs šlapalo ir ketoninių kūnų kiekis gali būti rizikos faktorius, lemiantis vaisingumo mažėjimą. Norint nustatyti fiziologinius veiksnius, turinčius įtakos laikui nuo apsiveršiavimo iki pirmosios ovuliacijos ir nuo ovuliacijos iki apvaisinimo, būtini tolesni tyrimai, taip pat ir progesterono analizė.

Raktažodžiai. Estijos holšteinų veislės karvės, vaisingumas, medžiagų apykaita, organizmo būklė, energijos balansas, kraujo medžiagų apykaitos produktai.

Introduction. Several factors and interactions of the factors like nutrition, metabolic and body condition 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; Knutti et. al., 1999; 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. In addition to blood metabolites as indicators of the metabolic status of dairy cows, body reserves of cows expressed by body condition score (BCS) and BCS change have been used to monitor the amount and mobilisation of body adipose tissue. Several studies have reported that health and fertility parameters are related to BCS at calving or BCS change during early lactation or both (Butler, 2000; Heuer et al., 1999; Broster and Broster, 1998, review).

The objective of our study was to analyse relationships between some metabolites associated with energy and(or) protein status of cow, body condition score (BCS) and some fertility parameters (interval calving to first service, service period) 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 until March. The cows were fed according to Estonian feeding standards; they were milked twice per 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.

Starting from about two weeks before calving the cows body condition was scored by two observers during a 140-day period after every 10 days on farm A (one observer) and after every 14 days on farm B (another observer). Body condition scoring was performed using a 5-point scale with quarter point divisions according to Edmondson's BCS chart (Edmondson et al., 1989). 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 by using everyday observations. Silage intake on farm B was estimated by 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 : $I = -14.2 + (0.00643 \times BW) + (0.149 \times SDM) + (0.319 \times SOM) + (0.112 \times ECM) + (0.291 \times CI)$. In all feeds the content of dry matter, crude protein, crude fibre, crude fat, ether extract and major minerals was determined in the chemistry laboratory of Department of Animal Nutrition, Institute of Animal Science, Estonian Agricultural University. Content of metabolizable energy (ME) and digestible protein (DP) in the feeds was calculated according to national feeding standards. Hygienic quality of ensiled feeds was evaluated. Cows on farm A received 260 MJ ME and 75.6 g of DP per kg of ECM milk; on farm B the respective figures were 182.9 and 73.5.

Control milking was performed once a month on farm A and twice a month on farm B. Data of control milking on milk fat content from milk laboratory of Estonian Agricultural Register's and Information Centre were used to calculate energy corrected milk production for the period preceding 14 days 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.

Blood samples were taken from jugular (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 to 4 days before calving - DBC), first 14 days of lactation (1 to 14 days after calving - DAC); 28 to 42, 63 to 77 and 117 to 151 days after calving (DAC). The concentrations of metabolites and the activity 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. The 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%.

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 signifycance ($P<0.1$) and not significant ($P\geq0.1$). Natural logarithms were used where (if) appropriate.

Results. Analysis of variance showed the significance ($P<0.001$) of the model regarding the farm and stage of gestation and (or) lactation as factors in the case of BCS, 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. The farm and stage of gestation and(or) lactation separately influenced BCS and 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 ($P<0.001$).

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 ($P<0.001$) (Table 1). On farm A positive correlation ($r = 0.24$; $P<0.05$) was observed between the interval from calving to first service and service period. On farm A 20 days *pre partum* BCS significantly influenced ($r = -0.39$; $P<0.05$) days to first insemination. Conception rate at the 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. Mean BCS of the cows and the curves of their changes during the 140-day period on farms A and B are shown in Figure 1. The average BCS 10 days before calving – 3.63 (2.75-4.5) – was slightly higher on farm B compared to 3.54 (2.75-4.75) on farm A. After parturition BCS decline was more drastic on farm B, where it declined up to 2.59 by 30 days in milk. The lowest BCS on farm B (2.49) was scored 70 days after calving, on farm A (2.66) – 100 days after calving. The overall BCS loss was larger on farm B (1.1 units) compared to farm A (0.9) units. At the same time BCS loss was more pronounced ($P<0.001$) on cows having BCS >3.5 before parturition (1.51 units) compared to cows with BCS <3.5 before parturition (0.89 units), in both cases the losses tended (P<0.1) to be bigger on farm B.

Table 1. **Number of cows, means and standard deviations of different reproductive indices on farms A and B.**

Farm	Parameter	Interval calving to first service (CFS)	Service period (SP)	Interval first service to last service (FSLS)
A	$\bar{x} \pm$ sd	72.0±18.26	100.8±44.63*	29.4±41.44**
	n	50	49	49
	Min–max	46–126	46–223	0–171
B	$\bar{x} \pm$ sd	76.1±18.1	146.7±77.3	70.1±75.01
	n	52	48	48
	Min–max	50–135	56–458	0–338

* – difference between the farms P<0.05

** – difference between the farms P<0.001

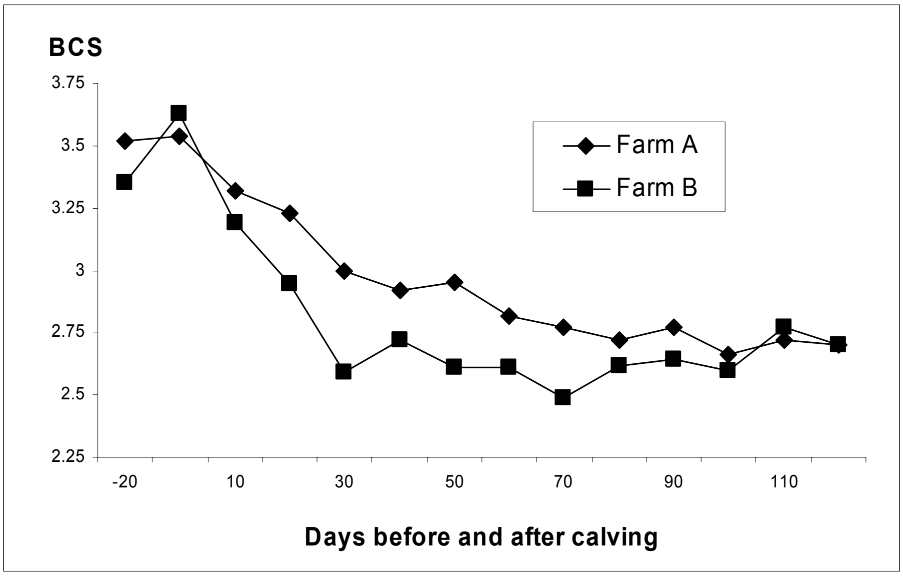

Figure 1. **Mean BCS during the investigated 140-day period on farms A and B**

During the first two weeks of lactation cows on both farms had nadir (-47 and -52MJ on farm A and B respectttively) 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.*

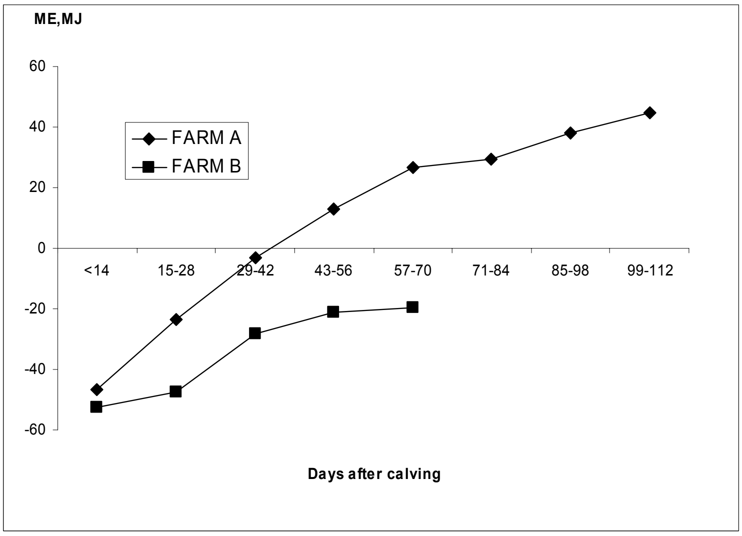

Figure 2. **Estimated energy balance of cows on farm A and B**

Amongst investigated enzymes, especially AST activities expressed differences between farms (Figure 3). 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. Only on farm A GLDH expressed increase. *Pre partum* activity on the farm tended to be lower, but exceeded farm B values 63 to 7 DAC.

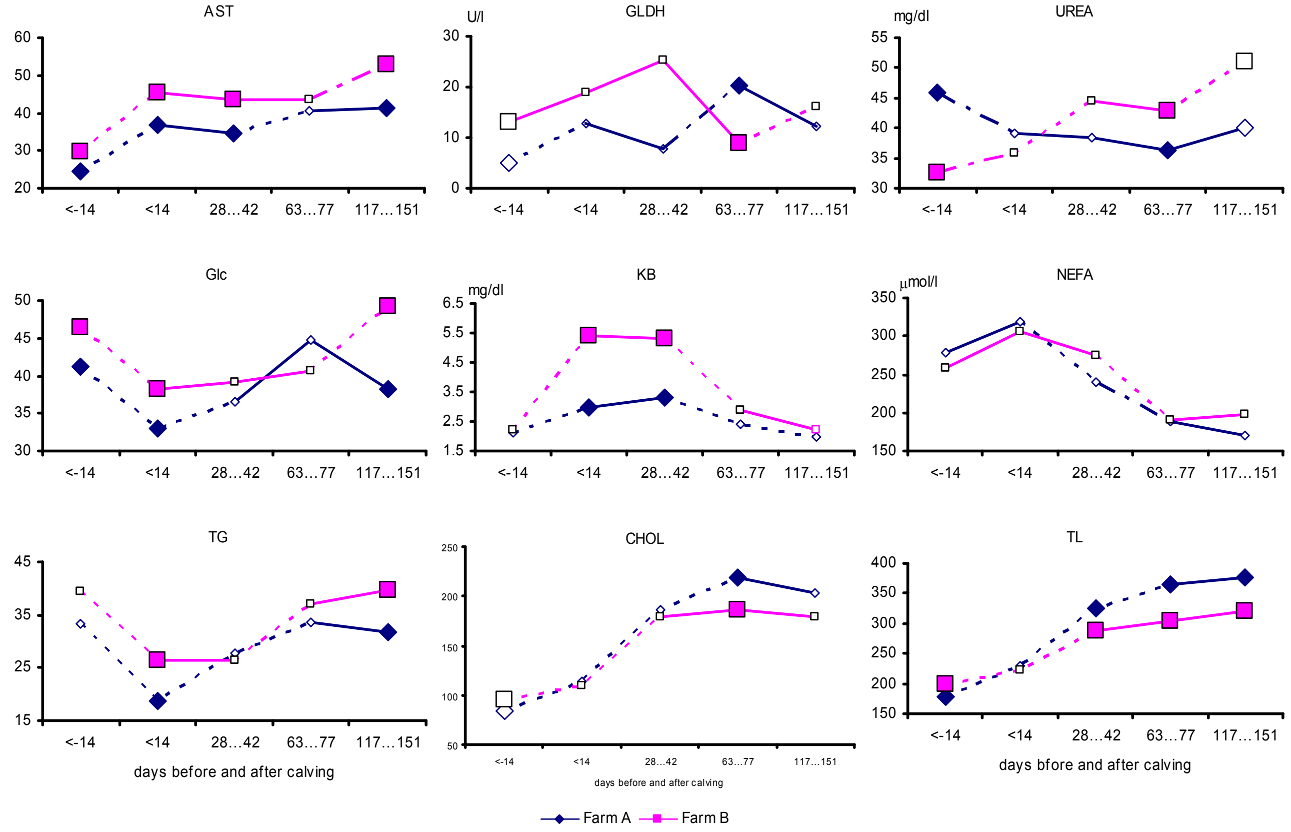

Figure 3. **Dynamics of enzyme activities and metabolites concentrations on Farm A and B. Dotted lines indicate significant differences between stages and dashed-dotted lines tendency to differ. Big legend markers show differences between farms (filled:P<0,05, not-filled: P<0,1). Small legend markers indicate insignificant differences.**

On two farms UREA concentration changes outlined clearly in a different way. Before calving UREA concentration was higher on farm A. The following increase on farm B and decrease at least up to 28 to 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 to 14 DAC and 28 to 42 DAC with concurrent KB topmost level 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. Curves NEFA and TG have similar opposite appearance as GLC and KB do. Concentration of NEFA had already risen *pre partum* and declined in the course of lactation. Triglycerides dynamics on farm A and B as well as differences between the farms and earlier recovery on farm A was very similar to GLC.

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

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 to 14 DBC correlated with interval CFS. The same tendency was seen in GLDH activity 63 to 77 DAC. UREA concentration 28 to 42 DAC had a trend to correlate negatively with interval CFS. During stages 1 to 14 and 28 to 42 DAC and between service period during 117 to 151 DAC DBC CHOL and TL correlated with interval CFS. Moreover, CHOL 63 to 77 DAC correlated with interval CFS. During the stages 28 to 42 DAC and 63 to 77 DAC GLC concentration had a tendency to correlate negatively with both investigated fertility parameters. There was a negative correlation between KB 1 to 14, and 28 to 42 DAC and CFS and between KB 1 to 14 DBC, 28 to 42 and 63 to 77 DAC and service period.

The most clear correlations between enzymes/metabolites and BCS were evident in case of KB. In several cases negative correlations appeared between the same or anterior and posterior time periods.

Discussion. We used various indicators simultaneously to characterize dairy cows fertility. On both farms CFS interval was similar, but most of other parameters providing information about fertility between farms A and

B differed significantly. First service conception rate was lower and the number of services per conception higher on farm B leading to significantly longer service period. In commercial dairy herd situations direct assessment of energy balance in individual cows is not possible but changes in BCS provide an indirect measure. With more extensive loss of BCS, the reduction in conception rate becomes greater. Many studies report that cows losing one unit or more BCS during early lactation are at greatest risk for low fertility with conception rates of 17 to 38 % (Butler, 2000). In our study BCS loss after calving was more abrupt on farm B, where BCS declined more than one unit during the first 30 DAC. That may be one reason for a low first service conception rate (28.6%) on the farm.

Table 2. **Correlation between interval calving to first service and blood enzymes/metabolites of cows on farm A and 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;
xx - P~0.1;
xx* - P<0.1;
xx** - P<0.05

Table 3. **Correlation between service period and blood enzymes/metabolites of cows on farm A and 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;
xx - P~0.1;
xx* - P<0.1;
xx** - P<0.05

In an earlier study we examined the repeatability of BC scoring between different observers. The results showing that 83% of the scores deviated only by either zero or a quarter point were in line with Ferguson et al (1994) giving confidence to use the subjective method.

The average BCS close to calving on the farms A and B was comparable to the results of other authors (Markusfeld et al, 1997) and recommended value 3,5 (Wattiaux, 1996). The decline in both BCS and energy balance was more pronounced on farm B. The bottom of the energy balance curve was deeper and the 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 was still negative 10 weeks after calving. 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 the 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 that on farm B.

The overall decline in BCS in 100 days on farm A was 0,9 units and on farm B in 70 days – 1.1 units versus 0.62 indicated for a 4-8 week decline in a US study (Domecq et al, 1997) and 0.55 in a 60–70 day decline specified as an average in a recent paper (Broster & Broster, review 1998). Seemingly 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.

In protein metabolism AST and especially GLDH play a key role. Their activity in blood may rise in case of insufficient or too abundant protein availability, as well as energy deficiency. As a criteria to evaluate energy/protein balance of the cows ration UREA concentration in blood or milk is often used (Moore, 1996; Carlsson, 1996). In our experiment AST activity and UREA concentration rose till between 117 and 51 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 energy/protein 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 some differences between farms also occurred. Reason for lower GLC level during all the investigated stages of gestation and/or lactation on farm A could be methodological - compared to farm B's longer interval between blood collecting and analysing. At the same time a rise from post-partum nadir began on farm A earlier than on farm B – from 1 to 14 DAC. Compared to farm A considerably risen level of KB with simultaneous GLC nadir during 1 to 14 DAC and 28 to 42 DAC was evident on farm B. Triglycerider like GLC on farm A started to rise from its 1 to 14 DAC nadir earlier than that 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 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 and 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. (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 to 42 DAC (r=-0.26); negative correlation between both fertility parameters and GLC level 63 to 77 DAC was not signifycant. 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* are (Table 2 and 3), described also by B. Clark et. al. (2000) and are interesting Negative correlations found between KB and BCS show that BCS beside GLC, TG and NEFA, can also reflect cows energy status quite objectively.

In our experiment CHOL level *pre partum* had a tendency to be lower and from 63 to 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 and 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 a 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, having 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).

Total lipids curves reflect CHOL dynamics due to big portion of CHOL amongt TL; its correlations with fertility parameters are also similar to CHOL (Tables 2 and 3).

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 potentiality in reproductive ability of the farms. Prolonged SP on farm B indicates to possible shortages in management resulting in impaired fertility.

Further nutritional and metabolic investigations, as well as progesterone profile analysis, are needed to differrentiate physiological factors influencing intervals from calving to first ovulation and from first ovulation to actual conception.

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References

1. Broster W. H., Broster, V. J. Body score of dairy cows. Review article. J. Dairy Res. 1998. Vol. 65. P. 155-173.

2. Butler W.R.. Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci. 2000. Vol. 2. P. 449-457.

3. Bösö A. R., Saukko T. M., Tesfa A. T., Lindberg L.-A. Fat Infiltration in Liver and Activity of Lecitin: cholesterol acyltransferase in Serum of Dry and Lactating Dairy Cows. Res. Vet. Sci. 2000. Vol. 68. P. 169-173.

4. Clark B. A., Chagas P. M., Gore B, Verkerk G. A. Prediction of post-partum anovulatory interval in dairy cows. Proceedings of the New Zealand Society of Animal Production 2000. Vol. 60. P. 15-18.

5. De Vries M. J., Veerkamp R. F.. Energy balance of dairy cattle in relation to milk production variables and fertility. J. Dairy Sci.. 2000. Vol. 83. P. 62-69.

6. Domecq J. J., Skidmore A. L., Lloyd J. W., Kaneene J. B. Relationship between body condition score and milk yield in a large dairy herd of high yielding Holstein cows. J. Dairy Sci. 1997. Vol. 80. P. 101-112.

7. Edmondson A. J., Lean L. J., Weaver L. D., Farver T., Webster G. A. Body condition scoring chart for Holstein dairy cows. J. Dairy Sci. 1989.Vol. 73. P. 68-78.

8. Feddersen E. Fütterung durch Harnstoffuntersuchergen der Milch überprüfen. Der Tierzüchter. 1994. Vol. 6. S. 69 -71

9. Ferguson J. D., David T., Galligan T., Thomsen N. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci. 1994. Vol. 77. P. 2695-2703.

10. Forshell K. P., Andersson L., Pehrson B. The relationships between the fertility of dairy cows and clinical and biochemical measurements, with special reference to plasma glucose and milk acetone. J. Vet. Med. 1991. Vol. 38. P. 608-616.

11. Carlsson, J. The value of the concentration of urea in milk as an indicator of the nutritional value of diets for dairy cows, and its relationship with milk production and fertility. Thesis, Skara, 1994.

12. Herdt T. H. Ruminant adaptation to negative energy balance. The Veterinary Clinics of North America: Food Animal Practice 2000. Vol. 16. P. 215-230.

13. Heuer C., Schukken H., Dobbelaar P. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. J. Dairy Sci. 1999. Vol. 82. P. 295-304.

14. Kruip T. A. M., Meijer G. A. L., Rukkwamsuk T., Wensing T. Effects of feeding in the dry period on fertility of dairy cows post partum. Reprod. Dom. Anim. 1998. Vol. 33. P. 165-168.

15. Larson, S. F., Butler, W. R., Currie, W. B. 1997. Reduced Fertility Associated With Low Progesterone Postbreeding and Increased Milk Urea Nitrogen in Lactating Cows. J. Dairy Sci., 80, 1288-1295.

16. *Lumsden J. H. "Normal" or Reference Values: Questions and Comments. Veterinary Clinical Pathology 1998. Vol. 27, 4. P. 102-106.*

17. Lutskii D. J., Žarov A. V., Šiškov V. P. Metabolic disorders of high-producing cattle. Moscow: Kolos. 1978. 384 P. (in Russian).

18. Markusfeld O., Galon N., Ezra, E. Body condition score, health, yield and fertility in dairy cows. Veterinary Record 1997. Vol. 141. P. 67-72.

19. Mihm M. Delayed resumption of cyclicity in postpartum dairy and beef cows. Reprod. Dom. Anim. 1999. Vol. 34. P. 277-284.

20. Moore D. BUN and MUN: urea nitrogen testing in dairy cattle. Food Animal 1996. Vol. 18. P. 712-720.

21. Mwaanga E. S., Janowski T. Anoestrus in dairy cows: causes, prevalence and clinical signs. Review article. Reprod. Dom. Anim. 2000. Vol. 35. P. 193-200.

22. Pehrson B. , Plym Forsell K., Carlsson J. The effect of additional feeding on the fertility of high - yielding cows., J. Vet. Med. 1992. Vol.39. P.. 187-192.

23. Pysera B., Opalka A. The Effect of Gestation and Lactation of Dairy Cows on Lipid and Lipoprotein Patterns and Composition in Serum During Winter and Summer Feeding. J. Animal and Feed Sci. 2000. Vol. 9. P. 411-424.

24. Reksen O., Gröhn Y.T., Havrevoll O.S., Bolstad T., Waldmann A., Ropstad E.,. Influence of concentrate allocation and energy balance on postpartum ovarian activity in Norwegian Cattle. Journal of Dairy Science. 2001. Vol. 84. P. 1060-1068.

25. Roxtröm A., Strandberg., Berglund B., Emanuelson U., Philipsson J. Genetic and environmental correlations among female fertility traits and milk production in different parities of Swedesh Red and White Dairy Cattle. 2000. Acta Agr. Scand. Sect A, Animal Sci. Vol. 51. P. 7-14.

26. Rukkwamsuk T., Wensing T., Geelen M. J. H. Effect of overfeeding during the dry period on regulation of adipose tissue metabolism in dairy cows during the periparturient period. J. Dairy Sci. 1998. Vol. 81. P. 2904-2911.

27. Trubka R. J. Methods to diagnose bovine ketosis. In: Prophylaxis and treatment of farm animals. Riga: Zinatne. 1974. P. 5-11(in Russian).

28. Villa Godoy A., Hughes T. L., Emery R. S., Stanisiewsky E. P., Fogwell R. L. Influence of energy balance and body condition on estrus and estrous cycles in Holstein heifers. J. Dairy Sci. 1990. Vol. 73. P. 2759-2765.

29. Villa-Godoy A., Hughes T. L., Emery R. S., Chapin L. T., Fogwell R. L. Association Between Energy Balance and Luteal Function in Lactating Dairy Cows. J. Dairy Sci. 1988. Vol. 71 P. 1063.

30. Wattiaux M. A. Reproduction and genetic selection. Technical Dairy Guide. The Babcock Institute for International Dairy research and Development. 1996. 162 P.