Influence of increased lipid content in diet in the form of treated rapeseed meal on the metabolism and milk yield of dairy cows in the first third of lactation

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ABSTRACT: The purpose of this study was to evaluate the influence of high lipid concentration in the diet, served as calcium salts of fatty acids from rape, on metabolism and the milk yield of dairy cows during the first third of lactation. 28 dairy cows were divided into experimental (E; n = 14) and control groups (C; n = 14) and monitored within 100 days of lactation since the day of parturition. The diet of both groups had a balanced content of energy and crude protein, while there was a difference in lipid content (C – 3.7% vs. E – 6.99% of dry matter in the diet). Blood and urine samples were taken at the end of 1st, 2nd and 3rd months of lactation. Evaluation of milk yield was carried out based on the results of monthly milk yield control, while the evaluation of reproduction was performed using data supplied by a farm livestock specialist. At the end of the first month, a higher degree of energetic metabolism disturbance was determined in group E in comparison with group C (beta-hydroxybutyrate 1.05 vs 0.51 mmol/l, $P \leq 0.05$; nonesterified fatty acids 0.68 vs. 0.27 mmol/l, $P \leq 0.01$), as well as a higher occurrence of liver damage (bilirubine 6.50 vs. 4.59 μmol/l, $P \leq 0{,}01$; aspartate amino transferase 1.66 vs. 1.39 μkat/l, $P \leq 0.05$; lactate-dehydrogenase 45.2 vs. 34.3 μkat/l, $P \leq 0.05$). During the entire experiment, the total concentration of cholesterol, HDL-cholesterol and vitamin E rose, and thus in the 3rd month, the values in the experimental group were almost double that of the control group (cholesterol 7.28 vs. 3.69 mmol/l, $P \leq 0.0001$; HDL-cholesterol 5.43 vs. 3.26 mmol/l, $P \leq 0.0001$; vitamin E 19.9 vs. 10.3 μmol/l, $P \leq 0.0001$). The proportion of HDL-cholesterol was lower in group E (3rd month 76.1 vs. 88.8%, $P \leq 0.001$). We also determined a higher total anti-oxidant status of serum in group E in the second (0.96 vs. 0.90 mmol/l, $P \leq 0.05$) and third months of lactation (1.02 vs. 0.94 mmol/l, $P \leq 0.05$), while other parameters of the anti-oxidation system (glutathionperoxidase, superoxiddismutase) did not differ between groups. The total production of milk within the 100 days of lactation in both monitored groups was similar. In group E the concentration of milk protein was lower (3.18 vs. 3.45%, $P \leq 0.01$), while the concentration of fat was insignificantly higher (3.55 vs. 3.21%) than in group C. The results of effect on reproduction did not differ significantly either, but the total percentage of gravidity was higher in the experimental group. Our results revealed that feeding of higher doses of lipid (6.99 %) fed in bypass form during the first month after parturition creates the health risk of a fatty liver, but no negative impact on the health of dairy cows was demonstrated during the peak period of lactation.

Keywords: TAS; cholesterol; vitamin E; SOD; T3; T4; reproduction; blood

Increasing milk yield of dairy cows demands higher requirements on nutrition and feeding, mainly in the first third of lactation, when dairy cows achieve the highest milk yield with limited dry matter intake capacity. Dairy cows frequently end up with a negative energetic balance, which has an adverse impact on their milk yield, health and reproduction (Bobe et al., 2004).

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Traditional sources of supplementary energy include starchy materials such as cereals, or fibrous materials as by-products, like sugar beet pulp. High starch diets can result in a rapid fermentation in the rumen and the low pH induced may inhibit forage-digesting bacteria, which are less tolerant of low pH conditions than amylolytic microorganisms. This results in lower feed intake and a reduction in the butterfat content of milk. For diets very high in energy, it is necessary to use fats. These have a gross energy content, about twice that of grass and cereals, metabolisable energy is about three times as high and net energy about four times as high (Garnsworthy, 2002).

In the Czech Republic, one of the frequently used lipid sources are rapeseed products, including Proenergol (ACCS Zichlinek, Czech Republic), made by physical-chemical methods from rapeseed meal. Energetic components in this preparation consist of calcium salts of fatty acids from rape, and in glycerol. Compared to non-treated rapeseed, it contains a higher percentage of non-degradable protein with high utilisation in the intestines, greatly reduced content of glucosinolates, higher calcium content, better stability and taste (Komprda et al., 2000).

The purpose of our experiment was to evaluate the impact of a high lipid concentration in the diet, supplied in the form of Proenergol, on the metabolism and milk yield of dairy cows in the first third of lactation, compared to a diet with a higher content of saccharides, without supplementary lipids.

MATERIAL AND METHODS

Description of farm animals

Monitoring was performed on a farm of high milk yield dairy cows, crossbred with Holstein, in eastern Bohemia. Milk cows were stabled in a cow-barn for 96 cows. Average farm milk yield amounted to 6 500 litres.

Twenty eight dairy cows were selected for the experiment, divided on the principle of analogous couples depending on breed, age and efficiency to the experimental group (E; n = 14) and control group (C; n = 14), and monitored during the first three months of lactation. Both groups received an identical basic diet (Table 1) and two types of production mixture (batched according to cow milk yield), directly to the trough. The diet had identical content of energy, crude protein and mineral elements, excluding calcium. There was a difference in the content of lipid (C – 3.7% vs. E – 6.99% of dry matter in the diet), fibre (C – 14.4% vs. E – 15.6% of dry matter in the diet) and calcium (C – 0.83% vs. E – 1.3% of dry matter in the diet). The source of lipid in the diet for the experimental group was Proenergol (ACCS Zichlinek, Czech Republic), consisting of rapeseed meal, treated according to Patent Czech Republic No. 285745 (1999). The treatment converts fatty acids to calcium salts, which are not fermented in the rumen. The content of nutrients in the diet for the milk cows with 30 l milk yield is shown in Tables 2 and 3.

Collection of samples and laboratory examination

Metabolism of milk cows was monitored via the examination of blood and urine samples taken at the end of the 1st, 2nd and 3rd months of lactation. Blood was taken by puncturing of *v. jugularis* into single-use test tubes, with the addition of heparin to acquire whole blood, and without anti-coagulants added to the serum. For the glucose test, samples were preserved in sodium fluoride.

Table 1. Composition of the diet for milk cows in control and experimental group for 30 l milk yield

Component (kg)	Experimental group	Control group
Clover haylage	14.0	14.0
Corn silage	15.0	15.0
Meadow hay	2.0	2.0
Experimental production mixture	9.1	–
Control production mixture	–	9.57
Mineral mixtures	0.45	0.42
Fodder calcium	–	0.03

The following parameters were determined for the blood serum: urea (U), total bilirubine (Bil), aspartate amino transferase (AST), γ-glutamyltransferase (GMT), lactate-dehydrogenase (LDH), triacylglyceroles (TG), total cholesterol (Chol), HDL-cholesterol (HDL-chol), nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHB), vitamin E, total antioxidant status (TAS), superoxiddismutase (SOD), thyrosine (T3), thyroxin (T4), calcium (Ca), phosphorus (P), and magnesium (Mg). Glucose (Glu) was measured in fluoride blood plasma. Glutathionperoxidase (GSH-Px) activity was measured in whole blood.

The values for parameters were determined by photometric methods (which used an automatic analyzer Cobas Mira (Roche, Switzerland)) and the following tests: total bilirubine (BIL 100, Cat. No. 1105309), TG (TGL 4 × 100, Cat. No. 1312983), GMT (GMT KIN 100, Cat. No. 1302082), urea (Urea UV KIN 4 × 50 test, Cat. No. 1307017), HDL-cholesterol-precipitation solution (HDL CHOL 250E, Cat. No. 1301302) all supplied by LACHEMA; glucose (^{L}Glukosa, Cat. No. 11601), total cholesterol (^{L}Cholesterol, Cat. No.10851), AST (^{L}AST, Cat. No. 10351), LDH (^{L}LDH, Cat. No. 12352), phosphorus (^{L}Fos for inorganics, Cat. No. 11352) supplied by BioVendor; NEFA (NEFA, Cat. No. FA 115), BHB (RANBUT, Cat. No, RB 1008), TAS (Total antioxidant status, Cat. No. NX 2332), SOD (RANSOD, Cat. No. SD 125), GSH-Px (RANSEL, Cat. No. RS 504) supplied by RANDOX.

Table 2. Composition of production mixtures

Component (%)	E	C
Rape seed	6.5	0
Treated rapeseed meal	21.8	0
Treated rapeseed oil	3.3	0
Glycerol	4.4	0
Molasses	2.2	0
CaO	2.0	0
Extracted soya groats	16.4	24.0
Corn	9.5	21.0
Wheat	11.3	25.0
Oats	6.7	5.0
Wheat bran	2.3	5.0
Flax seed	2.3	5.0
Beet sugar	3.7	8.0
Dried whey	7.6	7.0

Thyroid hormones were determined by means of a chemical-luminescent method, using an Immulite unit (DPC, Los Angeles, USA). We used the following tests supplied by BioVendor: T3 (LKT31), T4 (LKT41).

Calcium and magnesium were determined using flame atomic absorption spectrophotometry (F-AAS) using an AAS Solaar M6 unit. The concentration of vitamin E was determined by fluorometry, as described by Thompson et al. (1973) and Bouda et al. (1980), using a 204 Perkin-Elmer fluorescence spectrophotometer.

Health was monitored by attendants and a local veterinarian. Evaluation of milk yield was carried out based on results of the monthly milk yield control, while evaluation of reproduction was performed using the data supplied by the farm livestock specialist.

Statistical methods

Results were evaluated statistically using the *F*-test to evaluate variation in individual files and, depending on the result, by Students *t*-test for files with equal/unequal variation. Reproduction was evaluated, with regard to differences in individual values and the small frequency of files, using Wilcoxon's matched pairs rank test.

RESULTS AND DISCUSSION

Metabolism of dairy cows

Only 13 dairy cows from the experimental group were included in the evaluation of metabolism, since one dairy cow was slaughtered in the second month due to decreased milk yield after abomasum dislocation. Results of selected blood analyses are shown in Tables 4 to 7.

At the end of the first month, worsened state of energetic metabolism was determined in the experimental group. Cows with increased lipid supplementation showed a higher concentration of BHB and NEFA. The increase in NEFA under physiological limits (0.35 mmol/l) represents a high risk for the development of a fatty liver in dairy cows (Pechova et al., 1997). The NEFA:TG ratio indicates the risk of fat retention in the liver. In

Table 3. Nutrients in the diet for control and experimental group for 30 l milk yield

Component	Experimental group	Control group
Dry matter (g)	20 810.2	21 033.8
N-matters (g)	3 553.3	3 587.1
PDIA (g)	895.6	920.1
PDIN (g)	2 259.8	2 294.7
PDIE (g)	1 789.8	1 876.8
Lipid (g)	1 453.9	777.7
Fiber (g)	3 244.9	3 024.0
Starch (g)	1 292.9	2 414.6
NEL (MJ)	140.1	139.9
Calcium (g)	269.0	174.4
Phosphorus (g)	127.0	121.7
Sodium (g)	41.3	41.3
Magnesium (g)	56.2	53.7
Potassium (g)	348.6	361.2
Vitamin A (i.u.)	222 586	211 164
Vitamin D (i.u.)	32 614	33 786
Tocopherol (mg)	941.89	900.33

our experiment, this ratio was significantly higher in the experimental group when compared to the control group, in the first month after parturition. Higher occurrences of hepatopathy at the end of first month were also indicated, due to higher values of total bilirubine, AST and LDH in the experimental group.

Increased occurrences of fatty livers due to increased lipid concentration in the diet, corresponds with the results of previously published detailed monitoring (Lubojacka et al., 2005). At the end of the second and third months of lactation, no significant differences in liver parameters were determined, and BHB concentration differences gradually decreased as well. The risk of a negative impact from lipid supplementation, on the condition of liver parenchyma, especially in the peripartal period, was also found by Grum et al. (1996). Similar results were published by Skaar et al. (1989), where lipid supplementation within 17 days before parturition and 15 weeks after, led to increased TG in liver during the first lactation phase. Berttics and Grummer (1999) also report higher TG in liver, due to lipid supplementation during the 10-day period of restricted diet.

An important effect was seen in the parameter of lipid metabolism. Compared with a normal lipid diet (3.70% total lipid), a high lipid diet (6.99% total lipid) given during the first 3 months of the lactation increased the concentration of total cholesterol and HDL-cholesterol in the blood serum. The concentration of total cholesterol exceeds the physiological range which is 2.6–5.2 mmol/l (Vrzgula et al., 1990). Similar results were obtained by other authors (Grummer and Carroll, 1991; Weiss and Wyatt, 2003), which assumed that this is due to a stimulatory effect of the fat on cholesterol synthesis. Significant differences were also seen in the proportion of HDL-cholesterol to total cholesterol, which was during the 2nd and 3rd month, less in the experimental group, than in the control group. Our results indicate that high lipid diets in the dairy cows may have a risk on health, as it is well known in humans. There is evidence that the risk of coronary disease is directly related to the plasma concentration of LDL-cholesterol and inversely related to that of HDL-cholesterol. The risk is reduced significantly by lowering elevated serum cholesterol levels. There is some evidence that atherosclerosis in the uterine blood vessels could

Table 4. Selected parameters of energetic and liver profile in the experimental (E; n = 13) and control (C; n = 14) groups during experiment (mean ± standard deviation)

Month of lactation	1		2		3	
Group	E	C	E	C	E	C
Glucose (mmol/l)	3.24 ± 0.47	3.19 ± 0.90	3.12 ± 0.50	3.24 ± 0.57	3.07 ± 0.34	3.03 ± 0.41
BHB (mmol/l)	1.05 ± 0.70*	0.51 ± 0.14*	0.81 ± 0.34	0.58 ± 0.15	0.76 ± 0.19	0.62 ± 0.24
Urea (mmol/l)	4.66 ± 0.98**	5.91 ± 0.93**	5.14 ± 0.99	5.66 ± 1.13	5.91 ± 0.82	5.69 ± 1.39
Bilirubin (μmol/l)	6.50 ± 2.04**	4.59 ± 1.70**	6.72 ± 1.35	5.64 ± 1.66	4.68 ± 1.26	3.86 ± 0.81
AST (μkat/l)	1.66 ± 0.33*	1.39 ± 0.28*	1.46 ± 0.21	1.46 ± 0.44	1.25 ± 0.10	1.37 ± 0.22
GMT (μkat/l)	0.43 ± 0.15	0.38 ± 0.15	0.45 ± 0.15	0.40 ± 0.10	0.43 ± 0.11	0.41 ± 0.10
LDH (μkat/l)	45.2 ± 10.8*	34.3 ± 6.3*	37.0 ± 8.8	35.2 ± 6.1	36.3 ± 5.1	37.3 ± 5.1

*$P < 0.05$ comparing the experimental and control group
**$P < 0.01$ comparing the experimental and control group

be related to infertility (Koper et al. 1994). These authors examined 20 cows aged 5–7 years, which were culled from the breeding herd because of infertility. Postmortem examinations were made of the uterus in 10 cows with retained corpora lutea, and 10 cows with inactive ovaries. Atherosclerotic changes were seen in the lumen of the arteries, sometimes causing marked narrowing of the arterial lumen. More advanced changes were seen in aged multiparous cows. Kampl et al. (1990) found in high yielding cows with reproductive disorders, the mean serum total, esterified and free cholesterol values were higher than in healthy controls. More experimental works show a negative effect of low cholesterol in blood on fertility. Huszenica et al. (1994) found the lower total cholesterol level in acyclity or *corpus luteum* deficiency. Kampl et al. (1995) published results showing lower concentrations of VLDL-cholesterol in cows with mastitis, foot diseases and with reproductive problems than in healthy cows. Ruegg et al. (1992) demonstrated that cows conceiving with ≤ 2 services had higher serum cholesterol values than did cows requiring more services. The reason for infertility in cows with low cholesterol values in blood may be in the concentration of cholesterol within follicular fluid, which is about 45% of that in blood (Wehrman et al., 1991). These authors found out that feeding high lipid diet supplements to postpartum range cattle for 30 days increased the incidence of ovarian luteal activity by 18 %, compared with cattle fed the normal lipid diet. Granulose cells from preovulatory follicles of heifers fed a high lipid diet, secreted 2.1 to 3.5 – fold more pregnenolone and progesterone, than cells from heifers fed the normal lipid diet.

Our results indicate a positive effect of supplemental fat on the concentration of vitamin E in blood serum, which was almost two times higher in the experimental group than in the control group, on completion of the study. Vitamin E in blood is closely related to circulating lipoprotein. Only 3% of plasma vitamin E was not associated with lipoproteins. It can be concluded that higher amounts of lipid in the diet improves absorption and utilisation of vitamin E, because in the content of vitamin E in these two diets were very small differences (in the experimental diet there was only about 5% more vitamin E than in the control diet). Weiss and Wyatt (2003) monitored the influence of various vitamin E supplements in the diet with and without supplementary lipids. Concentrations of α-tocopherol in plasma were higher for cows fed fat, and concentrations increased linearly with increasing dietary vitamin E. Dietary fat supplementation affected plasma concentration of α-tocopherol expressed per litre, but did not affect plasma concentration per unit of cholesterol. In our study, the vitamin E:Chol ratio was lower in the experimental than in the control group. We suggest that this is due to capacity of the plasma lipoproteins ability to carry α-tocopherol and the amounts of vitamin E in the daily ration. In comparison with Weiss and Wyatt (2003), the amount of vitamin E in our daily ration was low. One of the positive impacts of higher vitamin E concentration in the blood serum consists in the protection of cells against oxidative damage.

Table 5. Selected parameters of lipid metabolism and concentration of vitamin E in the experimental (E; n = 13) and control (C; n = 14) groups during experiment (mean ± standard deviation)

Month of lactation	1		2		3	
Group	E	C	E	C	E	C
Triacylglycerols (mmol/l)	0.28 ± 0.06	0.27 ± 0.09	0.33 ± 0.07	0.31 ± 0.13	0.39 ± 0.11**	0.23 ± 0.13**
NEFA (mmol/l)	0.68 ± 0.43**	0.27 ± 0.10**	0.36 ± 0.11	0.27 ± 0.15	0.23 ± 0.06***	0.13 ± 0.05***
NEFA/TG	2.45 ± 1.47*	1.25 ± 0.82*	1.10 ± 0.33	1.01 ± 0.63	0.65 ± 0.27	0.85 ± 0.71
Total cholesterol (mmol/l)	5.57 ± 1.51***	3.31 ± 0.76***	7.00 ± 1.60****	3.73 ± 0.72****	7.28 ± 1.95****	3.69 ± 0.75****
HDL-cholesterol (mmol/l)	4.16 ± 0.82****	2.77 ± 0.46****	5.31 ± 1.06****	3.31 ± 0.58****	5.43 ± 1.06****	3.26 ± 0.59****
HDL-cholesterol (%)	76.6 ± 10.7	85.3 ± 11.2	76.5 ± 6.81***	89.4 ± 7.4***	76.1 ± 8.6***	88.8 ± 7.9***
Vitamin E (mmol/l)	15.3 ± 3.4**	10.8 ± 2.9**	16.1 ± 4.4***	10.2 ± 2.4***	19.9 ± 4.8****	10.3 ± 2.1****
Vitamin E/Cholesterol	2.88 ± 0.79	3.28 ± 0.52	2.33 ± 0.48*	2.76 ± 0.51*	2.79 ± 0.47	2.83 ± 0.52

*P < 0.05 comparing the experimental and control group
**P < 0.01 comparing the experimental and control group
***P < 0.001 comparing the experimental and control group
****P < 0.0001 comparing the experimental and control group

With the aim to determine whether higher doses of lipids influence the body's defenses against free radical damage, we also monitored other parameters – total antioxidant status of blood serum and the activity of glutathionperoxidase and superoxiddismutase. Significant differences in the total antioxidant capacity were found between the experimental and control groups at the end of the 2nd and 3rd months of lactation (values were higher in the experimental group). These results point to a higher capacity in the defense system, which may relate to a higher concentration of vitamin E, but other mechanisms may play their role as well. Decreasing the antioxidant capacity of muscle, due to vitamin E deficiency in sheep was discovered by Fry et al. (1993). TAS in relation to milk yield of dairy cows and their metabolic load was studied by Castillo et al. (2003, 2005). These authors determined lower values of TAS in cows during the peripartal period, but found no differences in TAS in relation to the milk yield of dairy cows. The dairy cows with high milk yield present higher plasma lipid hydro-peroxides, but this increase in oxidant compounds was not accompanied by higher levels in protective substances (TAS). As a single measure, total antioxidant status provides more biologically relevant information, which may more effectively describe the dynamic equilibrium between pro-oxidants and antioxidants in the plasma compartment (Ghiselli et al., 2000).

Table 6. Selected parameters of antioxidants and thyroid hormones in the experimental (E; n = 13) and control (C; n = 14) groups during experiment (mean ± standard deviation)

Month of lactation	1		2		3	
Group	E	C	E	C	E	C
TAS (mmol/l)	0.83 ± 0.09	0.82 ± 0.10	0.96 ± 0.07*	0.90 ± 0.07*	1.02 ± 0.09*	0.94 ± 0.09*
SOD (μkat/l)	261 ± 26.8	272 ± 36.8	260 ± 30.7	262 ± 37.5	247 ± 36.6	264 ± 30.5
GSH-Px (μkat/l)	605 ± 192	560 ± 179	569 ± 109	597 ± 231	613 ± 118	592 ± 112
T3 (nmol/l)	2.16 ± 0.84	2.13 ± 0.35	1.91 ± 0.43	1.96 ± 0.35	2.01 ± 0.38	2.29 ± 0.57
T4 (nmol/)	52.2 ± 18.1	63.5 ± 12.7	57.0 ± 13.2	62.5 ± 12.8	61.6 ± 13.7	65.5 ± 14.6

*P < 0.05 comparing the experimental and control group

Among other metabolism parameters, we monitored the concentration of thyroid hormones. Lower values of T4 (52.18 vs. 63.45, $P \leq 0.079$) were found at the end of the first month in the experimental cows, but the values gradually evened up in subsequent months, which shows that even when high doses of rapeseed were used, iodine metabolism was not disturbed. The treatment used eliminated the risk of a negative influence of glucosinolates sufficiently. The root cause of the discrepancy in the first month of lactation was probably due to the increased occurrence of an energetic deficit in the experimental group cows. These results are in accordance with our previous observations (Pechova et al., 2004), where decreased T4 values were determined in dairy cows with ketosis and liver alteration. There is a positive correlation between energetic balance and T4 concentration (Huszenica et al., 2002). Like we, Kokkonen et al. (2004) did not establish the influence of lipid content in the diet on thyroid hormones.

From the standpoint of the influence on metabolism of minerals, we observed an increase in concentration of calcium in the serum, due to the calcium supplementation in the form of calcium salts containing the fatty acids, which was significantly higher in the second and third months in the experimental group.

Milk production

The results of milk production for the first 100 days of lactation are shown in Table 8. Increased lipid concentration in the diet did not significantly influence either the total production of milk or the average daily milk yield. Insignificant differences were found in fat concentration, which was 0.34% higher in the experimental group. The influence of lipid in the diet on the concentration of fat in the produced milk was not uniform and was influenced by both the form and the composition of the diet (Onetti and Grummer, 2004). Lowering of the fat concentration was caused by the negative impact of lipid on rumen fermentation, when acetate and butyrate production was decreased, resulting in reduced lipid synthesis in the milk glands. In recent years, lipid synthesis depression has been explained by the biohydrogenation theory, based on the assumption of direct inhibition of milk lipid synthesis by trans-fatty acids, which are generated as intermediate products of biohydrogenation of unsaturated fatty acids in rumen (Bauman and Griinari, 2001). Conversely, milk fat concentration can be increased by using processed lipids, which are not fermented in rumen and thus increase the reserves of fatty acids for milk lipid synthesis. This mechanism probably played some role in our experiment, as we used calcium salts of fatty acids, which are not fermented in the rumen. Similarly Kokkonen et al. (2004) determined increased fat concentration in milk due to increased lipid content in the diet as a result of supplementation by calcium salts of palm fatty acids. Jones et al. (2001), on the other hand, established a decrease in fat concentration in milk as a result of supplementation of heat-treated rapeseed. This discrepancy could have been caused by different treatment, as the lipid was not sufficiently protected against biohydrogenation by rumen microflora.

To the contrary, concentration of protein in milk in the experimental group was lower by 0.27%. These results are in accordance with other authors, while protein depression is probably independent of lipid source. Garnsworthy (2002) explains depression of milk protein synthesis by a lack of glucose, whose synthesis may be influenced by lipid supplementation in several ways: (i) decreasing of

Table 7. Selected parameters of mineral metabolism in the experimental (E; $n = 13$) and control (C; $n = 14$) groups during experiment (mean ± standard deviation)

Month of lactation	1		2		3	
Group	E	C	E	C	E	C
Ca (mmol/l)	2.34 ± 0.18	2.35 ± 0.13	2.55 ± 0.15**	2.38 ± 0.12**	2.42 ± 0.15*	2.29 ± 0.11*
P (mmol/l)	1.79 ± 0.34*	2.09 ± 0.33*	1.87 ± 0.48	1.69 ± 0.33	1.97 ± 0.53	1.93 ± 0.34
Mg (mmol/l)	1.00 ± 0.15	1.01 ± 0.06	0.98 ± 0.10	0.95 ± 0.07	0.95 ± 0.09	0.97 ± 0.09

*$P < 0.05$ comparing the experimental and control group

**$P < 0.01$ comparing the experimental and control group

propionate production in starch replacement in diet by lipids; (ii) decreasing of microbial synthesis of protein if there is fermentable metabolisable energy in rumen, with the resulting lack of glucogenic amino acids; (iii) consumption of glucose in intestinal mucosa synthesis of glycerol-3P, which is necessary for the absorption of free fatty acids; (iv) increasing of glucose consumption for lactose synthesis during milk consumption. Another factor that might cause the depression of protein synthesis in our study was the higher occurrence of fatty livers in the experimental group, which may negatively influence gluconeogenesis in the long term. Long-term decreasing of protein concentration in milk was determined as a result of fatty liver during the post-parturition period (Illek et al., 1995).

Reproduction

Evaluation of reproduction, with regard to a rather small number of monitored animals, is for orientation purposes only – for results see Table 9. All dairy cows from the experimental group were included in statistics, as opposed to only 12 dairy cows from the control group, as 2 dairy cows did not become gravid until the end of 9th month of lactation, and they were removed from the farm. From the viewpoint of the total number of pregnant milk cows, the results were better in the experimental group (100% vs. 85.7%). In evaluation of individual monitored parameters, the experimental group showed a longer period of time to the first insemination and open days, but the insemination index was higher in the control group. We believe that the reason for the longer period between the first insemination and the subsequent pregnancy was the higher occurrence of fatty liver in the experimental group. During the development of fat cow syndrome, uterine involution is delayed and ovary activity starts later, due to a decreased synthesis of steroidal hormones and a negative energetic balance (Bobe et al., 2004). On the contrary, better results in the percentage of pregnant cows and lower insemination index in the experimental group might be explained by a positive effect of lipid supplementation in the diet. As with efficiency investigation, reproduction results in relation to lipid supplementation in the diet are not uniform, but numerous papers corroborate its positive effect. The mechanism by which dietary fat improves reproductive performance has not been elucidated. Several hypotheses have been proposed: (i) an amelioration of a negative energy status; (ii) an increase in steroidogenesis favourable for fertility; (iii) manipulation of insulin so as to stimulate ovarian follicle development; and (iv) a stimulation or inhibition of the production and release of $PGF_{2\alpha}$, which influences the persistence of the *corpus luteum* (Staples et al., 1998). On the other hand, there is a risk of worsened reproduction functions due to atherosclerosis, caused by the increased concentration of cholesterol in blood – this study revealed an increased presence of LDL-cholesterol.

Our results show that the main health risk related to the feeding of higher doses of lipid (6.99%) in the form of calcium salts of fatty acids from rapeseed, lies in the higher occurrence of liver steatosis in the first moth after parturition. Positive effects consist of an increased concentration of vitamin E, total cholesterol, HDL-cholesterol and total antioxidant status during the study in the lipid-supplemented group. Although rather high doses of rapeseed were used, concentration of thyroid hormones were not affected, which indicates that the processing removes the negative influence of glucosinolates. The

Table 8. Performance of dairy cows of the experimental (E; n = 13) and control (C; n = 14) groups during the 1st month of lactation and after 100 days of lactation (mean ± standard deviation)

Group	E	C
100 days lactation (kg)	2 810.0 ± 392.9	2 678.7 ± 458.0
Daily milk yield (kg/day)	28.10 ± 3.94	26.79 ± 4.57
Fat (%)	3.55 ± 1.07	3.21 ± 0.67
Protein (%)	3.18 ± 0.15**	3.45 ± 0.19**
Lactose (%)	4.99 ± 0.10	5.01 ± 0.12

**$P < 0.01$ comparing the experimental and control group

Table 9. Evaluation of reproductive functions in dairy cows in the control (C; *n* = 12) and experimental (E; *n* = 13) groups (mean ± standard deviation)

Group	E	C
Days to the 1st insemination	71.9 ± 20.9	62.4 ± 17.1
Days open	117.1 ± 43.4	103.67 ± 38.9
Insemination index	2.0 ± 0.6	2.3 ± 1.0

total production of milk in both monitored groups in the 100 days of lactation was similar. The concentration of fat was insignificantly higher, while the concentration of protein was significantly lower in the experimental group. Reproduction results did not differ significantly either, however the total percentage of pregnancies were higher in the experimental group of dairy cows.

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