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The effectiveness of rapeseed cake and glycerine in feeding dairy cows

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ABSTRACT

The aim of the study was to evaluate the effect of replacing cereal grains and soyabean oilmeal with rapeseed cake or glycerine in the rations of high-yielding cows. The experiment was carried out on 56 Polish HF cows allotted to 4 groups of 14 animals according to body weight, milk yield, and parity. The cows were fed rations differing in protein and energy content from 6 weeks before calving to 12 weeks of lactation. The basal diet of all groups was composed of silages, meadow hay and concentrate feeds. In the experimental groups, the cereal grains and soyabean oilmeal fed to the control group (C) were replaced by rapeseed cake (30%; groups RC and RC_{W6}) or glycerine (5%, group G).

For the entire experimental period, no statistical differences were found among the groups in the daily intake of dry matter and other nutrients, except PDIE. The control group cows produced about 1.2 kg/day less milk ($P>0.05$), but the contents of dry matter, fat, protein, lactose, and urea in milk were similar. The highest concentration of free fatty acids and β-hydroxybutyric acid were found in the milk of the control cows. Introducing 5% glycerol into the diet did not cause any significant changes in production parameters. The serum metabolite profile of cows fed the experimental diets suggests that these cows could have been at a lower risk of ketosis.

KEY WORDS: dairy cows, rapeseed cake, glycerine, performance, blood metabolites

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INTRODUCTION

Bio-fuel production by cold-press extraction of oil is intensifying and bringing increasing quantities of by-products, including rapeseed cake and glycerine, to the Polish market. The protein content of rapeseed cake can make it a good substitute for soyabean meal, while glycerine can be a good source of energy (Goff and Horst, 2001; De Frain et al., 2004; Chung et al., 2007). The use of rapeseed cake that has not undergone any additional processing makes sense primarily for feeding ruminants, which are less sensitive to antinutritional factors thanks to their ruminal ecosystem. In previous studies, the nutrient content, rate of ruminal protein degradation, and intestinal digestibility coefficient of rumen undegradable protein were determined, and the nutritive value of rapeseed cake containing 15% fat per kg feed, originating from both dark and yellow rapeseed, was determined on bulls (Strzetelski and Niwińska, 1997; Niwińska et al., 2001; Strzetelski et al., 2001a).

Fattening bulls weighing between 155 and 540 kg with a complete diet containing 23% rapeseed cake (15% fat) resulted in similar weight gains (about 1220 g/day) as feeding a control diet containing soyabean oilmeal (Stasiniewicz et al., 2000). High body weight gains (about 1300 g/day) were also obtained when bulls (180 to 530 kg body weight) were fattened with maize silage and a concentrate containing 29% rapeseed cake (Strzetelski et al., 2001b).

Nonetheless, feeding rapeseed cake to ruminants may impair rumen function and reduce feed digestibility when rations are improperly balanced because fat supplies no energy to ruminal microorganisms and feed intake may decrease as a result of glucosinolate content (Kowalczyk et al., 1977; Flachowsky et al., 1994). On the other hand, due to its fatty acid composition, rapeseed cake is a feed that can be used to modify the composition of milk and meat fat (Jahreis et al., 1996). In an earlier study, Stasiniewicz et al. (2000) reported that rapeseed cake contributes to increasing the proportion of polyunsaturated fatty acids (PUFA) in intramuscular fat, including conjugated linoleic acids (CLA), reducing the linoleic (C18:6 n-6)-to-linolenic acid (C18:3 n-3) ratio, and lowering the serum cholesterol level without affecting the physicochemical properties of meat. It was also shown that extended feeding of concentrates prior to calving may have a favourable effect on production and economic results (Osięgłowski and Strzetelski, 2006).

Glycerol, the main component of glycerine, is a glucogenic substrate for ruminants that can be converted to glucose by the bovine liver and provide energy for cell metabolism (Goff and Horst, 2001). Therefore, glycerine may be a good source of energy for high-yielding cows, especially in the transition period when the cow has a limited feed intake capacity and its addition to the ration may positively affect feed intake, blood metabolic profile and hepatic lipid metabolism, limit the energy deficit after calving, and prevent metabolic disorders. Boyd et al. (2011) reported that dietary glycerol may improve performance and efficiency in high-yielding dairy cows. Similarly, data presented by Carvalho et al. (2011) indicate that glycerol can be recommended to replace maize in rations fed to transition dairy cows up to about 11% of the pre- and post-partum ration DM, based on the lack of detrimental effects on feed intake, milk production or milk composition. There is insufficient research in this area on by-products obtained from the production of bio-fuels from local rapeseed.

The objective of the experiment was to determine the possibility of using rapeseed cake and glycerine in diets balanced for energy and protein according to IZ-INRA (2001) standards, and to examine the effect of an extended period of feeding a diet with rapeseed cake on milk yield and composition, the health-promoting value of milk, and blood metabolic profile.

MATERIAL AND METHODS

Experimental design, feeding and management of cows

The experiment was carried out on 56 Polish Holstein-Friesian cows, which were allotted by the analog method to 4 groups of 14 animals each according to body weight three weeks before calving, maximum milk yield in the previous lactation, and parity (from 2 to 4), and started 6 weeks before the planned calving.

Cows were kept in straw-bedded tie-up stalls equipped with individual feeding troughs enabling control of the intake of concentrates and roughages separately, automatic drinkers, and salt licks. Three or four weeks before predicted parturition, the cows were moved to a calving pen until the first week of lactation.

Table 1. Concentrate rations for cows before calving, kg·day^{-1}

Groups	Weeks before calving					
	-6	-5	-4	-3	-2	-1
C, control	-	-	-	1	2	3
RC, concentrate with rapeseed oil cake	-	-	-	1	2	3
G, concentrate with glycerine	-	-	-	1	2	3
RC_{W6}, concentrate as in RC group	1	1	1	1	2	3

The experiment was carried out until 12 weeks of lactation. Before calving from week (W) -3 to -1 and in the lactation period, all of the cows were fed according to IZ-INRA (2001) standards. Additionally, from weeks -6 to -4 before calving, the cows of group RC_{W6} received 1 kg of concentrate (Table 1). The basal diet for groups of cows was composed of whole plant maize silage, lucerne silage, sugar beet pulp silage, wet brewer’s grains silage, and meadow hay. The composition of concentrates is given in Table 2.

Table 2. Concentrate composition

Item	Concentrates for groups		
	C, control	RC and RC_{W6}	G
Maize, ground	50.0	50.0	13.0
Wheat, ground	21.0	9.0	55.0
Soyabean oilmeal	18.0	-	16.0
Wheat bran	5.0	5.0	5.0
Rapseed cake	-	30.0	-
Glycerine[1]	-	-	5.0
VITAMIX KW-ZZD[2](Polmass)	2.8	2.8	2.8
$Ca(H_2PO_4)_2$	1.2	1.2	1.2
Limestone	1.2	1.2	1.2
MgO	0.2	0.2	0.2
$NaHCO_3$	0.5	0.5	0.5
NaCl	0.1	0.1	0.1

[1] 99% gliceryne, Over Agro firm; [2] in kg; g: P 60, Ca 150, Mg 30, Na 10; mg: Cu 1000, Zn 12000, Mn 8000, vit. E 3000; IU: vit. A 1000000, vit. D 13000

Daily rations for cows before parturition contained 8.5 kg dry matter (DM) from the basal diet and from 0 to 2.7 kg DM from concentrate, whereas during lactation, from 14.2 to 18.1 kg DM and from 3.1 to 10.8 kg DM, respectively. Additionally, Aquablend, Supercynk, and Hydrovit (Aquablend, the Netherlands) mineral and vitamin additives were supplied in water in automatic drinkers. Possible calcium deficiencies in the ration were corrected with ground limestone as needed.

Cows were fed twice a day: silages from maize, sugar beet pulp, and brewer's grains were fed in the morning, whereas lucerne silage, whole plant barley and lucerne mixture silage, and meadow hay were provided in the afternoon. The concentrates were fed three times a day.

Measurements and sampling

The body weight of the cows was taken on days 21, 14 and 5-7 before expected calving and on days 7, 35 and 84 after calving. Refusals were collected and weighed daily before the morning feeding, beginning from week 3 pre-calving to the end of week 4 post-calving. During the remaining weeks of lactation, refusals were collected for 1 day every 2 weeks.

Milk yield was estimated daily using Milk Master equipment. Milk composition was determined in representative daily samples taken from each cow every two weeks throughout lactation. They were refrigerated at +4°C for no longer than 2 weeks.

Starting from 1 week before calving to 5 weeks of lactation, blood samples were taken from the jugular vein on the third day of each week about 4 h after the morning feeding; serum was preserved at -20°C until analysis.

Chemical analysis

The chemical composition of feeds and refusals was analysed according to AOAC (1990) methods. The volatile fatty acids (VFA) in silages were analysed by gas chromatography using a Varian 3400 apparatus and Auto sampler 8200CX, capillary column CP-Wax 58 (25 m x 53 mm x 1 mm). The initial furnace temperature of the column (80°C) was increased by 7°C/min until 270°C. Injection temperature was 200°C and detector temperature 260°C. Helium was used as the carrier gas at a flow rate of 6 ml/min. One ml of water extract from the silages was applied to the column. Silage pH was determined with the Auto Kjeldahl Unit K-370 (Bischi) using the pH determination function. Lactic acid was determined by high performance liquid chromatography (HPLC) after separation of water permeates with 24% met-phosphoric acid using a 250 cm Lichrocart Superspher RP 18 column, UV 210 nm detector, fluent (1 µl H_2O + 100 µl H_2SO_4, 1 ml/min) and injection 20 µl. The fatty acid profile in milk was determined by the same gas chromatography apparatus used for VFA in silage analysis, but capillary column was Rtx2330 (105 m x 0.32 mm x 02 µm), injection and detector temperature - 250°C, and helium flow rate was 3 ml/min. Temperature program was: 60°C - 10 min, up to 120°C - 20°C/min, up to 240°C - 3°C/min.

Milk composition was determined using a Milko-Scan FT 120 (Foss Electric, Denmark).

Non-esterified fatty acids (NEFA) in blood serum were determined colorimetrically using acyl-CoA synthetase, oxidase, and peroxidase (WAKO Reagents). D-3 hydroxybutyric acid (BHBA) was determined by a kinetic enzymatic reaction using a Cobas-Bio analyser (Roche) and a high-sensitivity reagent kit (RANDOX). Glucose was determined using a VITROS 950 analyser (Ortho-Clinical Diagnostic; Test Methodology Manual, 1997).

Calculations and statistical analysis

The energy and protein value of the feeds, as well as the composition of concentrates and diets were formulated according to IZ-INRA (2001) standards based on chemical composition using WINVAR 1.6 (2000) and INRAtion 2.63 (1998/99) software.

The results were subjected to one-way analysis of variance using the GLM procedure of SAS (2001). The differences between treatments were then estimated using the LSM method. $P<0.05$ was considered significant.

The curves showing changes in dry matter, energy and PDI intake were plotted using Excel. The curves showing changes in daily milk yield in subsequent days of lactation, depending on treatments, were calculated using Quick Statistica (1992), according to the equation:

$$Y = a \log x = bx + c$$

where: Y and x - mean daily yield and lactation day, respectively.

RESULTS

The difference between the predicted and actual day of calving averaged (days):1.4 (group C), 2.0 (RC), 2.2 (G), -0.3 (RC_{W6}).

The content of fat and crude fibre was higher in diets with rapeseed cake, whereas in the diet with glycerine the fat content was the lowest, and crude protein, PDI and energy contents were higher compared with the other groups (Table 3).

Table 3. Chemical composition and nutritive value of 1 kg DM

Feeds	Dry matter, g	Crude protein, g	Ether extract, g	Crude fibre, g	Ash, g	PDIN, g	PDIE, g	UFL
Maize, ground	896.10	93.40	38.20	20.30	13.00	85.10	106.90	1.28
Triticale, ground	868.00	139.90	12.00	32.40	21.10	161.20	108.90	1.23
Lucerne silage[1]	415.10	69.30	12.15	135.90	42.85	96.85	62.50	0.73
Soyabean oilmeal	890.9	460.60	23.60	33.50	66.20	368.3	249.40	1.22
Wheat bran	873.10	154.70	36.10	92.60	50.10	177.20	117.30	0.92
Rapeseed cake	929.50	333.30	123.40	129.50	60.70	358.60	216.40	1.24
Brewer's grains silage	245.80	60.70	23.70	39.30	10.70	246.9	183.50	0.94
Sugar beet pulp silage	196.30	20.80	1.9	34.50	10.30	106.00	64.50	1.01
Meadow hay	837.50	83.40	23.60	289.60	44.70	99.60	62	0.65
Glycerine (energy plus)	800							2.36
Concentrate for groups								
C	890.00	187.33	31.09	31.02	28.40	137	127	1.16
RC and RC_{W6}	904.00	184.75	65.27	62.55	32.21	123	103	1.17
G	872	195.54	19.66	34.92	30.26	135	120	1.20

[1] fermentation products (FP): propionic acid + lactic acid + acetic acid + butyric acid; FP of maize silage = 36.00 g/kg feed, pH =4.56; FP of lucrne silage = 36.80 g/kg feed, pH =4.79; FP of brever's grain silage = 13.70 g/kg feed, pH =5.04; FP of sugar beet pulp silage = 8.50 g/kg feed, pH =5.08

During the pre-partum period, no statistically significant differences were found between the groups in daily intake of nutrients and energy, although cows receiving additional concentrate from 6 to 4 weeks before calving (group RC_{W6}) ingested about 24±3% more concentrate per day compared with the other groups (Table 4). No significant differences were found either between the groups in daily nutrient intake during lactation, except PDIE. Cows in groups C and G ingested about 6.5% more PDIE ($P<0.01$) per day compared with animals fed the rapeseed cake diet (groups RC and RC_{W6}).

In some weeks of lactation, small differences did occur, however, in dry matter intake, particularly energy intake, in cows fed the diets containing rapeseed cake (groups RC and RC_{W6}) and receiving less ileum-digested protein for almost the entire lactation period (Figure 1).

Table 4. Feed and nutrients intake

Item	Group[1]				P	SE
	C	RC	G	RC_{W6}		
Before calving						
feeding days	43.43	43.00	44.17	41.45	0.40	0.67
total concentrate, kg·cow^{-1}	46.28[a]	45.08[a]	48.50[a]	61.73[b]	0.046	2.02
dry matter, kg·d^{-1}	9.22	9.32	9.32	9.53	0.14	0.05
crude protein, kg·d^{-1}	1.27	1.31	1.31	1.35	0.52	0.02
PDIN, kg·d^{-1}	0.81	0.82	0.82	0.85	0.61	0.01
PDIE, kg·d^{-1}	0.76	0.76	0.77	0.79	0.87	0.01
UFL, d^{-1}	6.41	6.69	6.65	6.93	0.68	0.15
From calving to 12th week of lactation						
concentrate offered in lactation, kg DM·d^{-1}	8.07	8.32	8.09	8.34	0.18	0.03
before and after calving, kg DM·d^{-1}	8.56	8.81	8.59	8.87	0.21	0,04
dry matter, kg·d^{-1}	22.69	22.76	23.14	22.81	0.36	0.09
crude protein, kg·d^{-1}	3.43	3.52	3.57	3.50	0.21	0.02
PDIN, kg·d^{-1}	2.29	2.24	2.32	2.23	0.08	0.01
PDIE, kg·d^{-1}	2.16[a]	2.02[b]	2.16[a]	2.02[b]	<0.01	0.02
UFL, d^{-1}	19.77	20.55	20.58	20.51	0.58	0.25

P>0.05 - differences are not statistically significant; [1] C - control; RC and RC_{w6} - with rapeseed cake; G - glycerine supplement

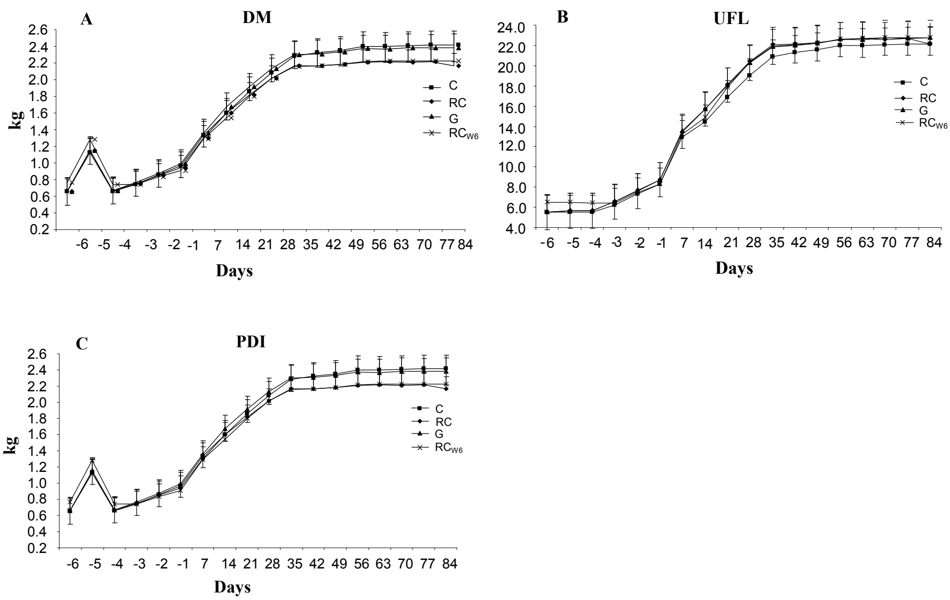

Figure 1. Changes of daily dry (DM) matter intake (A), energy (UFL) (B) and protein (PDI) (C) during experimental period

Compared with the other groups, cows in the control group produced about 1.2 kg less milk per day, but differences between the groups were not significant (Table 5). Neither were there significant differences between the groups in dry matter, fat, protein and lactose percentage and in urea concentration in cows' milk. The shape of lactation curves also indicates that compared with the control group, feeding the rapeseed-cake or glycerine diets, as well as the extended feeding of the diet from 6 week before calving, had a more beneficial effect on milk yield (Figure 2).

Table 5. Milk production and milk composition

Item	Group[1]				P	SE
	C	RC	G	RC_{W6}		
% HF	92.20	94.06	91.19	94.02	0.412	0.71
Parity	3.00	2.75	3.69	3.10	0.374	0.19
Total milk production, $kg \cdot cow^{-1}$	3046.7	3166.8	3142.4	3130.7	0.91	65.98
Milk yield, $kg \cdot d^{-1}$	36.27	37.70	37.41	37.27	0.92	0.79
Milk composition, %						
dry matter	12.05	11.76	11.84	11.95	0.86	0.12
fat	3.39	3.17	3.17	3.45	0.69	0.11
protein	3.17	3.15	3.25	3.33	0.27	0.03
lactose	4.79	4.73	4.71	4.70	0.65	0.03
urea, mg, l^{-1}	177.69	216.07	203.00	192.22	0.33	7.58

P>0.05 - differences are not statistically significant; [1] as in Table 4

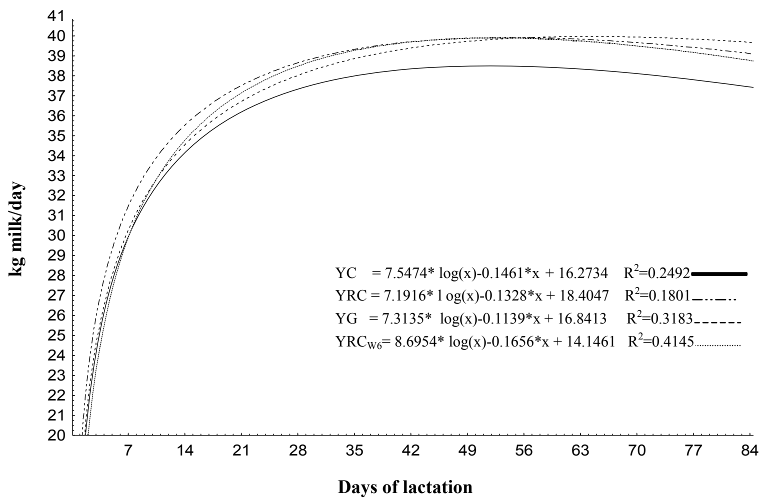

Figure 2. The changes in daily milk yield during lactation

The efficiency of diet and nutrient utilization per kg milk production was similar in all the groups (Table 6).

Table 6. Concentrate and nutrients utilization from 1 to 12 weeks of lactation

Item	Group[1] C	RC	G	RC_{W6}	P	SE
Concentrates, kg·1 kg milk						
in lactation	0.24	0.24	0.25	0.25	0.89	0.02
before and after calving	0.26	0.26	0.26	0.27	0.92	0.04
Conversion, 1 kg milk, kg						
dry matter	0.64	0.62	0.63	0.62	0.95	0.02
crude protein	0.095	0.093	0.095	0.094	0.98	0.001
PDI	0.059	0.053	0.058	0.054	0.12	0.001
UFL	0.55	0.56	0.56	0.55	0.99	0.02

P>0.05 - differences are not statistically significant; [1] - as in Table 4

On each measurement day, no statistically significant differences were noted between the groups in body weight and body condition (Table 7), but on day 84 compared with day 7 of lactation, the body weight loss of cows in group C was characterized by numerically greater values on average (32.71 kg) compared with cows in groups RC (29.75 kg), G (26.46 kg), and RC_{W6} (26.10 kg). During the same period, body condition score (BCS) loss followed a similar pattern, averaging 0.13, 0.11, 0.03 and 0.05 for groups C, RC, G and RC_{W6}, respectively.

Table 7. Liveweight and body condition score (BCS) before and after calving, kg

Day from parturition	Group[1] C	RC	G	RC_{W6}	P	SE
Liveweight , kg						
before calving						
-21	685.14	666.25	684.77	663.60	0.80	9.72
-14	691.00	676.08	698.23	673.00	0.80	10.06
-7	697.21	683.00	707.53	689.60	0.42	22.24
calving						
+7	658.28	637.66	648.61	636.20	0.68	9.45
+35	618.00	609.91	627.00	606.40	0.83	8.15
+84	626.71	607.91	622.15	616.10	0.68	8.48
BCS, points						
before calving						
-21	3.49	3.56	3.48	3.53	0.97	0.06
-14	3.62	3.66	3.60	3.65	0.97	0.05
-7	3.72	3.43	3.68	3.32	0.55	0.11
calving						
+7	3.33	3.28	3.25	3.25	0.86	0.04
+35	3.16	3.15	3.18	3.12	0.97	0.04
+84	3.20	3.17	3.22	3.20	0.85	0.03

P>0.05 - differences are not statistically significant; [1] as in Table 4

The highest serum concentrate of FFA and BHBA was observed in the control group, whereas cows in the groups receiving the glycerine (G) or rapeseed-cake diets from 6 weeks before calving (RC_{W6}) were characterized by a notably lower concentration of these metabolites compared with group RC (Figure 3).

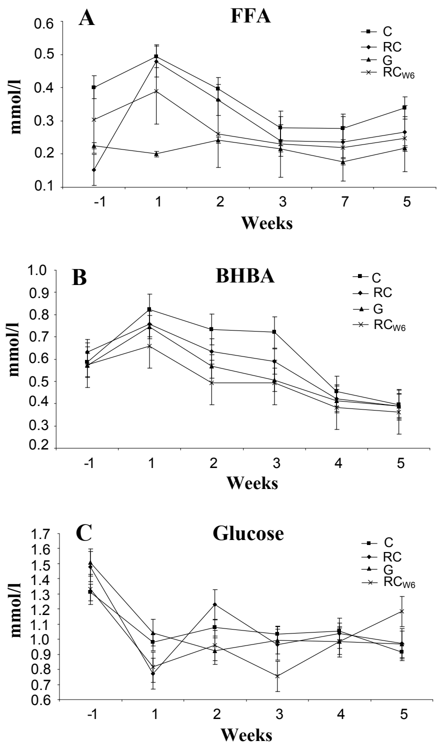

Figure 3. Changes of (A) free fatty acids (FFA), (B) β-hydroksybutyric acid (BHBA) and (C) glucose concentrations in blood serum

The rapeseed-cake diets had a favourable effect on the fatty acid profile of milk: fat SFA content tended to decrease and milk PUFA content increased, although the differences were not significant (Table 8). The total n-3 PUFA content of milk was higher in the experimental groups compared with the control group, but significant differences were noted in relation to group RC_{W6} only ($P<0.05$). The content of linolenic acid (C18:3) in the milk of cows receiving the rapeseed-cake diets from 6 weeks before calving was also significantly higher compared with the other groups ($P<0.05$) as well as a more beneficial n-6/n-3 PUFA ratio, which decreased significantly in this group ($P<0.05$). The cows fed the rapeseed cake diet showed a tendency towards a higher content of oleic acid (C18:1) and CLA in milk than of control cows.

Table 8. Fatty acids profile, g FA/100 g of milk fat

Fatty acid	Groups[1]				P	SEM
	C	RC	G	RC_{W6}		
c8	3.30	3.52	3.50	3.01	0.39	0.11
c10	4.39	4.51	4.94	3.74	0.14	0.18
c12	7.00	6.83	7.83	7.10	0.70	0.30
c14	19.09	18.44	18.83	16.80	0.08	0.35
c16	33.99	33.14	34.24	34.46	0.84	0.51
c16-1	2.40	2.23	2.50	2.24	0.83	0.11
c18	6.81	6.82	5.96	6.85	0.85	0.40
c18-1	19.71	20.26	18.35	21.59	0.52	0.75
c18-2	1.76	1.81	1.76	1.98	0.44	0.05
Gama18-3	0.01	0.02	0.01	0.02	0.23	0.00
c20	0.04	0.05	0.04	0.06	0.93	0.01
c18-3	0.26^{b}	0.28^{b}	0.27^{b}	0.35^{a}	0.01	0.01
Cc9-t11	0.72	0.95	0.81	0.80	0.31	0.04
Ct10c12	0.29	0.65	0.70	0.34	0.45	0.11
Cc9-c11	0.06	0.07	0.04	0.12	0.08	0.01
Ct9-t11	0.06	0.06	0.06	0.05	0.70	0.00
c22	0.00	0.00	0.00	0.01	0.17	0.00
c20-4	0.10^{ab}	0.13^{a}	0.11^{ab}	0.07^{b}	0.03	0.01
c22-1	0.00	0.22	0.03	0.40	0.07	0.06
EPA	0.01	0.01	0.03	0.01	0.26	0.01
DHA	0.00^{b}	0.02^{a}	0.00^{b}	0.01^{b}	0.01	0.00
SFA	74.62	73.31	75.34	72.02	0.57	0.86
UFA	25.38	26.69	24.66	27.98	0.57	0.86
MUFA	22.12	22.71	20.88	24.24	0.50	0.76
PUFA	3.26	3.99	3.78	3.74	0.40	0.15
PUFA-6	1.87	1.97	1.87	2.07	0.54	0.05
PUFA-3	0.27^{b}	0.30^{ab}	0.31^{ab}	0.36^{a}	0.05	0.01
DFA	32.19	33.51	30.63	34.82	0.55	1.03
OFA	67.81	66.49	69.37	65.18	0.55	1.03
UFA/SFA	0.34	0.37	0.34	0.39	0.59	0.02
DFA/OFA	0.48	0.51	0.45	0.54	0.59	0.02
MUFA/SFA	0.30	0.31	0.28	0.34	0.54	0.01
PUFA/SFA	0.04	0.05	0.05	0.05	0.49	0.00
PUFA 6/3	7.14^{a}	6.55^{ab}	6.19^{b}	5.70^{b}	0.02	0.18
CLA	1.12	1.72	1.61	1.30	0.35	0.13

[1] as in Table 4

DISCUSSION

The significantly higher intake of the concentrate with rapeseed cake during the 6 weeks before calving in group RC_{W6} compared with the other groups did not significantly affect the daily intake of dry matter, protein (CP, PDIN, PDIE) and energy (UFL) (Table 4). Neither did it cause statistically significant differences over the entire lactation period among the groups in the intake of these nutrients, except PDIE. The lower daily intake of PDIE by the cows in groups RC and RC_{W6} could possibly be attributed to the lower content of this component in the rapeseed-cake diets than in the diets for groups C and G that contained more cereal feeds. Although the average daily intake of dry matter by the lactating cows in different groups was similar, the animals from the control group, compared with the other groups, were characterized during the first weeks of lactation by a slightly lower intake of dry matter and energy, and cows receiving rapeseed cake, by a lower intake of PDI (Figure 1). This suggests that cows in the control group had a slightly greater energy deficit during lactation compared with animals in the other groups, and cows in groups RC and RC_{W6} had a greater protein deficit. Nevertheless, this resulted in no statistically significant differences among the groups in total and daily milk yield, or in the content of the analysed milk constituents, although cows fed the rapeseed cake and glycerine diets were characterized by a 1.2 kg numerically higher daily milk yield. Urea levels in the milk of cows in all the groups show, however, that the energy and protein balance in the rumen, which shows if the ration is balanced, did not deteriorate. Compared with the control group diet, the rapeseed cake, which contained 12.34% fat in DM, was an equivalent substitute for 18% soyabean meal and 12% ground wheat. Similar results were obtained for fattened bulls (Stasiniewicz et al., 2000; Strzetelski et al., 2001b) that received diets with rapeseed cake containing 17% fat in dry matter.

In a study by DeFrain et al. (2004), who gave cows liquid glycerol (a co-product of biodiesel production) to the extent of 0.86 kg/day (5.4% TMR dry matter) starting from 14 days before calving to 21 days of lactation, productivity and feed intake did not increase. Although cows in our study received pure glycerol, the daily glycerol ration was almost half the above and averaged 490 g/day, which accounted for 2.1% of traditionally fed ration dry matter. Ogborn (2006) found feed intake to decrease in cows receiving dried crude glycerol. The unfavourable effect of glycerol on feed intake may be due to the quality of this product, because substances such as salts and methanol that take part in glycerol synthesis may negatively affect the taste of the final product. It cannot be excluded that also the amount of feed and the feeding method (traditional or TMR) as well as lactation period may have significance. Donkin et al. (2009), who replaced maize grain with partially refined glycerine (99.5%) in the rations of cows in the second period of lactation at 5, 10 and 15% of dry matter, did not find any negative effects on the productivity of the cows and milk composition, but observed feed intake to decrease slightly when the ration contained 15% glycerine.

The use of the concentrate with 30% rapeseed cake, extended feeding of this diet from 6 weeks before calving, and introduction of the concentrate with 5% pure glycerine into the diet had no significant effect on the efficiency of diet and nutrient conversion per kg milk production.

The lack of differences between the groups on different days of measurements in cows' body weight and condition during the analysed period may be indicative that the rations adequately met the cow and foetal requirements. The absence of negative effects on body condition score (BCS) and body weight was also reported by other authors who fed cows a high energy diet for 6 weeks before calving (Mashek and Beede, 2001; Strzetelski et al., 2008). The changes in body weight did not correspond to the changes in BCS. One BCS point equates to a change of 82 kg in Holstein-Friesian cows (NRC, 2001). The discrepancy between body weight and BCS could be due to differences in water intake and hydration state in the cows (Donkin et al., 2009).

The shape of the curves for FFA and BHBA concentration suggests that feeding cows with the glycerine diet (group G) or with the rapeseed cake diet for 6 weeks before calving (group RC_{W6}) reduced the mobilization of the body's fat reserves and lowered the blood ketone content to a greater extent than in the blood of cows fed the rapeseed-cake diet (RC) from 3 weeks before calving, especially in comparison with the control group (C). The extended feeding of the rapeseed cake diet prior to calving could enhance the adaptation of rumen microorganisms to the digestion of high-starch diets after calving, while glycerol could act as a glucoplastic substance. The highest level of these metabolites in group C leads us to assume that the cows in this group, compared with animals from the other groups, were at a greater risk of ketosis. The lower serum concentration of BHBA may be indicative of a better energy balance. McNamara et al. (2003), who found a non-significant correlation between NEFA concentration in blood and the energy balance, suggested that NEFA is not accurate as an indicator of energy balance in early lactation cows. The same authors also showed a significantly negative correlation between blood BHBA concentration and the negative energy balance, and hold the view that BHBA is a more useful indicator of energy balance than NEFA. Whitaker (1997) suggest that glucose is not as sensitive an indicator of the cow's energy balance as NEFA and BHBA. The marked reduction in serum glucose concentration in all the groups post-partum, in relation to the pre-partum period, may be attributed to the increased requirement of the mammary gland for glucose needed for lactose synthesis (Ballard et al., 2001; Manderbru et al., 2003).

Feeding the lactating cows with rapeseed cake caused favourable changes in the fatty acid profile of milk fat. Although the rapeseed cake had no significant effect on the content of saturated fatty acids (SFA), SFA content tended to decrease, especially in the milk of cows from group RC_{W6}. This was due to a decrease in the level of different saturated fatty acids, including mainly C10 and C14. Similar findings were reported by Komprda et al. (2000), Kudrna and Marounek (2006) and Vaselý et al. (2009). Meanwhile, the significant increase ($P<0.05$) in the n-3 PUFA content of milk, especially in the group of cows receiving the rapeseed-cake diet from 6 weeks before expected calving, resulted from the increased content of linolenic acid (C18n-3) in the milk of cows from this group ($P<0.05$). Different results were obtained by Kudrna and Marounek (2006) and Vaselý et al. (2009). When feeding rapeseed cake to lactating cows, they found the C18:3 and n-3 PUFA content of cows' milk to decrease significantly compared with cows receiving soyabean meal in their diets. This difference is probably due to the greater proportion of rapeseed cake in our experiment (30%) compared with the studies conducted by the authors cited above (25% and 19%, respectively). In addition, the extended feeding of rapeseed cake (from 6 weeks before expected calving) can also be a contributing factor. The increased supply of linolenic acid (c18 n-3) in the diet caused a large part of it to avoid biohydrogenation in the rumen and to become incorporated into milk fat. From the nutritional point of view, it is also significant that the n-6/n-3 PUFA ratio decreased significantly, not only in the milk of cows in the group under discussion (RC_{W6}), but also in the milk of cows fed the glycerine diets, although in this case the result is difficult to comment. The use of rapeseed cake and glycerine in the diets of lactating cows did not have a significant effect on the CLA content of milk, although the concentration of CLA in milk tended to increase, especially in groups RC and G. This tendency was observed for both the content of the two main CLA isomers (*cis*-9, *trans*-11 c18:2 and *trans*-10, *cis*-12 c18:2) and the total CLA content of cows' milk. These results contradict those of Vaselý et al. (2009), who observed a reverse tendency. There is no conclusive explanation for these findings. It is well known that CLA is produced in the rumen by biohydrogenation of linoleic acid supplied in the feed, in the presence of the *Butyrivibrio fibrisolvens* and other bacteria from group A, or through endogenous synthesis from rumen-derived vaccenic acid under the influence of Δ-9-desaturase (Bauman et al., 1999). It is safe, however, to assume that many yet unknown factors could affect rumen metabolism, and thus the CLA concentration in milk fat.

CONCLUSIONS

The results obtained suggest that rapeseed cake with a 12% fat content is a valuable protein and energy source for high-yielding cows and can be added to the diets of high-producing cows up to 30% (instead of 18% soyabean meal and 12% cereals) without negative effect on dry matter intake, milk yield and milk composition. The addition of rapeseed cake to the diet positively affects the health-promoting value of milk fat. The introduction of 5% pure glycerin into the diet causes no significant changes in the analysed production parameters. Serum metabolite profile in cows fed the glycerine diet or the rapeseed cake diet from 6 diet causes no significant changes in the analysed production parameters. The serum metabolite profile in cows fed the glycerine diet or the rapeseed-cake diet from 6 weeks before parturition suggests that the cows in these groups could have been at a lower risk of ketosis.

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