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COMPARISON OF DIFFERENT DIETARY PROTEIN SOURCES FOR DAIRY COWS

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Abstract

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The aim of the experiment is to compare sunflower meal (SFM), as a protein source in rations of lactating dairy cows to rape seed meal, canola type (RSM), dry distillers grain with solubles (DDGS) from maize, and soybean meal (SBM). Twenty four multiparous Holstein cows averaging 51±19 days in milk at the start of the experiment and 603±48 kg body weight were randomly assigned in 4x4 Latin square design trials. Each period lasted 21 days. Weeks 1 and 2 were used for adjustment and week 3 for data collection. Diets for each period and treatment group consisted of 4.3 kg alfalfa hay and 22 kg maize silage (31% DM), and 12.2 kg compound feed. All ingredients were mixed and provided to the cows as total mixed rations (TMR). TMR contained 17.1% crude protein in dry matter, with 47 to 58% coming from 4 tested supplementary protein sources. During each period, the cows were offered 1 of 4 compound feeds containing: 1) 39% SFM, 2) 46.6% RSM, 3) 62% DDGS, and 4) 32% SBM. By adding sunflower hulls to diets with SBM, RSM and DDGS, and maize germ as source of fat to those with SFM, RSM and SBM all rations were equalized by net energy concentration, crude fiber and fat in dry matter. Therefore, the different protein source was the only main difference between the 4 diets. Dry matter intake tended to increase for diets with DDGS and SBM as protein supplement. Milk production was significantly lower for cows receiving SFM with diet (30.1 kg/day), compared to diets with SBM (33.2 kg/day) ($P<0.05$) and tended to be lower than in cows fed rations with RSM and DDGS. There were no significant differences ($P>0.05$) between SBM, RSM and DDGS as a protein sources. Milk protein yield per day was 1.08, 1.05, 1.04 and 0.96 for cows receiving diets with SBM, RSM, DDGS and SFM respectively, and respective percentages of milk protein were 3.24, 3.22, 3.19 and 3.18 ($P>0.05$). Fractions of true protein, casein and whey protein from total protein did not differ significantly. There was a tendency for lower true protein and casein content in milk of cows receiving diet with SFM. Non-protein nitrogen in milk from SFM diet was significantly higher, than in other diets. There were no significant differences in yield of fat, and milk fat percentage in cows receiving diets with 4 different protein feeds. Production of energy corrected milk from intake of one kilogram dry matter was the lowest in cows fed SFM diet (1.54 kg) ($P<0.05$), followed by DDGS (1.63 kg), RSM diets (1.67 kg), and SBM (1.69 kg). Less true protein in milk ($P<0.05$) was produced from 1 kg crude protein in ration with SFM compared to other rations. However, utilization of protein digestible in intestine for milk true protein production did not differ significantly between the four rations. Milk produced from cows fed ration with SBM had a farm gate price which was by 15 to 23% higher than those of milk produced by the other three rations. The cheapest milk was from the DDGS diet, followed by RSM and SFM

Key words: protein sources, dairy cows, sunflower meal, rape seed meal, soybean meal, dry distiller's grain, milk yield, milk composition

*Abbreviations***:** CP – crude protein, DDGS – dry distillers grain with solubles, DM – dry matter, NDF – neutral detergent fiber, SBM – soybeans meal, SFM – sunflower meal, RSM – rape seed meal, canola type

Introduction

A traditional protein source in South East Europe is sunflower meal (SFM), which is produced in the region and its cost is lower, compared to other protein sources. The recent expansion of biofuel production capacity in the world as well in the Balkan area has resulted in an increased availability of byproduct as dry distillers grain with solubles (DDGS) and

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rapeseed meal, canola type (RSM). Availability of those by-products has increased substantially and, consequently, the interest in using these feeds in dairy cattle diets has also increased. Recently many farmers use SBM or combination of SFM and SBM to improve amino acids profile of the ration for dairy cows. However, the price of SBM protein, which is 100% imported, is about 2 to 3 times higher compared to domestically produced protein feeds.

In the international literature there are many publications comparing RSM to SBM, or DDGS to SBM which are reviewed by Yildiz and Todorov (2014). In most of the production experiments with dairy cows the replacement of SBM with RSM was successful, as milk yield and composition are concerned. Differences in production level are not significant when a part of the SBM was replaced by the DDGS too.

There is only limited number of experiments comparing SFM to other available sources of protein. Milk production was similar when partially dehulled (Schingoethe et al., 1977), or fully dehulled (Parks et al., 1981) SFM replaced SBM in dairy cow rations. Cows fed an extruded blend of SFM and SBM had a more desirable amino acid balance than cows fed SBM, indicating that a blend of SFM and SBM proteins may be better than either protein source alone for high producing cows (Drackley and Schingoethe, 1986). Indeed milk production increased slightly when cows were fed a blend of SFM and SBM instead of SBM as the only protein supplement (Nishino et al., 1980). In Bulgaria Todorov et al. (2008) carried experiment with relatively low producing cows and replaced successfully compound feed with SFM by DDGS. SFM was a good source of protein for cows with relatively low milk yield in the experiment of Ockolic et al. (1972).

In the experiments of Magometovich (2011) cows receiving SFM have low milk yield compared to SBM and thermally treated lupine. Milk yield is higher when the protein source in ration for dairy cows is RSM than SFM (Agapov, 2010).

Therefore, milk yield in some production experiments is equal for diets with SFM and SBM, but in other trials SFM was worse compared to SBM or RSM. Experiments comparing SFM with other protein sources for high yielding cows are not recently available. On the other hand it is important to know whether it is possible to decrease the expensive imported SBM or to exclude it from rations of high producing dairy cows by proper combination of locally available SFM, RSM and DDGS.

The aim of this experiment was to compare milk yield and composition when soybean meal, rapeseed meal, sunflower meal and dry distillers' grain with soluble were included as additional sources of protein in the ration of dairy cows.

Materials and Methods

Twenty four multiparous Holstein cows (51±19 DIM) were used in a replicated 4 × 4 Latin square design with 21-day periods. Weeks 1 and 2 of each period were for adjustment to diets and week 3 - for data collection. The cows were divided in four groups with 6 cows in each group equalized as much as possible according to days in lactation, live weight, and milk yield in previous lactation and from calving to beginning of the experiment.

The shorter experimental period allowed keeping milk yields at high level during the different periods. All diets consisted of 4.3 kg alfalfa hay, 22 kg maize silage with 31% DM (Table 1) and 12.2 kg different concentrate mixtures (Table 2).

The diets contained 18% alfalfa hay, 31% corn silage, and 51% concentrate on a DM basis. Dietary treatments consisted of 4 different protein sources SFM, SBM, RSM and maize

Table 1
Data from analyses of chemical composition of forages

Nutrients	Alfalfa hay		Maize silage	
	as fed	in DM	as fed	in DM
Dry matter, %	86	100	31	100
Crude protein, %	13.5	15.7	2.4	7.7
Ether extracts, %	2.2	2.6	0.9	2.9
Crude fiber, %	27.9	32.4	7	22.6
Nitrogen free extracts, %	35.3	41	19	61.3
Neutral detergent fiber, %	44.9	52.2	8.6	27.7
Non fiber carbohydrates, %	18.3	21.2	17.4	56.2
Ash, %	7.1	8.3	1.7	5.5
Calcium, %	1.1	1.3	0.11	0.38
Phosphorus, %	0.21	0.24	0.07	0.22

DDGS, which supplied 47, 46, 50 and 58% of the total dietary protein respectively. The diets were formulated to be isonitrogenous at 17.1% CP, isolipogenic at 4.5% fat and with equal content of crude fiber at 18.2% of DM. Maize germ were added to SBM, RSM and SFM diets, to maintain approximately equal ether extract concentration with DDGS diet. Maize germ is the most natural and close to quality of fat in DDGS source of fat to equalize ether extracts content of different diets. The experiments of Kelzer et al. (2009) showed similar feed intake, production and rumen parameters when dairy cows received diet with DDGS or with maize germ. Abdelqader et al. (2009) found that fat from corn germ may by relatively protected with no adverse effect on DM intake, milk production and milk composition when fed up to 14 % of diet DM. Sunflower hulls was added to diet with SBM, RSM and DDGS to equalize crude fiber in different rations, because SFM contains a significant quantity of hulls and more crude fiber, compared to other protein feeds. Sunflower hulls are the same source of crude fiber as in the ration with partly dehulled sunflower meal. In such a way we were able to exclude to the maximum extent other factors except for the quality of proteins and nutrient contents in the four

Table 2
Composition of experimental compound feeds

Ingredients, %	Diets with ♦			
	SFM	SBM	RSM	DDGS
Maize, ground	14.2	12.1	15.9	12.5
Maize germ	15	15.2	13	0
Wheat, ground	15	15	10	10
Barley, ground	15	14.8	10	10
Sunflower meal (SFM)	39	0	0	0
Sunflower hulls	0	8.7	3	3.7
Soybean meal (SBM)	0	32	0	0
Rape seed meal (RSM)	0	0	46.6	0
Dry distillers grain with solubles (DDGS)	0	0	0	62
Limestone	0.85	0.75	0.55	0.85
Monocalcium phosphate	0	0.5	0	0
Common salt	0.7	0.7	0.7	0.7
Premix*	0.25	0.25	0.25	0.25
Energy and nutrient content in 1 kg compound feed:				
Dry matter (DM), g	883	875	883	889
Feed units for milk (FUM)**	1.14	1.12	1.11	1,16
Crude protein (CP), g	207.2	206.1	207.2	207.3
Protein digested in intestine (PDI)**, g	104.2	123.4	112.3	124
Balance of protein in rumen (BPR)**, g	48.3	33.4	51.7	6.9
Lysine digestible in intestine**, g	7.16	8.92	7.96	6.2
Methionine digestible in intestine**, g	2.53	2.5	2.63	2.45
Crude fibre (CF), g	93.9	93.2	94	94.1
Neutral detergent fibre (NDF), g	158.6	181.9	196.3	159.4
Ether extracts (EE), g	53.2	53.2	53.3	53.2
Non fibre carbohydrate (NFC), g	462	427.1	422.3	473.1
Calcium (Ca), g	4.9	4.8	5	4.9
Phosphorus (P), g	6.5	4.9	5.5	5.9

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles

* Guaranteed analysis for 1 kg mixture: Zn 150 mg, Cu 30 mg; Co 0.6 mg; I 1.95 mg; Se 0.6 mg; Mn 150 mg, vitamin A 15000 IU; vitamin D 2000 IU; vitamin E 62.5 mg.

** Calculated data for degradability of protein feeds determined by authors and for other feeds and parameters according to Todorov et al. (2007). One FUM is equal to 6 MJ net energy for lactation.

compared diets. The net energy and nutrients were formulated to meet the requirements according to Todorov et al. (2007). However, some unavoidable small differences in ratio of neutral detergent fiber (NDF) and non-fiber carbohydrates content of the four diets remained (Table 3).

Level of crude protein was equal to requirements (Todorov et al., 2007) to avoid the effect of excessive protein on comparison of the four tested protein sources. Scheme of experiment is shown in Table 4.

Cows were kept tied in a barn and individually fed diets as a total mixed ration (TMR) for *ad libitum* intake two times daily (07:00 AM and 7:00 PM). Feed intakes were recorded daily. Refusals were kept between 2 and 5% of offered TMR, by changing slightly TMR quantity. After drying up of refusals, the different components were separated and subtracted from given ration.

Samples of forages were collected for 3 consecutive days at the end of each period. Samples were composite by period and dried at 55°C for 48 h. Composites were ground through a 1-mm screen. Samples were corrected to 100% DM by drying an aliquot of the composite at 105°C for 24 h.

Samples of forages, grains, SFM, SBM, RSM, and DDGS were analyzed for CP, ether extract (with petroleum ether), crude fiber, ash (according to AOAC, 2007). Neutral detergent

Table 3
Average daily intake of feedstuffs and nutrients of one experimental cow ♣

Item	Diets with♦			
	SFM	SBM	RSM	DDGS
Feedstuffs intake per day:				
Alfalfa hay, kg	4.1	4.24	4.2	4.15
Maize silage (31% DM), kg	20.8	21.1	21	20,9
Compound feed, kg	11.9	12.1	12	12
Nutrients intake per day:				
Dry matter, kg	20.5	20.8	20.7	20.7
Feed units for milk*	22.6	22.7	22.5	23.1
Crude protein, g	3518	3572	3558	3549
Protein digestible in intestine (PDI)*, g	1981	2250	2099	2234
Lysine in % of PDI*	7.04	7.26	7.18	5.73
Methionine in % of PDI*	2.38	2.12	2.33	2.09
Balance of protein in rumen*, g	486	316	532	-6
Crude fiber, g	3722	3792	3775	3776
Ether extracts, g	911	927	921	920
Neutral detergent fiber, g	5518	5919	6047	5605
Non fibre carbohydrates* , g	9254	8397	8831	9380
Calcium, g	128	129	131	130
Phosphorus, g	100	81	89	93

♣ Data are for intake feeds, without refusals. Differences between groups were not significant (P>0.05)
♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles
* Data are taken from Todorov et al.(2007) or calculated

Table 4
Scheme of experiment

Period	1 group	2 group	3 group	4 group
	Diets with supplemental protein ♦			
First	SFM	DDGS	SBM	RSM
Second	SBM	RSM	SFM	DDGS
Third	RSM	SBM	DDGS	SFM
Fourth	DDGS	SFM	RSM	SBM

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles fiber was determined with sodium sulphite and α-amylase (Van Soest et al., 1991). Composition of the TMR was calculated based on analyses and concentrations of nutrients in different feeds (alfalfa hay, maize silage and compound feed) in the diets.

Particle size of diets was determined on all treatment diets using the Penn State Particle Separator during third week of each period for the total mixed rations delivered by feed mixer to the cows. Content of each sieve and bottom was dried to determine the percent on a DM basis, as recommended by Kononoff et al. (2003).

Cows were **milked 3 times** daily at 06:00, 14:00, and 21:00 h and yields were recorded in each milking. Milk samples were collected for each milking on 2 consecutive days during the last week of each period. Samples were mixed by gentle inversion and composites by weight corresponding to the respective milking for each cow on sampling day were made. These samples were sent to laboratory for composition analysis where fat, protein, and lactose were determined using mid-infrared spectroscopy. Concentrations of milk urea were determined using urease methodology as per Angelov et al. (1999)

Body condition scores on a scale of 1 to 5 (Todorov, 1999) and body weights were recorded approximately 3 h after feeding for 3 consecutive days at the start of the experiment and at the end of each period.

Rumination and chewing were observed for 24 hours for each cow and period. Consistency of feces and percent of the particles with size above 1.5 mm in faeces were determined at the end of each experimental period according to Todorov et al. (2015).

Means of dry mater intake, milk yield, and milk composition were used in statistical analysis. Analysis of variance was conducted using the mixed procedure of SAS (SAS Institute Inc., Cary, NC). Significance was declared at $P < 0.05$ and tendencies noted at $P < 0.10$.

Results and Discussions

Dry matter and nutrients intake. The average values of DM intake by cows were in narrow ranges (20.5 to 20.8 kg per cow per day) for the four diets. This allowed very equal consumption of net energy (Feed units for milk, FUM), crude protein, fat and crude fiber from cows fed the four tested diets (Table 3). CP in all diets did not exceed the requirement (Todorov et al., 2007), which is important for evaluation of the quality of the different protein sources.

Milk production differed significantly ($P<0.05$) only between cows receiving diet with SFM (30.1 kg per day) and diet with SBM (33.2 kg /day) (Table 5). The energy corrected milk of cows fed diet with SFM was lower compared to cows receiving SBM and RSM ($P<0.05$) (Table 6).

The results of other experiments comparing SFM to SBM are equivocal. In the experiments of Schingoethe et al. (1977) and Parks et al. (1981) milk production was similar for SFM and SBM in dairy cow rations. Blend of sunflower and soybean meal was better than either protein source alone for high producing cows (Drackley and Schingoethe, 1986). Produc-

Table 5
Milk yield and composition, body weight and condition score of experimental cows

Item	Diets with ♦			
	SFM	SBM	RSM	DDGS
Milk yield, kg/day	30.1a	33.2b	32.7	32.5
Fat, %	3.81	3.84	3.86	3.79
Fat, g/day	1147	1275	1262	1232
Protein, %	3.18	3.24	3.22	3.19
Protein, g/day	957a	1076b	1053b	1036
Lactose, %	4.12	4.13	4.11	4.12
Lactose, g/day	1240	1371	1344	1339
Milk urea, mg/dL	30	26	29	29
Body weight (BW)*, kg	611	609	610	612
BW, g of change/ day	-340	-390	-400	-360
Body condition score (BCS)*	2.9	2.8	2.9	3
BCS, change/period	-0.2	-0.25	-0.26	-0.2

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles
* At the end of period
ab Average values with different letters differed significantly at $P<0.005$

tion increased slightly when cows were fed a blend of SFM and SBM instead of soybean meal as the only protein supplement (Nishino et al., 1980). In the experiments of Cheipin (2006) and Magometovich (2011) cows receiving SFM had lower milk yield compared to SBM.

Results in this trial did not show significant difference in milk yield when SBM, RSM and DDGS were the supplementary sources of protein for dairy cows (Table 5). There were neither significant (P>0.05) differences in energy corrected milk yield (Table 6), and milk crude protein yield (Table 5) or milk true protein yield (table 6) between the three protein sources. In many experiments reviewed by Yildiz and Todorov (2014) there was an equal or even slightly better milk yield when RSM was compared to SBM in dairy cows diets. Comparison of SBM and DDGS also showed equal milk yield in cows (Sasikala–Appukuttan et al., 2008; Christen et al., 2010; Ranathunga et al., 2010; Mjoun et al., 2010b). Meta-analysis of Martineau et al. (2013) shows small advantages of SBM in comparison with RSM, as sources of protein for dairy cows. Ipharraguerre and Clark (2005) in a review of published experiments found that it is difficult to improve milk yield by feeding different protein supplements to replace SBM.

Improved milk production observed (Brito and Broderick, 2007; Brito et al., 2007). with RSM is attributed to the amino acid profile in the bypass fraction of RSM being complementary to microbial protein (Brito et al., 2007). The post-rumen supply of total amino acids, essential amino acids, branched-chain amino acids, and limiting amino acids (methionine, lysine, histidine, and threonine) when RSM is used as a protein supplement is numerically higher or at least comparable to that when diets are supplemented with SBM or cottonseed meal (Brito et al., 2007).

Satisfactory results with DDGS as a protein source in dairy cows diets are obtained by other researchers too. In the trials of Liu et al. (2000) there was no change in milk production when feeding DDGS versus a blend of SBM, DDGS and fishmeal. However, Nichols et al. (1998) and Anderson et al. (2006) observed an increase in milk production when feeding DDGS versus SBM-based control diets. However those studies did not balance the fat content of all diets; thus, part of their response might be attributed to the increased fat content of the DDGS diets. Hubbard et al. (2009) also observed increased milk production when DDGS was fed instead of SBM.

Piepenbrink et al. (1998), Brito and Broderick (2007) and Sanchez and Claypool (1983) observed similar milk production when feeding RSM as a replacement for SBM. However, Brito and Broderick (2007) and Sanchez and Claypool (1983) reported an increase in dry matter intake when RSM was fed as an alternative of SBM. Mulrooney et al. (2009) observed similar production when DDGS or RSM was fed and slightly higher production with blends of the two protein supplements.

Froidmont et al. (2011) replaced SBM with combination of RSM, SFM and DDGS and reported similar intake and similar milk production and weight gain for both diets. Mulrooney et al. (2009) compared the effect of replacing 0, 33, 66 and 100% of DDGS with RSM and reported similar DM intake, milk production, milk protein and milk fat concentration in all diets. However the protein yield tended to be greater in rations with increasing amount of RSM. Feed efficiency, concentrations of ammonia and volatile fatty acids in rumen contents was similar for all rations. Lysine was the first limiting amino acid for milk synthesis for diets with DDGS and

Table 6
Dry matter, net energy and protein efficiency

Items	Diets with ♦			
	SFM	SBM	RSM	DDGS
Energy corrected milk, kg/day*	31.6a	35.1b	34.6b	34.1
True protein in milk, g/day	885.2	1009.8	981.4	964.5
Efficiency of utilization of dry matter and energy:				
Energy corrected milk/ Intake of dry matter	1.54a	1.69b	1.67b	1.63b
Energy corrected milk/ Feed units for milk	1.40a	1.55b	1.52b	1.48
Efficiency of utilization of protein:				
True protein in milk/ Intake of crude protein	0.25a	0.28b	0.28b	0.27ab
True protein in milk/ Protein digestible in intestine	0.44	0.45	0.47	0.43

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles
* Energy corrected milk (to 3.5% fat contents) = 0.327 × milk (kg) + 12.95 × fat (kg) + 7.20 × protein (kg)
. ab Average values with different letters differed significantly at P<0.005

RSM, but for diets with both DDGS and RSM, the first limiting amino acid was methionine.

Composition of milk. Milk fat percentage (Table 5) was approximately similar in all diets. Kalscheur (2005) observed that milk fat content was lower only in diets supplemented with wet or dried distiller's grains with soluble that contained less than 50% forage and 22% forage neutral detergent fiber (NDF). The similar milk fat percentage with the SBM and RSM diets agreed with data from others researches (Sanchez and Claypool, 1983; Piepenbrink et al., 1998; Brito and Broderick, 2007; Mulrooney et al., 2009).

There was tendency for lower yield of fat in cows fed SFM because of the lower milk yield (Table 5).

Protein content in milk of cows receiving SFM as supplementary protein in a ration tended to be lower compared to other tested protein sources. However, the yield of protein was significantly lower for SFM diet (957 g/day) vs. SBM, RSM and DDGS diets (1036 to 1076 g/gay). This could be connected with not well balanced essential amino acids (EAA) in the ration with SFM, especially with lysine limitation. Improving EAA balance in the diet of dairy cows resulted in increase of milk protein content and milk yield (Agovino et al., 2012; Lemasquet et al., 2012). Zelenina (2011) also concluded that milk protein percentage depended on the level of metabolizable protein in the ration and balance of EAA.

The slight decrease of percentage of crude protein in milk when cows received DDGS observed by Nichols et al. (1998) did not agree with this experiment. The difference in content of crude protein in milk compared to SBM and RSM was below 2%. However, Abdelqader and Oba (2010) found no difference in milk production and milk composition when fed DDGS or RSM. Mjoun et al. (2010b) reported increased lysine extraction efficiency by the mammary gland for DDGS diet (76.1%) versus SBM diet (65.4%), which can help for increasing milk yield when DDGS is fed to dairy cows. In the experiments of Mjoun et al. (2010a and 2010b) despite the apparent deficiency of lysine, milk protein percentage was increased in cows fed DDGS diet.

Sanchez and Claypool (1983) observed no change in milk protein percentages when feeding RSM or SBM, but an increase in protein percentage when RSM was fed.

Christen et al. (2010) reported equal DM intake, milk yield, protein and fat yield, and percent of protein in the milk when 38% of total protein in the ration of cows was supplied by SBM, RSM, DDGS and high protein DDGS. Milk fat percentage was lower for DDGS and RSM diet compared to SBM.

There are no significant differences in the content of different protein fractions in the milk of the four groups of experimental cows (Table 7). Only non-protein nitrogen was significantly higher in the milk of cows receiving SFM vs. the other diets.

There was no difference in lactose content of milk from the different groups of cows, but the yield of lactose tended to be lower for SFM diet, because of the lower milk yield (Table 5).

Milk urea concentrations were similar across all treatments (Table 5).

Live weights of cows were very close for the different groups and experimental periods. All cows decreased their live weight during the experiment. The changes of body weight of cows during the experiments were practically small for the four experimental diets; therefore this factor did not have any effect on the data evaluation of feed and protein efficiency for production of 1 kg milk.

Body condition scores of cows and during the different experimental periods did not differ significantly (Table 5).

The efficiency of utilization of dry matter and net energy for milk production, without subtracting the energy and protein for maintenance, which were almost equal for all groups of cows, because of the equal live weight, is shown in Table 6. The efficiency of the diet with SFM was significantly lower ($P<0.05$), than that of other diets. The probable reason was the lower milk yield of cows.

The efficiency of utilization of crude protein in the ration was lower in cows fed SFM diet, than that of other rations ($P<0.05$). However, there were no significant differences in utilization of protein digestible in the small intestine between the four groups of cows.

The amount of DM retained on the top sieve of the particle separator was between 13 and 15% (Table 7). The four diets were similar in particle size distribution and within normal expected ranges.

Cows receiving different diets spent approximately the same time ruminating per day (Table 8).

There were no significant differences in the consistency of feces of cows receiving rations with different protein sources (Table 8). This was probably connected with small differences in starch and fiber content of the different diets.

Cost of milk produced by different rations is shown in Table 9. Alfalfa hay, maize silage and all grain were produced in the farm, other components of ration were purchased. The production of compound feed was in a farm's mill, and all expenses (except for feed cost) were included in total farm expenses. Therefore, costs of forages and compound feeds were relatively lower than the price of commercial feeds. However, this did not affect the comparison of costs of different ration and milk produced by experimental rations. The farm gate price of 1 kg energy corrected milk under the Bulgarian economic and farming conditions was by 15, 21 and 23% more

Table 7
Average content of milk protein fractions in experimental cows

Items	Diets with ♦			
Percent in milk:	SFM	SBM	RSM	DDGS
Total protein*, %	3.18	3.24	3.22	3.19
True protein*, %	2.94	3.04	3	2.97
Casein*,%	2.49	2,58	2.56	2.54
Whey proteins*, %	0.45	0.46	0.44	0.43
Percent of total nitrogen:				
- true protein nitrogen	92.5	93.8	93.2	93.1
- casein nitrogen	78.3	79.6	79.5	79.6
- whey protein nitrogen	14.2	14.2	13.7	13.5
- non protein nitrogen**	8.5[a]	6.2[b]	6.8[b]	6.9[b]

** Difference total nitrogen minus true protein

ab Average values with different letter differed significantly at P<0.005

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles

Table 8
Feed particle size of diet, ruminating time and feces consistency*

Items	Diets with ♦			
	SFM	SBM	RSM	DDGS
Diet particle size, DM basis:				
>19 mm, %	13	15	14	15
8 of 19 mm, %	36	37	34	36
< 8 mm, %	51	48	52	49
Average ruminating time:				
Total time of chewing, min./day	476	465	471	459
Feces evaluation				
Score (1 hard, 5 liquid)	2.8	2.9	2.9	3
Particles > 1.5 mm, % of DM	14.3	13.8	15	14.8

* Average values did not differed significantly at P<0.005

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles

Table 9
Cost of the experimental rations and 1 kg milk

Items	Cost	Diets with ♦			
	Leva/ton	SFM	SBM	RSM	DDGS
Feedstuffs in diet:		Feed cost per cow per day, leva			
Alfalfa hay	180	0.74	0.76	0.76	0.75
Maize silage	75	1.56	1.58	1.58	1.57
Compound feed with SFM*	373.1	4.44	0	0	0
Compound feed with SBM*	517.1	0	6.26	0	0
Compound feed with RSM*	391.6	0	0	4.7	0
Compound feed with DDGS*	373.3	0	0	0	4.48
Total cost of diet, leva**/day		**6.74**	**8.6**	**7.04**	**6.8**
Milk yield per day and cost of 1 kg:					
Milk yield, kg/day		31.1	33.2	32.7	32.5
Energy corrected milk (ECM), kg/day		31.6	35.1	34.6	34.1
Feed cost of 1 kg milk, leva		0.217	0.259	0.215	0.209
Feed cost of 1 kg ECM, leva		0.213	0.245	0.203	0.199
Farm gate cost*** of 1 kg milk, leva		0.425	0.508	0.422	0.41
Farm gate cost*** of 1 kg ECM, leva		0.418	0.481	0.398	0.391
Farm gate cost of 1 kg ECM, %		100	115	95.2	93.5

♦ SFM – sunflower meal, SBM – soybean meal, RSM – rape seed meal, DDGS – maize dry distillers grain with solubles

* Price of supplementary protein sources are SFM 340 leva/ton, SBM 865 leva/ton, RSM 400 leva/ton and DDGS 395 leva/ton

** One lev is equivalent to 0.51 euros

*** Feed cost is 51% of farm gate costs of milk expensive for SBM ration, compared to rations with SFM, RSM and DDGS (Table 9). The cheapest milk was that of cows fed ration with DDGS as supplementary protein sources, followed by RSM ration and SFM ration.

Conclusions

The recently available in Bulgaria rape seed meal (canola type), and corn dry distillers grain with solubles are good sources of supplementary protein for dairy cows. When fed to lactating cows, the feed intake, milk yield and milk composition, as well as the efficiency of utilization of energy and protein, were practically equal to diet with soybean meal as supplementary protein. The use of sunflower meal as a single source of supplementary protein in the ration of dairy cows with milk yield above 30 kg per day led to significantly lower milk and protein yields, compared to rations with soybean meal, and rape seed meal. The percentage of non-protein nitrogen from total nitrogen in milk of cows fed sunflower meal was higher than in the milk of cows receiving soybean meal, rape seed meal and dry distillers grain with solubles. Under the Bulgarian economic and farming conditions the cheapest farm gate milk was produced when the diet contained DDGS as supplemental protein source, followed by RSM diet, SFM diet and SBM diet. The price of milk produced by SBM diet was by 15 to 23% higher than from the other three diets.

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