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# THE COMPARISON OF THE MAIN PROTEIN SOURCES FOR DAIRY COWS. A REVIEW

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# Abstract

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Review of now-a-day knowledge was made for nutritive value and effect on milk yield and composition of the main protein sources – soybean meal (SBM), sunflower meal (SFM), rapeseed (canola) meal (RSM), and dried distillers grain with solubles (DDGS) – in diets of dairy cows. Independent of low lysine content of DDGS and RSM and some inconsistent data, there are many group production experiment with dairy cows showing equal or even higher milk and protein yield, compare to rations with SBM. There is limited number of published experiments to compare SFM with SBM, but in part of them there are not significant differences in milk production and composition, while in other trials SBM have advantage. In spite of significant differences in rumen degradability, intestinal digestibility of rumen undegadable protein, and amino acid composition of different protein sources in many trials milk and protein production is not corresponding to nutritive characteristics. Authors are mentioning several suggestions to explain discrepancies between theoretical requirement of cows for rumen undegradable protein and for lysine and experimental production results which are supported in some experiment, but scientific evidences are not enough so far. It is emphasized needs of further experiment to compare SFM with other protein sources, and to find biologically and economically optimal combination of different protein sources in ration of dairy cows, especially for region of Shout East Europe.

*Key words:* soybean meal, sunflower meal, rapeseed meal, dried distiller's grain with solubles, degradability, digestibility, milk production, protein yield, lysine deficit

*Abbreviations:* **ADF** – acid detergent fibre, **ADFN** – nitrogen in ADF, **c-DDGS** – corn dry distillers grain with solubles, **CP** – crude protein, **DDG** – dry distillers grain, **DDGS** – DDG with solubles, **DG** – distillers grain, **DGS** – DG with solubles, **DM** – dry meter, **EAA** – essential amino acids, **FUM** – feed unit for milk (= 6 MJ NEL), **MP** – metabolizable protein, **NDF** – neutral detergent fibre, **NDFN** – nitrogen in NDF, **NEL** – net enery lactation, **RSM** – rapeseed (canola) meal, **RUP** – ruminally undegradable protein, **SBM** – soybean meal, **SFM** – sunflower meal, **w-DDGS** – wheat DDGS, **WDG** – wet DG, **WDGS** – wet DGS

# Introduction

Increasing pressure in the dairy industry to produce milk more efficiently given impetus to devising cheaper diet formulation and feeding strategy to meet these challenges. Optimizing energy and protein level and quality in the rations is one approach to enhance milk yield and efficiency of production.

Improving the efficiency of MP utilization by dairy cows remains a key issue in dairy industry that can be achieved by manipulation the EAA profile of dietary proteins. This contributes to cost-effective production.

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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 come to conclusion that percent of milk protein depend on level of MP in the ration and balance of EAA.

Lemasquet et al. (2012) find that balancing the EAA profile by lysine, methionine, and leucine in dairy cows ration improved MP utilization from 48 to 52%. Dietary energy source (starch or NDF) can also modify the nitrogen utilization (Cantalapiedra-Hijar et al. 2012).

The price of feed dominates the cost of the production of animal products, because feed ingredient costs were 50 - 60% of production

costs of dairy cows. Therefore, it is important, optimization of available feed ingredients that minimize costs while still providing dietary nutrient levels that maximize production rates and product quality.

Traditional protein source in South East Europe is SFM, which is produced in the region and it cost is lower, compare to other protein sources. The recent expansion of biofuel production capacity in the world as well in Balkan area has resulted in an increased availability of byproduct as DDGS and RSM. Availability of those by products has increased substantially and, consequently, the interest in using these feeds in dairy cattle diets has also increased.

Because of shortage of EAA especially of lysine in SFM and DDGS, and partly in RSM, in ration for high producing cows and rapid growing calves is necessary to include some source of lysine. Most often it is SBM. Difference in price of the different protein sources is significant. Ignoring some tentative price variations, the average approximate cost of one metric ton in is 230 \$ for SFM with 35% CP, 340 \$ for RSM with 35% CP, 350 \$ for DDGS with 28% CP and 700 \$ for SBM with 48% CP. The cost of one 1 ton crude protein is respectively 657, 971, 1250 and 1458 \$.

The big price difference of protein needed careful optimization of quantity of inclusion in diets of different protein sources, available in the region. To contribute in solving problem of optimal inclusion of different protein sources in diet for dairy cows is aim of this review. It is limited to the main protein sources– SFM, RSM, SBM and DDGS.

#### Sunflower Meal as Source of Protein for Dairy Cows

#### Nutritive characteristics

The sunflower seed has rough and thick hulls part, which are removed before extraction of oil. Depending on degree of dehulling of sunflower seeds content of CP in the SFM varied in wide range, usually from 23 to 39% as an air dry feed (Todorov et al., 2007). The content of crude fiber varied from 11 to 29%. There is relationship between content of crude fiber and CP in by-product from processing sunflower seeds (Smith, 1968). According Todorov et al. (2007) CP in SFM (%) = 49.6285 – 0.9013 Crude dibre (%).

According to Todorov et al. (2007) feed units for milk (FUM) in 1 kg SFM varied depending on fiber content from 0.70 to 0.98 (4.3 to 5.88 MJ NEL). This value is lowest compare to other sources of protein - 1.13 to 1.15 FUM in 1 kg SBM, 1.1 - 1.2 FUM in DDGS and 0.9 - 1.05 FUM in RSM.

Amino acid content of SFM is characterized with relatively high content of sulphur containing amino acids methionine and cystine, but low content of lysine.

Data for amino acid content does not show real quantity of amino acids available for metabolism in animal organism and for milk and muscle synthesis. Other important factor is degradability of amino acids in the rumen of animals and digestibility of RUP in the small intestine. If accepted that all amino acids have the same degradability and digestibility in the small intestine, according average data from the literature (Todorov et al., 2007) we have calculated quantity of available lysine and methionine in 100 g CP from different protein feedstuffs (Table 1).

Percent of different EAA from sum of the EAA for rumen microbes (MCP) and for feeds calculated as a percentage of respective amino acid as percent of sum of the EAA of milk protein is more accurate comparison. In such comparison first probably limiting amino acid in MCP is histidine (78%), for RSM isoleucine (79%), for SBM methionine (58%), for SFM lysine (50%), for c-DDGS phenylalanine (34%) and for w-DDGS lysine (40%)

However, in reality only part of EAA available for metabolism into cow organism is coming from RUP. In high yielding cows maximum 50% of protein (amino acids) is coming from RUP. Usually animals are receiving more than halves of protein from protein synthesize by rumen microbes. We have calculated EAA supply in percent of milk protein composition if 60% of MP is coming from microbial protein and 40% from RUP of four sources of feed protein. In this situation first limiting amino acid is 85% (lysine) for SFM, 80% (leucine) for RSM, 78% (Leucine) for w-DDGS, 77% (methionine) for SBM, and 73% (methionine) for c-DDGS. This data show that percents of first limiting EAA from requirements for milk protein synthesis are significantly higher compare to data for content of EAA different protein sources. Differences between 5 compared protein sources are not very big and varied from 73% to 85% supply of requirement of amino acids for milk synthesis. First limiting amino acid is not the same when taking in account only amino acid composition or mix of amino acid in by-pass protein and protein synthesized into the rumen.

There are other important factors for amino acid balance, as effect of other feeds in the ration, sources and type of carbohydrate, concentration of energy in diet, rate of passage of rumen content etc, which can overcome at least partly deficit of some amino acids.

SFM suppling at least 85% of EAA for milk protein synthesis is on the best position among compared protein sources. In reality we may expect better balancing of available amino acids in ration with RSM, SBM, w-DDGS and c-DDGS because grains, which usually accompany protein sources in rations for dairy cows content enough methionine. Leucene and phenylalanine also are rarely first limiting amino acid in experiments with dairy cows carried out up to now. For solving problem about impact of different protein sources on milk production and composition are important results from feeding trials.

Table 1Data for different protein sources

Item	SFM	SBM	RSM	w- DDGS	c- DDGS
Degradability	0.78	0.63	0.69	0.62	0.56
RUP digestibility	0.89	0.95	0.77	0.85	0.85
MP*	0.1958	0.3515	0.2387	0.323	0.374
Available lys*	0.724	2.144	1.265	0.646	0.935
Available met*	0.431	0.492	0.477	0.581	0.636
*g/100 g CP					

\*g/100 g CI

Combination of RSM and c-DDGS, or SFM with RSM or C-DDGS is expected to be successful replaces of SBM (Table 2). Combination of SFM, RSM and DDGS in dairy cow diets also is promising.

#### **Inclusion of SFM in Rations of Dairy Cows**

The production experiments with dairy cows show that SFM is relatively good source of supplemental protein in dairy rations. 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). Milk production increased slightly when cows were fed a blend of SFM and SBM instead of only SBM as the protein supplement (Nishino et al., 1980).

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

Although SFM is widely used in dairy cows ration in East Europe there are little researches reported on the protein value of SFM compared to SBM or other protein sources. The reason for that situation probably is very limited use of SBM in the past in dairy cows diet in former USSR, Ukraine and Balkan Peninsula area where SFM was the main source of protein for not very high producing dairy cows.

The sunflower expeller, rich of oil, is an excellent feed source for dairy cows as well. Most modern dairies supplement fat in rations for lactating cows. The high fat sunflower expeller eliminates the need for some or all of the fat supplement requirements. Supply availability of the high fat content sunflower expeller is however limited.

SFM is also well utilized in young calves and growing heifers (Parks et al., 1980; Schingoethe, 1981). Weight gains and feed con-

#### Table 2

Amino acid supply of combination of microbial (MCP) and feed protein as percent of milk protein <sup>#</sup>

EAA	MCP+ RSM	MCP+ SBM	MCP+ SFM	MCP+ c-DDGS	MCP+ w-DDGS
Arg	180	178	203	148	167
His	95	91	90	95	93
Ile	93	97	96	96	98
Leu	80	83	99	92	78
Lys	98	100	85	80	81
Met	85	77	94	88	95
Phe	98	106	104	74	120
Thr	126	113	113	115	137
Trp	97	89	91	83	116
Val	90	87	92	94	91

# Assuming that RUP provided 40% and MCP remaining 60% of MP

sumption were similar for calves and heifers fed SFM or SBM. Digestion trials indicated that protein digestibility was the same for both meal rations (79 %) but energy digestibility was slightly less for SFM rations (73% vs. 78% for SBM) because of the low digestibility of sunflower hulls (Nishino, et al.,1980; Stake et al.,1973).

SFM is generally quite palatable without anti-nutritional factors. SFM and SBM were equally palatable by all ages of cattle ranging from young calves to milking cows. In studies with beef cattle, SFM and cottonseed meal were also equally palatable (Richardson and Anderson, 1981).

**In summary** SFM is characterized by 1. Variable protein (23 to 39% CP) and fiber (11 to 29% crude fiber or 12 to 22% ADF) content. 2. High degradability of protein into rumen (approximately 78%). 3. High methionine content of protein. 4. Low lysine content. 5. Relatively low and widely varying energy value (4.3 to 5.9 MJ/kg NEL). 6. Good palatability, lack of anti-nutritional component and no upper limits for inclusions in the rations of ruminants when partly dehulled. 7. Milk yield in some production experiment is equal for diets with SFM and SBM, but in other trials SFM was worse compare to SBM or RSM. There are a few recent experiments to compare SFM with other protein sources for high yielding cows.

# Rapeseed Meal (RSM) as a Protein Source for Dairy Cows

#### Nutritive characteristics

The term "canola" (canadian oil) was used in America in order to differentiate it from old variety of rapeseed. In Europe, is used the term "double-zero rapeseed" to identify canola seed, oil and meal. In this review we use abbreviation RSM for canola or doublezero rapeseed, which was bred to have low levels of erucic acid (< 2%) in the oil portion and low levels of glucosinolates ( $< 30 \mu mol/g$ ) in the remaining RSM.

Seeds of rape, canola type, contain approximately 40% oil and 22% CP. Remaining after extraction of oil RSM contain between 32 and 40% CP depending on variety of rape, growing condition and processing the seeds. Remaining oil in RSM varied from 2 to 4%, and crude fiber varied from 11 to 14%.

The quality of the meal can be both enhanced and diminished by altering the processing conditions in the crushing plant. Some temperature treatment is needed in order to deactivate the myrosinase enzyme which, if not destroyed, will break down glucosinolates into their toxic metabolites (aglucones) in the animal's digestive tract. According to Daun and Adolphe (1997) the rapeseed processing could also cause thermal degradation of part of glucosinolates in the meal. However, if temperatures are too high or applied too long period of time, then the protein quality of the meal can decrease.

The research by Newkirk et al. (2000) suggests that the commonly used temperatures in the desolventizer-toaster stage of 105°C cause some protein damage, which can be avoided at 95°C. Correct processing requires 6 to 10% moisture content of the rape seeds for effective break down of glucosinolate. Prior to the late 1970s, the use of this oilseed processing by-product as an animal feed was limited by the presence of glucosinolates in the seed. Glucosinolates are considered anti-nutritional factors in low erucic acid RSM. On their own they are innocuous, but when cells of the seed are ruptured glucosinolates come in contact with myrosinase. The myrosinase enzyme hydrolyzes the glucosinolates releasing sulphur, glucose and isothiocyanates. The isothiocyanates are goitrogenic, reducing the ability of the thyroid to absorb iodine (Downey, 2007). These metabolites of glucosinolates can affect animal performance and can be toxic to the liver and kidneys (Tripathi and Mishra, 2007).

Heating during processing of the meal eliminates most of the myrosinase, but is not completely effective in eliminating the effects of glucosinolates because some intestinal microflora also produces myrosinase (Tripathi and Mishra, 2007). Isothiocyanates are bitter compounds, and can also reduce palatability. Content of glucosinolates in RSM also can decrease intake (Smith et al., 2006)

Low erucic acid rapeseed contains several phenolic compounds. Sinapine is the choline ester of sinapic acid and is the principle phenolic compound. Levels in the meal have been reported to be in the range of 0.7-1.1% for North American and European plant varieties (Kowslowska et al., 1990), and 1.5% in Australian varieties (Bonnardeaux, 2007). Sinapine is metabolized and excreted by ruminants without harmful effects.

Tannins are more complex phenolic compounds that can bind proteins and some complex carbohydrates and can reduce digestibility. Levels in low erucic acid rapeseed are typically 1-3% (Kozlowska et al., 1990). Content of eight different glucosinolates in canola type RSM is under 30  $\mu$ mol/g, which is safety for animal (Bell, 1995; Bell et al. 1998; Newkirk et al. 2003). Therefore, low erucic acid RSM can be used as the sole protein supplement for ruminants.

RSM contains significant quantity of **crude fiber** (9.5%), ADF (20.8%) and NDF (30.1%) (Anonymous, 2011). Crude fiber in RSM can be decreased by dehulling seed (Mustafa et al. 1997) or cultivation of varieties with yellow seed cover, which is thin. High content of crude fibre, reduces its net energy value for ruminant animal to 1.76 Mcal/kg DM at 3 time maintenance feeding level and 1.66 Mcal/kg at 4 time maintenance level (NRC, 2001). This is equal respectively to 1.22 and 1.16 FUM in 1 kg DM or 1.06 and 1.00 FUM at 87% DM, according to Bulgarian net energy system. The net energy value of RSM is some higher compare to SFM.

**Fat content** of RSM varied widely depend of technology of oil extraction. With increase of fat content increased energy value of RSM. Fat of RSM is rich of linoleic acid (approximately 20%) and linolenic acid (approximately 10%) (Seberry at al. 2009), which can be transformed in omega- 3 fatty acids and in cis-9, *trans*-11conjugated linoleic acid (CLA). Because the oil is highly unsaturated, the amount that can be added to a ration may limit the use of meal from low erucic acid RSM high in residual oil (*i.e.* that has been cold-pressed (Downey, 2007)

RSM is a high-protein feed, containing typically approximately 34% protein, i.e. approximately 75% that of SBM. There is variation in **amino acid composition of RSM** depending on cultivar,

climate and processing of rapeseed (Newkirk et al., 2003; Fickel, 2005; CCC, 2009)

The amino acid profile of RSM is higher than that of the majority of other vegetable proteins. Content of first limiting amino acid lysine is lower, while methionine in RSM is higher compare to SBM (Bell and Keith, 1988). Ratio of lysine to methionine is 3, exactly as in milk protein, which is advantage for milk synthesis. Higher temperature treatment decreased amino acid availability in RSM (Newkirk et al., 2003a; 2003b). After 12 h incubation in the rumen digestibility of amino acids in intestine is 85% (Kendall et al., 1991). Percent of NDIN of total protein content in RSM is used as an index for protein availability (Newkirk et al., 2000). Values under 10% correspond to above 85% available lysine. Digestibility of RUP of RSM in small intestine is 75% (NRC, 2001).

The rumen degradability of RSM protein has been studied extensively. Table 3 provides a summary of the effective degradability of the DM and CP fractions of RSM assuming a rumen turn-over rate of 5% per hour. Increasing the ruminal turnover rate from 2 to 5% and 10 %/hour reduced effective degradability from 79.3 to 65.2% and 56.9% respectively (Sadeghi and Shawrang, 2006). Therefore, it is important when evaluating such results for ration formulation purposes to consider the type of diet into which the protein supplement is to be incorporated. Crude fiber content of RSM does not influence rumen degradability (Mustafa et al., 1997). Considerable variation was noted between samples and between amino acids in the proportion degraded ruminally or absorbed postruminally.

According data summarized by NRC (2001) rumen degradability of CP is 73% at low DM intake and 64% at 4 kg DM intake/100 kg body weight.

For carbohydrate composition of the RSM is characteristic relatively high content of sugars (6.7%) and oligosaccharides (2.2%).

#### Table 3

The effective rumen degradability (in percent) of RSM DM and protein fractions (rumen outflow rate 5% per hour, except data of Sadeghi and Shawrang, 2006)

Reference	DM	СР
Ha&Kennelly (1984) Trial 1	57.1	68.5
Ha&Kennelly (1984) Trial 2	57.7	65.5
Kirkpatrick&Kennelly (1987) 1	63.0	63.2
Kirkpatrick&Kennelly (1987) 2	54.2	72.0
Kendall et al. (1991)	53.5	61.5
Cheng et al. (1993) Trial 1		74.9
Cheng et al. (1993) Trial 2		72.3
Cheng et al. (1993) Trial 3		62.5
Piepenbrink&Schingoethe (1998)	65.1	53.1
Woods et al. (2003)	60.5	66.8
Sadeghi& hawrang (2006)2%/hr	78.1	79.3
Sadeghi& hawrang (2006)5%/hr	66.5	65.2
Sadeghi& hawrang (2006)10%/hr	59.5	56.9

Starch level is about 5.2%. The levels of starch, free sugars and soluble non-starch polysaccharides in RSM totals about 15% (Bell, 1993; Slominski and Campbell, 1990) which should result in a significant contribution to digestible energy and microbial protein synthesis.

In experiments of Yosifov (2013) measuring purine derivatives in urine of sheep, microbial protein reaching small intestine was almost equal for diets with SFM or w-DDGS, and slightly less for c-DDGS. However purine derivatives were about 30% more in diet with RSM as protein source, compare to SFM and DDGS as source of protein in diets with equal net energy and CP level. A comparative study investigating RSM, cottonseed meal and SBM as protein supplements for high producing dairy cows also demonstrated numerically higher post-rumen flow of microbial protein in cows fed RSM compared to those fed cottonseed meal and SBM (Brito et al., 2007).

RSM is an especially good source of phosphorus (1.06%), sulphur (0.83%) and selenium (1.1 mg/kg) (Sauvant, 2004). RSM contains some quantity of biotin, choline, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin and vitamin E (Bell, 1995; Hickling, 2001)

RSM is a highly **palatable** source of protein for ruminant animals. Sporndly and Asberg (2006) examined the relative palatability of common protein sources by comparing eating rate and preference in heifers. When fed a mash diet, heifers consumed 221g of RSM in the first three minutes, while those fed SBM only consumed 96 g, demonstrating the highly palatable nature of RSM. The reasons for the high degree of palatability may be related to the high sucrose content. However, high levels of glucosinolates can reduce feed intake. Ravichandiran et al. (2008) examined the impact of feeding rapeseed or mustard meals with varying levels of residual glucosinolates to five-month-old calves. Calves receiving a concentrate containing low glucosinolate RSM (<20  $\mu$ mol/g) consumed the same quantity as the control without RSM (1.10 vs 1.08 kg, respectively). However, calves fed a concentrate containing high glucosinolate mustard meal (>100  $\mu$ mol/g) only consumed 0.76 kg.

#### Including rapeseed meal in the dairy rations

RSM is an excellent protein supplement which is widely used in rations for lactating dairy cows. In a summary of 21 research trials with RSM (Table 4), the mean milk production response was +1.0 kg/d when compared to SBM. Recent research with cows producing  $\geq$ 40 kg/d (Brito and Broderick, 2007) clearly indicates that, even at high levels of production, RSM is still a superior protein supplement when compared with SBM or cottonseed meal.

RSM is an excellent source of histidine, methionine, cystine and threonine. The abundance of these amino acids and the extent to which they supplement amino acids from other protein sources may, in part, explain the consistent milk yield response found when RSM is included in dairy cow rations (Table 4).

Improved milk production that is 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, EAA, 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). Unequivocal research data indicates that when it is used to supplement dairy cow diets, RSM can meet the rumen degradable protein and RUP requirements of dairy cows, which is reflected by the increase in milk production.

Meta-analysis on lactational responses of substitution of different protein source by RSM in 49 isonitrogenous experiments in dairy cows show positive effect. Increase of milk yield was half less when RSM was substituted for SBM compared with substitution of other protein feeds then SBM (Martineau et al., 2013). Positive effect is partly related to an increase of percentage of milk protein content. These data indicate an underestimation of MP value supply by RSM by National Research Council (NRC, 2001).

Studies have shown that RSM can be effectively used in combination with DDGS to restore amino acid balance and maximize animal performance. Mulrooney et al. (2008) examined the potential to use RSM in combination with DDG in the rations of lactating dairy cows. Diet containing c-DDGS and RSM tended to produce the highest level of milk production

#### Table 4

1	1	, 0, 0	
References	SBM	RSM	
Ingalls and Sharma (1975)	23.0	23.7	
Fisher and Walsh (1976)	24.4	23.0	
Laarveld&Christensen (1976)	24.9	26.4	
Sharma et al. (1977)	20.7	20.9	
Sharma et al. (1977)	21.5	21.8	
Papas et al. (1978)	24.3	25.2	
Papas et al. (1978)	23.9	24.6	
Papas et al. (1979)	21.8	22.2	
Laarveld et al. (1981)	26.4	27.7	
Sanchez and Claypool (1983)	33.4	37.7	
DePeter and Bath (1986)	39.8	41.4	
Vincent and Hill. (1988)	28.5	28.6	
Vincent et al. (1990)	25.1	26.7	
McLean&Laarveld (1991)	28.9	30.7	
MacLeod (1991)	17.7	16.9	
Emmanuelson et al. (1993)	21.0	21.9	
Dewhurst et al. (1999	24.0	24.5	
Dewhurst et al. (1999)	23.7	25.5	
Whales et al. (2000)	21.8	22.7	
Maesoomi et al. (2006)	27.0	28.0	
Brito and Broderick (2007)	40.0	41.1	
Average milk yield	25.78a	26.70b	
a b Difference between data with different later are significant at $D<0.05$			

a,b Difference between data with different later are significant at P<0.05

Including RSM in ration for dairy cows increased milk yield (P<0.05), and improved conception rate and health status of cows, compare to SFM (Agapov, 2010).

Froidmont et al. (2011) replacing SBM with combination of RSM, SFM and DDGS reported similar intake level for both diets (23 kg DM/cow/d), similar milk production and weight gain (320 g/d) for both diets. No significant difference in major components of the milk and significant increase (P < 0.011) of the milk unsaturated fatty acid content with the diet with combination of RSM, SBM and DDGS. Mulroones et al. (2009) compare effect of replacing 0, 33, 66 and 100% of DDGS with RSM and reported similar DM intake, milk production, milk protein and fat concentration in all diets. However 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 first limiting amino acid for milk synthesis for diets with DDGS and RSM, but for diets with both DDGS and RSM first limiting amino acid was methionine.

In conclusion RSM has well balanced amino acid composition. Protein is with slow degradability. In the same time significant contain of easily fermentable carbohydrates in RSM enhance microbial protein synthesis, which favored intestinal passage of protein. In canola (double zero) varieties content of anti-nutritional factors is low enough to effect consumption and productivity of ruminants. RSM is widely used in dairy cows diets, and most of studies elsewhere have shown that it can replace almost all of the SBM in the rations, including very high yielding, dairy cows.

# Dried Distillers Drains with Solubles (DDGS) as Protein Feed for Dairy Cows

### Production

Two technologies are applied for production and are known as method of wet milling and dry milling method. In the traditional dry-milling process, whole grain is ground and fermented to produce ethanol. Residue from this process is call stillage with 5–10 % DM content. Next process is centrifugation to separate part of water and increase DM content to approximately 30%. Different part of liquid portion (thin stillage) can be returned to the cooking and distillation processes, to be sold directly as high moisture cattle feed or to be dehydrated to produce condensed distiller's solubles (CDS) with DM content approximately 30%. Some of CDS is used directly in total mixed ration (TMR) to increase moisture, protein and other nutrient in the rations. Other way of utilisation of CDS is by mixing WDG and used for animal feeding as WDGS with 30% DM, or dried to 90% DM (DDGS).

From 1 tonne DM of wheat by dry milling and good production technology are obtained 387 L of ethanol (x 0,789 = 305 kg ethanol), 372 kg w-DDGS (330 kg DM), electricity from the heat, and 365 kg of carbon dioxide (CO<sub>2</sub>). In wet milling of 1 tonne DM of maize are produced about 655 kg of starch (for ethanol, sweeteners and other purposes), 253 kg maize gluten, 52 kg maize gluten meal and 34 kg maize oil.

Development of process that aims to improve ethanol yield continued. Two products are currently being evaluating and are starting to be marketed: high-protein dried distillers grains with solubles (**HPDDG**) from the corn endosperm, which is approximately 45% CP (Kelzer et al., 2008; Hubbard et al., 2009; Tedeschi et al., 2009), and a low-fat (defatted) DGS, produced by solvent extraction of fat for use in biodiesel, that is approximately 35% CP (Mjoun et al., 2010b). The bran and germ are high in both fibre and fat, and their removal and use only endosperm for fermentation the resulting residue is HPDDGS. The main DG by-product is DDGS. In this review is discussed only DDGS produced from corn (c-DDGS) or wheat (w-DDGS), and their use in dairy cattle nutrition.

Ham et al. (1994) and Klopfenstein et al. (2008) reported lower net energy value of DDG compared with WDG. However the difference is not large (Schingoethe et al., 2009).

Wheat and barley DGS are usually higher in protein but lower in fat and energy than corn DGS, because of composition of used raw material.

Dry products are with relatively low volume weight (390 to 500 kg/m<sup>3</sup>), slightly hygroscopic and slowly increase its moisture, thus reducing their flowing ability and increases the formation of arches in the bunkers (Ganesan et al., 2009). Pelleting largely overcomes these problems and facilitates storage, transport and feeding of DDG (Rosentrater, 2007).

#### **Nutritional Characteristics of Distillers Grains**

To the DG passed all protein, fat, fiber and minerals, contained in the grain, plus yeast, developed during fermentation, and small quantities of remainders of any additives from process. The residue of the starch in DG varied, and usually is from 2.5 to 9.5% of DM (Cozannet et al., 2011). There is tendency for decreasing starch in DG with improving technology of processing. The concentration of individual nutrients in DM of DG can be assessed approximately by the equation (Todorov and Kozelov, 2011):  $X_{DG} = Xg / (1 - 0.95 \text{ Sg})$ ; *where*:  $X_{DG}$  - nutrient in the DM of DG, as part of unit; Xg - nutrient content in the DM of the grain, as part of unit; Sg - starch in the DM of the grain, as part of unit; 0.95 - coefficient reflects the residue of starch and added substances, including developed in the fermentation yeast cells.

It can be assumed that approximately 1/3 of DM of the grain turns into ethanol, 1/3 goes into DG and 1/3 volatilized as carbon dioxide (Saunders et al., 2009; Kim et al., 2010).

As a result of processing some changes occur in different nutrients.

The protein in the grain under the influence of temperature on drying is denaturized, thus reducing its solubility and degradability in the rumen (Table 5). Nuez (2010) and Walter et al. (2010) reported for increasing percent of RUP in wheat two times in w-DDGS. This is connected with an increase of ADFN and NDFN percentage of CP in DDGS compare to original grains (Kononoff and Christensen, 2007). When condensed solution in the product increase, as a sequence increased degradability and digestibility in small intes-

# Table 5Degradability of protein (RDP) in the rumen and digesti-bility of undegraded protion in the small intestine (dRUP)

Authors	c-DDGS		
Autions	RDP,%	dRUP, %	
Firkins et al. (1984)	46.0	n.a.	
Carvalho et al. (2005)	63.3	50.5	
MacDonald et al. (2007)	48.7	88.8	
Kononoff et al. (2007)	57.0	86.2	
Kleinschmit et al. (2007a)	22.0-36.5	n.a.	
Schingoethe et al. (2009)	47.0-64.0	n.a.	
Cao et al. (2009)	38.0	64.0	
Mjoun et al. (2010c)	n.a.	92.4	
Kelzer et al. (2010)	43.7-66.9	91.9-92.1	
Schingoethe et al. (2009)	45.0	n.a	
Oba et al.(2010)	69.3	n.a.	

tines (Nuez and Yu, 2010). Degradability of wDDG has a higher than c-DDG (Mustafa et al., 2000).

Despite the large variation found in degradability and digestibility of RUP in the intestine, which apparently is due to the regime of drying, it is clear that degradability is relatively low and this is beneficial in ruminant's feeding. It's unfavorable the reaction of some amino acids with carbohydrates, especially first limiting amino acid (lysine), which make amino acids unavailable for absobtion and metabolism (Nuez, 2010; Walter, 2010). The degree of loss of lysine depends on the drying process.

RUP and rumen degradable protein fractions of dietary protein are important considerations in formulating diets, especially for high-producing dairy cows. c-DDGS is a good source of RUP, usually ranging between 47 and 64% of the CP. For WDGS usually RUP is 5 to 8% lower then RUP in dried DGS (Firkins et al., 1984; Kleinschmit et al., 2007a). Intestinal digestibility of most amino acids in DDGS excided 93% and are slightly lower than for SBM except for lysine, where the digestibility was 84.6% for DDGS compared with 97.3% for SBM and other soybean product.

Lysine is the first-limiting amino acid in corn and wheat DGS, although DGS is a good source of methionine. Limited data (Kleinschmit et al., 2006, 2007a,2007b) indicate that higher quality DGS products may contain more available lysine than lower quality products. In fact, a recent data (Schingoethe et al., 2009; Mjoun et al., 2010c) indicated higher concentrations of amino acids, and especially lysine (3.15% of CP) in DDGS compared with that (2.24% of CP) listed in NRC (2001), and higher intestinal digestibility of amino acids (Mjoun et al., 2010c). This may indicate an overall improvement in the ethanol industry processing methods that minimize heat damage to DGS.

The fiber is not strongly impregnated with lignin and has good digestibility. *In situ* degradability of NDF in DDGS is quicker and higher compare to NDF in grains (Kononoff and Christensen,

2007). Finely grinding of DG doesn't provide physiologically active fiber in the rations (Li et al., 2011). Kleinschmit et al (2007) reported for 3.4 to 19.8 percent physiologically active NDF. Cyriac et al. (2005) found that the stepwise reduction of roughages in the ration from 55 to 34% and replacement by DDGS in which NDF were on the same level in all rations, fat content of milk decreases from 3.34% to 2.85%. Therefore, despite of high levels of crude fiber, DG can not be considered a complete substitute for part of forage, especially in dairy cows.

The energy value of DG is relatively high. The high fat concentration (approximately 4 - 6% for w-DDGS, and 8 - 12% for c-DDGS) and relatively ready digestible fibre (approximately 38% NDF content) contributed to the high energy content in DDGS. DG contains large amounts of NDF but low amounts of lignin, which allows the NDF in DGS to be quite digestible (62 to 71% digestibility according to Birkelo et al., 2004 and Vander Pol et al., 2009). NRC (2001) data (1.97 Mcal NEL in 1 kg DM) seems to underestimated net energy value, because more resent review of Schingoethe et al. (2009) shows 2.25 Mcal/kg DM, which is about 10% higher then energy value of corn. This reflects the improved fermentation efficiency of the new generation ethanol plants (Spiehs et al., 2002). The DGS of today contain more protein, energy, and available phosphorus than did DG from older ethanol plants. DG from new-generation plants contain virtually no starch, compared with as much as 5 to 10% starch in DG from older, less-efficient ethanol plants. Energy value of DG, calculated indirectly as a results of production experiments, in most cases exceeds that of an equivalent quantity of the DM of rolled grain (Klopfenstein et al., 2008).

Birkelo et al. (2004) based on 45 balance experiments with cows found that WDGS has 15-20% higher net energy value in DM, than corn grain. The presence of partially protected unsaturated fats and reduced degradability (which reduced fermentation losses) explain part of higher energy value, but not at all (MacDonald et al., 2007; Klopfenstein et al., 2008).

Firkins et al. (1985), Ham et al. (1994) and Vander Pol et al. (2009) emphasize the favorable effect of DG on fermentation processes in rumen. This may be associated with reduced amount of starch, the presence of small amount of fermentation products and other factors. Presence of yeast in DDGS (Castillo-Lopez et al. 2010) although in small quantity has favourable effect on rumen fermentation and probably on microbial protein synthesis.

**Starch** is approximately 65% of DM in wheat and 70% in maize fermented. As a result of fermentation DM of DG contained 2 to 10% starch. Replacing part of grain with the DG allows to reduce the starch in the rations. In fast-growing and fattening cattle and cows with high milk yield, receiving high levels concentrated feed, replacing part of concentrate with DG reduces the risk of acidosis (Li et al., 2010), decreased fiber digestibility, laminitis, anorexia, displacement of abomasum and other digestive disorders.

High **phosphorus** content is typical for DGS. Phosphorus in DGS is with relatively high biologically available (Mjoun et al., 2008). Phosphorus is apparently not a problem in the presence of

enough calcium in the rations. High levels of phosphorus can be an advantage to meet the requirements of animals and phosphorous fertilization. Simultaneously this fact may prevent the use of large amounts of DG (which also applies to nitrogen and sulfur) to avoid pollution of the environment (Schmit et al., 2009; Spiehs and Varel, 2009). High phosphorus or sulfur content in the DGS usually comes via the solubles (Cao et al., 2009). A high phosphorus concentration in DGS usually indicates that more than normal amounts of solubles were blended with the distillers grains.

**Sulfur**-containing compounds (sulfuric acid, sodium sulphide or other sulfur-containing substances) are often used for controlling pH and for cleaning equipment during various stages in the ethanol plant operation, and these compounds often end up in the solubles. Thus, DGS typically contains more sulfur than is present in the starting grains. Feeding more than 30% DGS that contain higher than normal amounts of sulfur coupled with high-sulfur water or other feeds high in sulfur may result in diets that approach the recommended dietary maximum of 0.4% sulfur in the total ration DM (NRC, 2001; Neville et al., 2010).

Some researchers report content of 0.3 to 1.0% sulfur (in most cases 0.5 to 0.7%). This can lead to poliencephalomalacia (PEM) in ruminants (Gould, 1998). To prevent PEM some researchers added to the rations thiamine (Buckner et al., 2007; Hulls et al., 2008; Schauer et al., 2008).

High levels of sulfur affects the absorption and utilization of copper and selenium, especially in the presence of molybdenum. At high sulfur content may require restriction of DG levels in order to maintain sulfur in the diet of an acceptable level (below 0.3% in concentrate ration and 0.5% of DM in the high roughage rations, NRC, (2005).

Experiments with finishing lambs fed 60% DDGS and more than 0.7 percent sulfur in DM of diet indicate that PEM does not occur, and rate of growth and carcass quality are normal (Schauer et al. 2008; Neville et al. 2010). Uwituze et al. (2011) find that DDG with high sulfur content, fed in high levels of beef cattle reduced DM intake, growth and carcass quality.

In some batches of DG **copper** can be greatly increased (using copper equipment) and this problem should be considered when feeding DG, particularly in sheep diets.

Vitamins of group B increased due to multiplication of yeast in the process of fermentation (2 - 5% of DM to DG is yeast mass). Although these vitamins can be synthesized by bacteria in the rumen, their presence in feed is often useful for fermentation in the rumen and for animals at all. Carotene and vitamin E in the grain is partially destroyed during processing, especially during drying.

**Mycotoxins**, molds, and other potential contaminants are considered potential problems. Ethanol plants routinely sample and test all loads of grain coming into the plants and reject contaminated loads. This is important because mycotoxins are not destroyed during the ethanol fermentation process or during the production of DG (Schaafsma et al., 2009). Thus, contaminated DGS could pose a risk to human health because a metabolite of mycotoxins can transfer to milk (<u>Garcia et al., 2008</u>). Any antibiotics used in ethanol plants (usually virginiamycin) are approved products and are ultimately destroyed or inactivated during processing (Shurson et al., 2003).

# Variability of composition and nutritional value, and their checking

The technology of producing ethanol is elaborated, better enzymes are used and this affects the quality of DG, including the utilization of protein. Olentine (1986) describes in details all the factors that lead to a change in the composition of the DDGS. The strongest influence had the quality of cereals (the differences are increasing in DDGS), mixing ratio of solid and liquid fraction (latter contains almost 2 times more fat and minerals, but approximately two times less fiber and protein from the solid fraction), chemicals and other additives in processing, and differences in temperature and in duration of drying. The effect of quantity of solubles mixed with solid part of stillage is shown by Martinez-Amezcua et al.(2007) and Cao et al. (2009). The residual starch from 2 - 16% in advanced technologies reduces strongly and this is part of the reasons for variation (Schingoethe et al., 2009).

Ganesan et al. (2006) found that fat level in DDGS increased from 8.8% up to 11.8% of the DM when the condensed distillers solubles increased from 10 to 25%. Noll et al. (2006) found that with increasing of the liquid fraction increases the particle size (due to conglomeration), and the product is darker, with more fats and minerals.

Significant variation was reported between different production batches (Spiehs et al., 2002; Nuez and Yu, 2009; Cozannet et al., 2009; Tumuluru et al., 2010; Walter. 2010; Nuez, 2010). The temperature of drying DG has significant influence on feeding value of DDGS (Cromwell et al., 1993; Nuez and Yu, 2009). Temperature of pelleting, diameter of pellet, and hardness of pellet may change feeding value too (Tumuluru et al., 2010; Nuez, 2010).

CP content varied in vides range 26 to 35% (Belyae et al., 2004, Pedersen et al., 2006; Fathi and Afifi, 2008). DDGS from wheat has higher CP content compare to corn DDGS (Gibb et al., 2008). CP in maize grain increased in result of processing from 7.4–10% to 23–32%, and for wheat grain from 8.5–14% to 26–38% (Aldai et al., 2009).

Analyses by Gamage et al. (2012) of metabolic characteristics of protein in different batches from the same plant of w-DDGS by Fourier transforming infrared spectroscopy showed significant variation in content of MP from 153 to 182 g/kg DM and balance of degradable protein in rumen from 145 to 181 g/kg DM.

Cozannet et al. (2011) indicated a rather variable amino acid profile of different samples of DDGS. Lysine was the most affected amino acid with contents ranging between 0.83 and 3.01 g/100 g CP. In addition, only 76 to 50% of total lysine were free and utilizable by animals. Low content of CP and low availability had also high occurrence of Maillard reaction and darker colour.

Ergul et al. (2003) established the true digestibility of lysine in the DDGS with bright color in birds from 59 to 83%, and Cozannet et al. (2011) – from 52 to 89%. According to Stein et al. (2005) standardized ileal digestibility of lysine in pigs is from 44 to 63%. Drying regime and color of DDGS correlate with the digestibility of lysine. The great variability in the digestibility of lysine and protein requires finding the faste methods to characterized DDG. Some idea of the damage of the lysine can be obtained with determining the color and smell of DDG. The **smell** of high quality DDGS must be pleasant, fermentable and sweet. Overheated products have a smell of burnt or smoked.

**Color** of c-DDGS varies from light yellow to dark brown and may be determined by spectrophotometer of Hunter or Konica Minolta companies. It depends on the raw material, the quantity of liquid fraction (Noll et al. 2006) and the regime of drying. In the dark DDGS reduces the content of lysine, without essential changing to other amino acids. There is a correlation of color (r = 0.74 to 0.86) with the digestibility of amino acids (Ergul et al., 2003; Fastinger and Mahan, 2006; Batal and Dale, 2006). Powers et al. (1995) reported that milk production of cows is lower when fed a dark colored DDGS than light colored DDGS.

Although a golden-yellow color may be a good indication of quality for c-DDGS, research data from Belyea et al. (2004) indicated that color is sometimes (e.g., Powers et al., 1995) but often not (Kleinschmit et al., 2007a) an accurate indicator.

ADIN is the method typically used by feed testing laboratories to estimate heat-damaged protein. DDG can be high in ADIN, ranging from 10 to 40 percent of CP (Chase, 1991). Nuez and Yu (2010a) and Kleinschmit et al. (2006) found correlation between quality of DG and content of ADFN. Nakamura et al. (1994) reported low correlation between NDFN and the apparent or true digestibility of protein in the DDGS (r = 0,24), and Ham et al. (1994) show that NDFN is a bad indicator for energy and protein value of the DG. *In vitro* degradability with pepsin and pancreatin or IDEA (Immobilized Digestibility Enzyme Assay) (Wang et al. 2007) can also be used to characterize DDGS. Recently were achieved good results (r > 0.8) in determining the digestible lysine by NIRS-analysis (Liu and Liu, 2007). Significantly better results (r = 0,993) are obtained by fluorescent analysis using so-called FFF-method (Front Face Fluorescence) (Urriola, 2006).

Existing big variations illustrate the importance of obtaining analytical data on the specific product received from a supplier and the importance of suppliers providing uniform, standardized products.

#### Using distillers grains in feeding lactating dairy cows

In the USA, where it produces the most ethanol, 87.5% of byproducts are used in dairy and beef cattle, 7.3% for pigs and 5.2% in the birds' diets (AgMRC, 2011). Several authors recommend DG to be included in dairy cows rations to a maximum levels by the 20% of DM (Nichols et al., 1998; Liu et al., 2000; Schingoethe, 2001; Lardy, 2003; Schroeder, 2003; Donkin et al., 2006). Hippen et al. (2004) think that there is not benefits of feeding more than 25% DG of DM in rations for dairy cows. Several studies however show that this levels may be exceeded. However Kalscheur (2005) and Schingoethe et al. (2009) reviewing literature come to conclusion that nutritionally balanced died can be formulated with 30% in DM or more of total diet DM as DDGS. Kalscheur (2005) published meta-analysis of 23 publications with 96 experimental groups (Tables 6 and 7). **DM consumption** increases with 0.7 kg at inclusion of 20 - 30% DDGS in the diet (P<0.05). This can be explained by the small particle size of feed, leading to accelerating the passage of feed particles through rumen (Beauchemin and Yang, 2005). When feeding more than 30% of ration DM consumption is the same as feeding rations without DDGS. When WDG is fed there is a increase of intake at 4 to 20% of DM inclusion in the diet and a negative effect when WDG is more than 30% (Table 6).

The review by Kalscheur (2005) (Table 6) indicated that **milk production** was maintained with increasing amounts of DGS in the diet and numerically was the highest when fed at up to 30% of diet DM as dried DGS. For WDGS, the highest production was achieved at 20% of diet DM. Anderson et al. (2006) found that WDGS gives better results than DDGS.

In several experiments with cows milk yield was higher when receiving DDG compared to other protein sources (Owen and Larson, 1991; Powers et al., 1995; Anderson et al., 2006; Kleinschmit et al., 2006; Sasikala-Appukuttan et al., 2008; Penner et al., 2009). Schingoethe (2004) compare results of 8 trials in which diet with c-DDGS gave more milk than diets with SBM or other proteins.

Kleinschmit et al. (2006) compare three c-DDGS, produced by different temperature regime, and included at 20% of DM of diet of dairy cows, as replaces of part of ground corn and SBM in the ration. Feeding diets containing DDGS had grater yield of milk (34.6 vs. 31.2 kg/day), fat corrected milk (32.7 vs. 29.6 kg/day), and energy corrected milk (35.4 vs. 32.3 kg) compared to diet with SBM. Feed efficiency, milk fat yield and milk protein yield were higher compare to diet with SBM.

Part of the additional production due to DGS may have been attributable to slightly more energy from a slightly higher fat content in DGS diets. However, in experiments such as those by Pamp et al. (2006) that compared DGS to SBM as the protein supplement, production was similar or higher for DGS, even when two diets were equal in RUP and fat.

Ranathunga et al. (2008) demonstrated that replacing diet without DDS with 29% starch by diet containing 21% DDGS and only 19.9% starch in DM had no effect on milk production or composi-

#### Table 6

# DM intake and milk yield of cows fed with different levels of DG (Kalscheur, 2005)

DG, %	DM inta	ke, kg /d	Milk yield, kg /d	
of DM	DDG	WDG	DDG	WDG
0	23.5c	22.2b	33.2	31.4
4 - 10	23.6bc	23.7a	33.5	34.0
10 - 20	23.9ab	22.9ab	33.3	34.1
20 - 30	24.2a	21.3ab	33.6	31.6
над 30	23.3bc	18.6c	32.2	31.6

abc- the lack of equal letter in a column means P < 0.05. In milk production there is not significant differences (P > 0.05)

tion, but tended to improve feed efficiency. All diets contained 49% forage and were balanced for fat content (4.7% of DM) therefore the response measured was to DGS fiber versus corn starch.

Research showed no decreases in **milk fat concentration** when diets contained wet or dried DGS at any level, even as high as 40% of DMI (Table 7). The small particle size of DGS make it fiber less effective (as measured by ability to stimulate chewing or rumination, and to maintain milk fat) compare to the forage fiber. However, in some experiments the high fat concentration in DG can result in milk fat percent dropping (Diaz-Royon and Garcia, 2012).

Abdelqader et al. (200 9) examine effect of fat in the DDGS on milk yield and composition using isolipidic diets. Ration with 30% DDGS contains 6% fat. To increase fat content of other rations to 6% was used corn germ, ruminally inert fat, or corn oil. Dietary treatment had no effect on milk yield, and energy corrected milk. DM intake was decreased with addition of corn oil to the ration, but no difference was observed between other three rations. Feeding DDGS and corn germ increased vaccenic and *cis-9*, *trans-*11 conjugated linoleic acid (CLA) in milk fat. DDGS and corn oil tend to decrease milk fat yield.

Bauman and Griinari (2001) demonstrated that there are two conditions which can reduce milk fat. One of them is the presence of unsaturated fatty acids (UFA) in the rumen, the other is an altered rumen environment that would cause incomplete bio-hydrogenation. Under certain conditions, the pathways for rumen biohydrogenation are altered and through alternative routes, intermediaries are produced, some of which, like *trans*-10, *cis*-12 conujugated linoleic acid (CLA), are potent inhibitors of milk fat synthesis in the mammary gland (Griinari et al., 2001). The concentration of the UFA in the rumen can be a key factor that contributes to changes in microbial population and an increase in the CLA isomer *trans*- 10, *cis*-12 (Jenkins et al., 2009).

In addition to the degree of fatty acid (FA) unsaturation, the rumen concentration of free fatty acids (FFA) should also be considered. The FFA content in the oil extracted from corn grain was 2.28%. In DDGS, however, the average FFA content increased to 9.1% (Moreau et al., 2011). When the fatty acids (FA) were supplied as FFA, there was an increased production of propionic acid, and a reduction in the production of acetic, butyric, and total volatile fatty acids.

#### Table 7

# Influence of quantity of DDG or WDG on the milk composition (Kalscheur, 2005)

% of diet DM	Fats, %	Protein, %
0	3.39	2.95a
4 - 10	3.43	2.96a
10 - 20	3.41	2.94a
20-30	3.33	2.97a
over 30	3.47	2.82b

abc - the lack of equal letter after averages in a column, means significance at P < 0.05

The high concentration of UFA in DG, together with high FFA content, can sometimes lead to milk fat depression in dairy cattle fed diets that include high levels of DG. In a meta-analysis of 24 experiments, Kalscheur (2005) found that use of DG caused milk fat depression only when diets had less than 50% forage or less than 22% NDF from forage.

The high content of UFA in the grain and consequently in DG leads to an increase of these FA in the organism of fed ruminants (Vipond et al., 1995; Koger et al., 2010) and in milk of lactating animals (Schingoethe et al., 1999; Leonardi et al., 2005; Anderson et al., 2006). The reason is that the fats of the feed are partially protected and are not fully hydrogenated in the rumen. Vander Pol et al. (2007) found an increase in UFA content of the duodenum when giving WDGS, compared to diet with corn or corn + corn oil in which animals receive equal fats with WDGS. Reduced biohydrogenation result in increases of the digestibility of fats (Vander Pol et al., 2007).

Leonardi et al. (2005), Anderson et al. (2006), Sasikala-Appukuttan et al. (2008) and Hippen et al. (2010) also reported some increases in the *cis-9*, *trans-*11 conjugated linoleic acid (**CLA**) and its precursor, vaccenic acid (*trans-*11 C18:1) in milk, that are beneficial to humans for improved health status (Bauman et al., 2006). However, they observed little change in concentration of *trans-*10, *cis-*12 CLA that are often associated with milk fat depression (Baumgard et al., 2002). There is also an increase of *cis-9*, *trans-*11 CLA in meat (Dugan et al., 2010).

The lysine limitation in DGS may cause a slight decrease in milk protein content (Nichols et al., 1998; Kleinschmit et al., 2007b). This effect may be more noticeable in diets that contain more than 30% DGS (Kalscheur, 2005; Kononoff and Christensen, 2007), reflecting the high RUP lysine limitation in DGS. Kleinschmit et al. (2007b) observed no differences in milk protein content or yield when feeding DDGS in diets where the forage varied from all alfalfa to all corn silage. However, the amino acid balance was improved with the alfalfa diet indicating a more desirable blend of amino acids in the diet compared with diet with corn silage. Anderson et al. (2006) found, that higher percent of milk fats and protein in partial replacement of corn grain and SBM with DDGS or WDGS in levels 10 or 20% of DM of the ration. But the protein content decreased significantly (P < 0.05) when DG is more than 30% of DM of the ration. In ration with DDGS as a source of supplementary protein the three first limiting amino acids are lysine, methionine and phenylalanine (Christen et al., 2010)

Attempts by Nichols et al. (1998) show a positive effect of addtion protected lysine in the diets. In some experiments lack of decrease of milk protein content and yield probably is due to giving to the cows more than minimal requirements of protein (Kononoff and Christensen, 2007).

Gehman and Kononoff (2010) found that the WDG provides more bacterial protein in rumen, higher yield of milk, protein and fats, compared with control ration. However Janicek et al. (2008) reported ratio of creatinine to purine derivatives in urine was not significantly different between control and DDGS rations. Therefore, there is not difference in microbial protein synthesis into rumen. Mjoun et al. (2010b) reported for increase of 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 experiments of Mjoun et al. (2010a and 2010b) despite the apparent deficiency of lysine, milk protein percentage was increased in cows fed DDGS diet. In experiments of Pamp et al. (2006) rations with DG and SBM were equalized on RUP and fats. Despite of that there is tendency for greater milk yield when using DG.

Kelzer et al. (2009) reported only slight, not significant, increase of DM intake and milk production when 15% of DDGS was included in the rations of dairy cows compare to control ration in two experiments. There were not changes in milk content of fat, protein, milk urea nitrogen and in digestibility of DM, organic matter and NDF. Acetate in rumen content was higher in control rations.

Zhang et al. (2010) replaced barley silage and barley grain with DDGS in dairy cows diets. Results indicated that a partial replacement can improve the production of lactating dairy cows without negatively affecting rumen fermentation and milk fat production. In the next experiments of Zhang et al (2010a) partially replacement of barley silage with DDGS improved productivity of lactating cows but decreased chewing time, rumen pH and milk fat concentration. Inclusion of alfalfa hay in the ration not alleviates mentioned changes.

Sasikala-Appukuttan et al. (2008) reported tendency of increasing of milk production, without changing milk fat and protein percentage when SBM was replaced by DDGS or DDGS plus condensed corn distiller's solubles. Milk urea decreased in DDGS diets. Long-chain and polyunsaturated fatty acids and conjugated linoleic acid (CLA) in milk are greater for diet with DDGS. Molar proportions of ruminal acetate increased with DDGS diet.

Christen et al. (2010) reported for 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 is supplied by SBM, RSM, DDGS and high protein DDGS. Milk fat percentage was lower for DDGS and RSM diet compare to SBM. Feed and protein efficiency not differed between diets. Molar proportion of volatile fatty acids (VFA) and ammonia concentrations was similar for all diets.

Ranathunga et al. (2010) replaced corn starch and SBM protein with NDF from DDGS plus soybean hills without affecting milk yield milk, fat and protein percentage or yield, milk fatty acids profile and milk urea nitrogen, volatile fatty acids in rumen contents, blood glucose and  $\beta$ -hydroxybutirate.

Abdelqader and Oba (2012) reported similar feeding value of wheat, and corn DDGS. Rations with both DDGS type increased milk and fat production compare to ration with RSM However, milk protein concentration was lower in milk of cows receiving DDGS, compare to those receiving SBM. There is some decrease of feed conversion ratio when w-DDGS was replacing c-DDGS. However Mulrooney et al. (2009) reported that increasing replacement of RSM with DDGS tended to decrease milk yield without affecting milk protein and fat concentration, whereas protein yield tended to be greater when increasing amount of RSM in the diet. Chibisa et al. (2012) reported than increasing iclusion of 10, 15 and 20% w-DDGS linearly increased DM intake. The addition of w-DDGS in place of RSM resulted in a 1.2 to 1.8-kg increase in milk yield and feed efficiency. Treatments did not differ for milk fat, protein, and lactose concentrations. Ruminal fermentation characteristics did not change except that the inclusion of 20% w-DDGS resulted in a decrease and a tendency for a decrease in molar concentrations of isobutyrate and total volatile fatty acids, respectively. Omasal flow of total bacterial nonammonia N (NAN) and bacterial efficiency were not different among diets; however, feeding w-DDGS resulted in a increase in nonammonia nonbacterial N flow at the omasal canal.

Several researchers estimate a **better utilization of DM** of the rations with DG (Firkins et al. 1985; Ham et al. 1994; Schingoethe et al. 1999; Birkelo et al. 2004, etc.).

**In long-term trials** higher yield of milk, fat and protein and better feed conversion was estimated with diets with DGS as protein source instate SBM throughout lactation (Mpapho et al. 2006;Mpapho et al. 2007). Health and reproduction processes were similar in both groups.

Summarizing DG has following advantages: 1. High protein content (26 - 28%) and high energy value (2.03 Mcal/kg NEL = 1.41)FUM), which are needed for higher milk production. 2. High content of bypass protein (55% of CP). 3. Removed starch reduces potential of high energy rations to cause acidosis and other related diseases. 4.The contain of dried yeast cells (3 - 4% of DM) that provides B vitamins, promotes palatability, increase fibre digestion and microbial protein production. 5. Highly digestible fiber (NDF), which increased energy value and stimulates rumen microorganisms, 6. Hgher methionine content offers opportunity to blend with lower methionine feeds. 7. Partly protected unsaturated fat (10% for corn and 5% for wheat). 8. Ready available and cheap phosphorous (0.7 - 0.9%). 9. Leak of anti-nutritional factors. 10. Production experiments with dairy cows show very comparable performance to SBM and RSM in spite of low content of lysine in DG. Lower lysine content moves the ratio of lysine to methionine as % of MP closer to the recommended 3:1, and is not reflected in low milk protein.

### Soybean Meal as Protein Source for Dairy Cows

#### Nutritive characteristics

SBM has long been considered an outstanding source of supplemental protein in diets for livestock. In fact, SBM is sometimes referred to as the "gold standard" because other protein sources are often compared to it. SBM is rich in highly digestible protein, and the protein is composed of a superior blend of amino acids, the building blocks of body and milk protein of the livestock.

One way of comparing the quality and nutritional value of the protein in different sources is to compare their lysine content as a percentage of the protein. The reason for using lysine as the comparator is that lysine is generally considered the first limiting amino acid for animals. Soy protein contains approximately 6.5% lysine

which is not greatly different from the lysine content of muscle and milk protein (it ranges from 6.5 to 7.0% lysine). Other oilseed meals have considerably less lysine in their protein. For example, the lysine content of RSM protein is 5.8%, cottonseed meal -4.2%, peanut meal -3.4%, and SFM -2.8%.

There are many **anti-nutritional factors** is soybeans, which are destroyed during modern processing to oil and SBM. Therefore in SBM there are not substances limiting it rate of inclusion in animal's diet.

Degradability of protein of properly toasted SBM is moderate and digestibility of RUP is relatively high (NRC, 2001; Todorov et al., 2007).

According to equations developed from extensive analysis of a large number of SBM samples over many years by the Degussa Corporation, lysine increases by 0.064 percentage unit, threonine by 0.038 unit, tryptophan by 0.012 unit, methionine by 0.014 unit, and methionine + cystine by 0.026 percentage unit for each one percentage unit increase in CP (Fickler et al., 1995).

Soybean protein is highly digestible. The standardized ileal digestibility coefficients (SID) in pigs for lysine (NRC, 2012), are approximately 90% for SBM. The SID coefficients for the other essential amino acids range from 85 to 94%. The SID coefficients for lysine (89 to 90%) are higher in SBM than for other oilseed meals (RSM – 78%; cottonseed meal – 64%; SFM – 83%); or for cereal grains (corn – 78%; barley – 79%; wheat – 81%).

The protein digestibility index (PDI) is a sensitive measure for SBM quality. PDI values changed linearly with the amount of heat processing. A urease level had value in measuring SBM with insufficient heat-treatment, but was not a good measurement of over-toasting or heat damage. The protein solubility in KOH was a sensitive measure of over-heating process, but pepsin digestibility was not sensitive enough to measure differences in heat-treatment. It is advisable to combine PDI and solubility in KOH to predict protein quality of SBM (Whang and Kwang-Youn. 2003).

Soybeans and SBM from different regions of the world have been shown to differ significantly. Analysis of 71 SBM samples collected from nine countries indicated that SBM processed in the United States was superior to the other major meals in terms of their absolute content of the top five key amino acids as well as their estimated digestible amino acids (Baize, 2000)

It would appear that US high-pro SBM may supply as much as 5.8% more digestible protein than Brazilian high-pro SBM and 16.6% more digestible protein than Argentine high-pro SBM. US low-protein SBM would appear to provide more digestible protein than all of the meals analyzed except US high-protein SBM and EU SBM. The worst SBM in terms of likely digestible protein was Argentine low-pro meal (Baize, 2000).

Globally, SBM accounts for nearly 69% of all protein sources used in animal feeds. However, in the U.S. SBM accounts for approximately 92% of the total oilseed meals fed to livestock.

Therefore, SBM is the excellent protein ingredient that complements feed grains to produce optimum livestock performance. Its amino acid balance complements the amino acid pattern of grains to produce diets to support optimum economic performance. SBM has been widely studied for most of the past century. No other feed ingredient has been so thoroughly studied as SBM. It is a highly available source of digestible amino acids and energy. Few other protein ingredients have consistently high levels of digestible amino acids needed for optimum animal production.

#### Soybean meal in diets for dairy cows

SBM is most widely used source of protein for animal nutrition with good reputation as source of protein for all species of farm animals. Reasons are high concentration of protein (44% to 49%), the protein is highly digestible, and the amino acids that are released from the protein following digestion consist of a blend that is close to ideal for animals. In other words, the digestible amino acid profile of soybean protein more closely matches the amino acids requirements of animals than any other oilseed meal. SBM is a rich source of lysine, tryptophan, threonine, isoleucine, and valine – the amino acids that are seriously deficient in cereal grains that are commonly fed to animals. However, the amino acids are not perfectly balanced, they tend to be low in methionine and cystine. It is used as a main protein supplement in countries having high milk yield of dairy cows. SBM usually is used as standard for comparison with other protein sources.

Advantages of SBM in comparison with other plant protein sources can be seen from meta-analysis of Marineau et al. (2013) showing twice higher difference when RSM replaces other protein sources then SBM. The part of this effect is related to higher percentage of milk protein in cows receiving SBM. Stanislavovna (2011) also find increase of protein content in milk when cows received balanced amino acids in ration according to requirements. In this case transaminase (ALT and ACT) in blood decreased.

In isonitrogenous diet SBM protein is a little bit worse only to fish meal as a protein source (Abu-Ghazaleh et al., 2001; Korhonen et al. (2002)). The reason is relatively low methionine content of SBM compare to milk protein (NRC, 2001)

Ipharraguerre and Clark (2005) in review of published experiments found that is difficult to improve milk yield by feeding different rumen undegradable protein supplements, compare to milk production when SBM is fed. Response in milk yield was -2.5 to +2.75%, which mean that is not possible to improve milk production significantly by using other protein sources than SBM.

Magometovich (2011) find highest milk yield in dairy cows receiving protected SBM, compare to SFM and thermally treated lupine. In other Russian research (Cheipin, 2006) also found higher milk yield of dairy cows receiving SBM compare to SFM

Brito et al. (2007) found practically equal non ammonia nitrogen flow to the omasum of dairy cows receiving isoprotein rations with SBM or RSM as a source of supplemental protein.

Abdelgader and Oba (2012) estimate that replacing of SBM with DDGS cause some increasing DM intake, but trend to decrease of CP digestibility. However, concentration of ruminal ammonia nitrogen, plasma urea nitrogen and milk urea nitrogen were higher when DDGS are fed. Milk production tends to be higher, but concentration of milk protein was lower for cows fed the DDGS diets. In other experiments for replacing RSM by w-DDGS, Chibisa et al. (2012) find also increase of DM intake and milk yield. The tendency for decries of feed efficiency is established when w-DDGS replaced SBM. However it was not observe decreasing of protein percentage in milk of cows fed DDGS. Mutrooney et al. (2009) however do not found difference in DM intake when DDGS was replaced by SBM, but milk yield tended to by higher in cows fed SBM. Milk protein yield tended to be lower in cows fed DDGS, compare to those fed diet with SBM, however there is not differences in milk fat content and yield. Feed efficiency (DM intake/energy corrected milk) was equal for different rations. Conversely Mjoun et al. (2010a and 2010b) reported higher feed efficiency for cows fed diet with DDGS, compare to diet with SBM.

Mjoun et al. (2010b) comparing isoprotein, isoenergy diet with SBM and DDGS do not find difference in yield of milk, milk fat and lactose, but despite the apparent deficiency of lysine milk protein percentage and yield were higher for DDGS diet.

Laarveld and Christensen (1976) reported that RSM, and SBM were equal as protein supplements to dairy cows. Study of Korhonen et al. (2002) shows the significant effect of degradability of protein into the rumen for milk production of cows. In there experiments milk yield was higher in cows receiving corn gluten meal compare to SBM, independently of better amino acid composition of SBM.

SBM is only slightly better supplier of digestible essential amino acids compare to DDGS and other plant proteins. However, SBM is better source of intestinally digestible lysine (Mjoun et al., 2010c).

Many experiments to compare milk yield and composition, as well as rumen parameters and microbial protein synthesis into rumen by using SBM or SFM, RSM and DDGS as a protein sources for dairy cows are described in other part of this review. To avoid repletion we are not reporting them again in this section.

Therefore, soybean meal, produced by proper processing method is an excellent source of high quality protein with high digestibility and relatively well balanced amino acid matrix for animals. Despite of that in many experiments with dairy cows are received equal, even higher milk and protein yield with other plant protein sources in the rations. This mean that is possible to find combination of protein feed in ration of dairy cows without SBM, which allowed achieving equal production and feed efficiency with ration with SBM. However, this possibility should be proven in exact experiments with dairy cows under definite conditions.

### Conclusions

Nutrient content and feeding characteristic of the reviewed four protein sources, varied, which reflect on dairy cow's production and feed efficiency result. In part of experiments rations with SFM ensured equal milk yield with rations with SBM. In another trials milk and protein yield is higher for SBM than for SFM. However experiments to compare SFM and other protein sources are limited for certain evaluation of justifiable inclusion rate of SBM in high yielding dairy cows rations.

There are equivocal results of considerable number of experiments comparing RSM, DDGS and SBM as protein sources for dairy cows. In most of the trials milk production and protein yield are equal for three protein feeds, with tendency in part of trials for slightly better result when SBM is the main source of protein in the diets. However production trial involves many factors and their interactions lead to additional variations in results which some times are even not easy to be explained.

Especially it is difficult to explain equal or better milk production in some trials with lactating dairy cows when SBM is replaced by DDGS, RSM or even with SFM, in spite of deficits of lysine in replacing protein sources. There are several suggestions to explain discrepancy, shown in some trials, but no one is supported with enough experimental data. 1. Higher content of available lysine in some batches of protein sources, especially those produced by new improved processes (Kleinschmit et al., 2007a; Mjoun et al., 2010c). 2. Higher microbial synthesis of protein into the rumen, which is reach in lysine (Gerham and Kononoff, 2010). 3. Improved fermentation conditions into rumen and probably higher microbial protein synthesis when SBM is replaced by other protein sources in the diet of dairy cows. 4. Better utilization of lysine by mammary gland when DDGS is source of RUP in ration (Mjoun et al., 2010b). 5. Small differences in level of net energy and metabolisable protein in comparing rations (Jonker et al., 2002; Kononoff and Christensen, 2007) which gave possibilities of cows to compensate small deficit of some amino acids in rations with SFM, DDGS or RSM.

Therefore, there is need of further clarification of possibilities and degree of replacement SBM with other protein sources. Especially for the condition in the South East Europe it is necessary to estimate is it possible and in what extend to decrease expensive SBM from the ration with SFM, RSM and DDGS or combination of those cheaper protein sources. In other words it is necessary to estimate what is optimal protein combination from production and economic point of view.

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