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RUMEN DEGRADABILITY OF DRY MATTER AND PROTEIN IN FOUR PROTEIN SOURCES AND THEIR RELATIONSHIPS WITH MILK PROTEIN YIELD IN DAIRY COWS

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Abstract

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This study evaluated the *in situ* ruminal degradability of sunflower meal (SFM), soybean meal (SBM), rapeseed meal, canola type (RSM) and dry distillers grain with solubles from maize (DDGS). The same feeds are used as a sole supplementary protein sources in rations of dairy cows to estimate their effect on true milk protein yield. *In situ* dry matter (DM) and crude protein (CP) degradability was estimated following Orskov and McDonald (1979) method using three rumen-fistulated cows at 0, 2, 4, 8, 16, 24 and 48 h rumen incubation time for totally 6 samples of each feed and incubation interval. Ruminal disappearance of DM of SFM and DDGS was much slower, and after 8 h of rumen incubation differences were significant ($P < 0.05$) compared to DM disappearance of SBM and RSM. Disappearance of DM of SBM and RSM was very similar and didn't differ significantly at any time of rumen incubation. Disappearance of CP of DDGS was slower and after 8 h of incubation differences were significant ($P < 0.05$) compared to other three protein feeds. SFM had faster ($P < 0.05$) protein degradability during the first 8 hours of rumen incubation compared to the other three feeds. On the opposite, SBM and RSM protein degradability was slower than that of SFM for the first 8 h of rumen incubation, but later SBM degradability was slightly better than that for SFM and RSM. There was no correlation between DM and CP values of the rapidly disappearing fraction *a* in different feeds. The rapidly degradable DM fraction *a* had higher value than protein fraction a in all tested feeds. SFM and DDGS had significantly ($P < 0.05$) higher easily degradable CP fraction *a*, compared to SBM and RSM. Rate of degradation of DM and CP of DDGS was much lower ($P < 0.05$) than in other feeds. Effective degradability values independently of passage rate of rumen content of DM in SFM and DDGS were significantly lower, than for SBM and RSM. Effective degradability of CP was significantly lower ($P < 0.05$) for DDGS than for the other three feeds. Effective degradability of SFM was the highest, followed by SBM and RSM but the differences were not significant ($P > 0.05$). There was a relatively high correlation between protein digestible into intestine (PDI) (equal to metabolizable protein) calculated from average data for degradability published in literature ($r = 0.72$) or from real degradability determined in this experiment ($r = 0.75$). Differences between two correlation coefficients were not significant. Correlation coefficient between milk true protein yields and lysine digestible in small intestine, calculated from literature data for CP degradability into rumen was 0.57, whereas the value calculated from real rumen degradability of supplementary protein was 0.48. There was a big discrepancy between lysine supply to cows and milk protein yield especially for the diet with DDGS. Presumably microbial protein synthesis into rumen was increased when DDGS was used as source of protein in the ration. A second possibility is the better mammary extraction of amino acids which are in small shortage compared to requirements.

Key words: rumen degradability, dry matter, crude protein, relationship with milk protein yield, cows
Abbreviations: DDGS – dry distillers grain with solubles from maize**,** DM – dry matter, PDI – protein digestible into small intestine or metabolizable protein, SBM – soybean meal, SFM – sunflower meal, RSM – rapeseed meal canola type

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Introduction

The main source of amino acids required by ruminant animals is protein produced by the microbial population in the rumen. The second source is dietary protein escaping microbial degradation in the rumen. Microbial protein is the larger portion and of better quality than most of dietary ingredients commonly fed to animals (Schwab, 1996). However, microbial protein does not meet all protein needs in ruminants with normal and especially high level of productivity. Many experiments have demonstrated increase of milk yield when the supply of rumen undegradable protein is increased (Feldet et al., 1991; Broderick, 1992; Brito and Broderick, 2007; Yildiz et al., 2014 etc.).

Matching protein digestible in small intestine (PDI) (equal to metabolizable protein) and amino acids digestible in small intestine (AADI) from microbes with PDI and AADI from undegraded dietary protein will allow dairy cows to reach a high level of milk yield with lower protein level in rations.

Rumen protein degradation is a very complex process that depends on nitrogen solubility, feed particle size, many microbes and enzymes, passage of different protein fractions through the rumen which is affected by ratio of forage and concentrate and of level of intake, as well as of other factors. Estimation of rumen degradability of different nutrients is usually done by *in sacco* methods (NRC, 2001) because *in vivo* determination is expensive and subject of considerable variations. The *in sacco* method is subject to number of imprecisions including non-digestion losses of soluble and small particle associated nutrients from the bag, restricted access of microbes of feeds encapsulated within the bag, and need to quantify microbial contamination of the feed residues remaining within the bag (Alexandrov et al., 1995, Broderick and Cochran, 2000). The *in sacco* values are more than 25% greater than those obtained by omasal sampling, probably related to the rather rapid passage rate used in the *in sacco* method (Broderick et al., 2010). Contrary to the expectations, Huhtanen et al. (2008) and Rinne et al. (2009) improved prediction of ration protein degradability by *in sacco* method through application of constant ruminal degradation value for forage protein. A meta-analysis of Huhtanen and Hristov (2009) showed that ruminal protein degradability predicted by the *in sacco* method was not a significant factor for prediction of milk protein yield and protein efficiency in dairy cows.

The aim of this paper was to estimate degradability of dry matter and protein in the four most often used protein feeds in Bulgaria– sunflower meal, rapeseed meal canola type, dry distillers grain whit solubles from maize and soybean meal. The second object was to estimate relationships between protein degradability and milk protein yield of dairy cows fed the tested protein sources.

Materials and Methods

Samples of protein feeds

For testing DM and protein degradability into rumen samples of SFM, RSM canola type (sunflower and rapeseeds are produced in Bulgaria), DDGS from maize (Vinprom Peshtera's ethanol plant in village of Katunitza, Bulgaria) and SBM imported from Brazil were taken from production experiment (Yildiz et al., 2015) to compare the effect of mentioned four protein sources on milk and milk true protein production of dairy cows. The chemical composition of tested feeds is determined by standard methods (AOAC, 2007) and is shown in Table 1.

Table 1

Chemical composition of tested feeds, g.kg^{-1}

Nutrients	SFM	RSM	DDGS	SBM
Moisture	111	108	99	112
Crude protein	346	335	267	428
Ether extract	16	26	77	18
Crude fiber	181	116	93	59
Nitrogen free extracts	273	344	424	364
Ash	73	71	40	19

Animals and diet

Three non-lactating dry cows of the Black and White Breed with rumen fistula were used for determination of dry matter and crude protein degradability into rumen. The cows were fed alfalfa hay (54.1%), oats hay (27.0%), compound feed (18.0%) and molasses (0.9%). The compound feed consisted of 38.5% barley, 10% maize, 28.5% maize gluten feed, 10% sunflower meal, 8% dry distillers grain with solubles and 5% minerals and vitamins supplements. Diet contained 13.2% crude protein. The cows were fed twice per day in 8:30 and 17:00 h. Experiment started after 14 days adaptation period to the ration. Bags with samples were inserted into rumen before the morning feeding, except for those for 16 hours incubation time in the rumen, which were inserted at 16:00 hours.

Bags

Polyester Saatifil PES 38/3 tissue manufactured by Saatitech S.p.A (Ventiano, Como, Italy) was used to prepare a bag with size 12.7 by 8.5 cm for incubation of approximately 3.2 g of feed samples. The tissue pore size was 38 μm. Bags were sealed with round corners. After filling with samples of feeds the bags are sewed with sewing machine.

Sample preparation

Samples were ground in a mill with a screen size of 2 mm. The bags with samples were pre-soaked prior to incuba-

tion, including samples for zero hours, for 20 minute at 39°C in tab water, without agitation. The bags were incubated in ventral rumen tied on 50 cm from the fistula.

Incubation interval

The samples were incubated for 0, 2, 4, 8, 16 and 48 h. From each feed 2.9 to 3.4 g of samples in 6 replications (two samples for each cow) were incubated into the rumen for measurement of degradability of dry matter and crude protein.

Rinsing

Bag removed from the rumen, and zero hour bags were first rinsed in cold tap water, without any manipulation, before machine washing. Washing in machine was at 25°C with several removals of water. After washing, bags with samples were dried at 65°C for 48 hours and then weighed after equilibration at room temperature. Remaining samples were analyzed for dry matter and nitrogen by methods described in AOAC (2007).

Calculations

The degradability of dry matter and crude protein were calculated by the equations of Ørskov and McDonald (1979):

$$y = a + b * (1 – Exp(-c * t)),$$

where: y = degradability, a = rapidly degradable fraction, b = slowly degradable fraction, c = rate of degradation of slowly degradable fraction, t = time of incubation, hours.

Effective degradation (EP) was calculated by the equation:

$$EP = a + [(b * c)/(c + k)],$$

where: k = rate of passage of particles

Production experiments with dairy cows

Twenty four multiparous Holstein cows were used in a 4 × 4 Latin square design within 21-day periods. The four diets were formulated to contain equal net energy, isonitrogenous at 17.1% CP, isolipogenic at 4.5% fat and with equal content of crude fiber at 18.2% of DM. Milk and true milk protein yield were recorded apart other parameters. Details of the production experiment with dairy cows are published by Yildiz et al. (2015).

Correlations between PDI and lysine digestible in intestine in the ration with true protein yield by milk were calculated both on the basis of literature and real degradability data.

Statistical analyses

Analysis of variance was conducted using the MIXED procedure of SAS (SAS, 2003). Significance was declared at $P < 0.05$ and tendencies noted at $P < 0.10$.

Results and Discussion

Data for disappearance of dry matter and protein during incubation in rumen

Results of *in sacco* degradability of dry matter of sunflower meal, DDGS, soybean meal and rapeseed meal are presented in Table 2, and degradability of protein in Table 3.

Table 2

Percent of disappearance of dry matter after different time of rumen incubation

Feedstuffs	Rumen incubation, h						
	0	2	4	8	16	24	48
Sunflower meal	26.36^{a}	39.06^{a}	42.59^{a}	55.10^{a}	65.18^{a}	71.21^{a}	79.55^{a}
DDGS (maize)	35.92^{c}	40.07^{a}	42.06^{a}	50.81^{a}	60.02^{a}	68.16^{a}	86.34^{a}
Soybean meal	32.78^{bc}	37.62^{a}	46.19^{a}	61.69^{b}	81.04^{b}	92.67^{b}	98.80^{b}
Rapeseed meal	29.94^{ab}	38.10^{a}	47.82^{a}	63.00^{b}	82.11^{b}	91.40^{b}	96.94^{b}

$^{a, b, c}$ Data in the same column differ significantly at $P < 0.05$ if don't have the same superscripts

Table 3

Percent of disappearance of protein after different time of rumen incubation

Feedstuffs	Rumen incubation, h						
	0	2	4	8	16	24	48
Sunflower meal	23.31^{b}	41.81	42.12	61.27	72.62^{a}	82.17^{a}	97.43^{ab}
DDGS (maize)	27.07	31.70^{a}	35.56^{a}	42.72	49.34	57.68	78.41
Soybean meal	17.63^{a}	26.70^{a}	35.87^{a}	50.42^{a}	74.91^{a}	91.11	99.25^{a}
Rapeseed meal	20.32^{ab}	28.12^{a}	36.19^{a}	49.68^{a}	71.08^{a}	81.54^{a}	92.00^{b}

$^{a, b}$ Data in the same column differ significantly at $P < 0.05$ if don't have the same superscripts

Disappearance of dry matter of sunflower meal and DDGS was much slower, and after 8 h of rumen incubation differences were significant ($P < 0.05$) compared to DM disappearance of SBM and RSM (Table 2 and Figure 1). The fast degrading fraction of dry matter was significantly higher in dry distillers grain with solubles, compared to other three tested feeds (Table 4, and Figure 3).

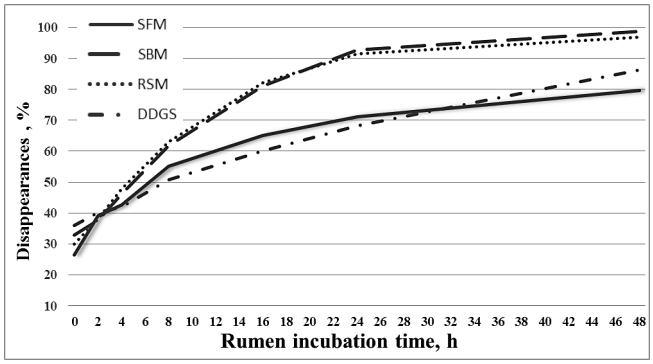


Fig. 1. Comparison of rumen disappearance curve of dry matter of the different protein sources (SFM, SBM, RSM and DDGS).

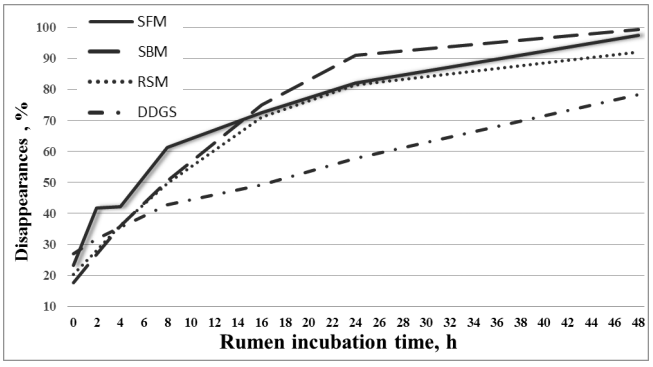


Fig. 2. Comparison of rumen disappearance curve of crude protein of the different protein sources (SFM, SBM, RSM and DDGS)

Disappearance of dry matter of soybean meal and rapeseed meal was very similar and didn't differ significantly at any time of rumen incubation. Slower degradability of dry distillers grain with solubles especially when it is produced from maize is well known and results from slow degradability of maize and the influence of DDGS drying process .

Disappearance of crude protein of DDGS was slower and after 8 h of incubation differences were significant ($P < 0.05$) compared to other three tested protein feeds (Table 3 and Figure 2). SFM had faster ($P < 0.05$) protein degradability during the first 8 hours of rumen incubation compared to the other three protein feeds. On the opposite, SBM and RSM protein degradability was slower than that of SFM for the first 8 h of rumen incubation, but later SBM degradability became slightly better than those of SFM and RSM.

Published data for degradability of protein in feeds which were within the scope of our experiments are quite variable and our data were in framework of reported literature variations.

Degradability parameters of supplementary protein sources

On the basis of data for disappearance of dry matter and crude protein after different time of rumen incubation values of different dry matter and protein fractions were calculated. Using the equation of Orskov and McDonald (1979) effective degradation values were calculated at different rates of passage (k). Results are presented in Table 4.

There was no correlation between dry matter and protein values of fast disappearing fraction *a* in different feeds (Figure 3). The fast degradable DM fraction **a** had higher value

Table 4

Data for different degradability parameters of dry matter and protein in feeds

	Dry matter				Crud protein			
Parameter	SFM	DDGS	SBM	RSM	SFM	DDGS	SBM	RSM
a (%)	28.37 [b]	36.15	29.74 [b]	26.36[b]	27.28 [b]	28.71 [b]	15.77 [a]	16.95[a]
b (%)	50.69	69.84 [b]	72.85 [b]	72.09 [b]	70.88	83.23 [a]	88.85	77.94[a]
c (h^{-1})	0.087 [b]	0.026	0.074 [b]	0.088 [b]	0.069 [b]	0.019	0.068 [b]	0.072 [b]
Effective degradability, %								
k = 0.045	61.72 [b]	61.87 [b]	74.97[a]	74.13[a]	71.18[a]	53.42	69.24[a]	64.91[a]
k = 0.06	58.31 [b]	57.40 [b]	69.89[a]	69.30[a]	66.19[a]	48.73	62.97[a]	59.46[a]
k = 0.08	54.71 [b]	53.40 [b]	64.67[a]	64.20[a]	61.10[a]	44.68	56.59[a]	55.84[a]

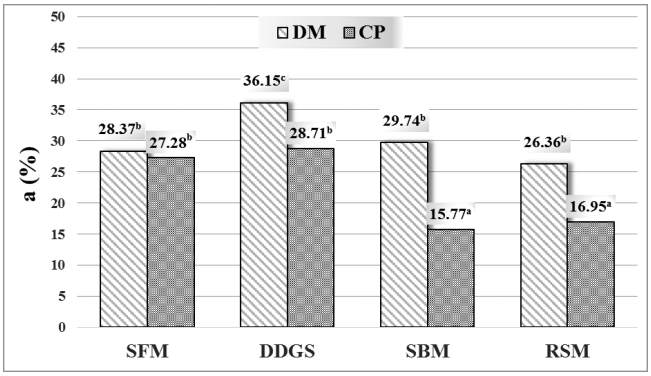
[a, b] Data in the same row differ significantly at $P < 0.05$ if don't have the same superscripts

Table 5

Correlations coefficients of metabolisable protein and lysine supply by rations and true protein yield by cows milk

Items	Supplementary protein				Corelation (r) with TPm
	SFM	SBM	RSM	DDGS	
True protein in milk (TPm), g/day	885.2	1009.8	981.4	964.5	
Ration parameters calculated by degradability from Todorov et al. (2007)					
Protein digestible into intestine, g/day	1981	2250	2099	2234	0.718
Lysine digestible into intestine, g/day	139.4	163.4	150.7	128.0	0.568
Ration parameters calculated by real degradability					
Protein digestible into intestine, g/day	2170	2250	2256	2278	0.754
Lysine digestible into intestine, g/day	145.0	163.4	157.0	131.0	0.484

than the protein fraction *a* in all tested feeds. DDGS had the highest easily degradable DM fraction *a* than other feeds ($P < 0.05$)**.** SFM and DDGS had significantly ($P < 0.05$) higher easily degradable protein fraction **a,** compared to SBM and RSM.

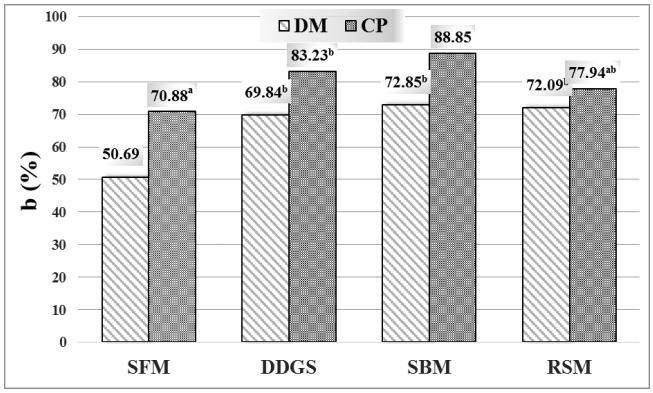


[a,b,c] Data for a given nutrient differ significantly at $P < 0.05$ if don't have same letter

Fig. 3. Comparison of data for soluble dry matter and crude protein fraction (fraction a) in four tested feed (SFM – sunflower meal, DDGS – dry distillers grain with solubles, SBM – soybean meal, and RSM – rape seed meal)

Potentially degradable fraction *b* for protein was bigger compared to the same dry matter fraction in examined feedstuffs. The difference between dry matter and crude protein fractions *b* was much bigger for sunflower meal compared to the other three feeds. The sunflower meal had significantly ($P < 0.05$) smaller potentially degradable fraction *b* of protein compared to other feedstuffs (Figure 3). The protein fraction b in SBM was higher then in other three feeds ($P < 0.05$).

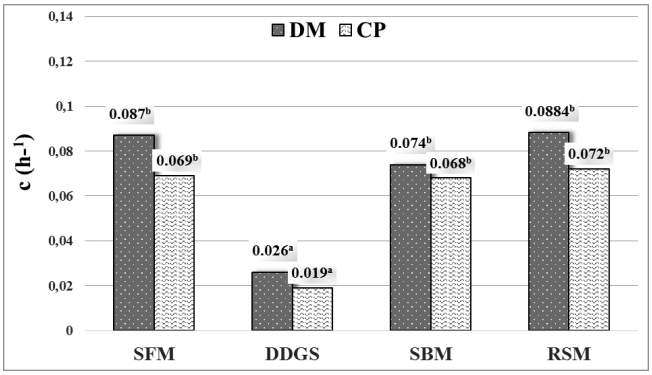
Rate of degradation of dry matter and crude protein of dry distillers grain with solubles was much lower ($P < 0.05$) than in other feeds (Figure 5). This was connected both to the character of maize as a raw material in DDGS production, and with temperature of DDGS drying of process. There were no significant differences among the other three feedstuffs.



[a, b] – Data for a given nutrient differ significantly at $P < 0.05$ if don't have same letter

Fig. 4. Comparison of data for dry matter (DM) and crude protein (CP) potentially degradable fraction (fraction b, %) in the four tested feedstuffs (SFM – sunflower meal, DDGS – dry distillers grain with solubles, SBM – soybean meal, and RSM – rape seed meal)

The data of our trial about the easily degradable fraction a, as well as about the rate of degradation of sunflower meal are relatively lower compared to data of NRC (2001). However, most of NRC (2001) data are from the USA where production of sunflower seed and its processing is not widely spread and only limited number of determinations is available. In Bulgaria, in an extensive study of dry matter and crude protein degradability of sunflower meal Alexandrov (1997) found out higher degradability values for DM and CP compared to our data. Effective protein degradability of soybean meal in his experiments was also higher than data from this study.

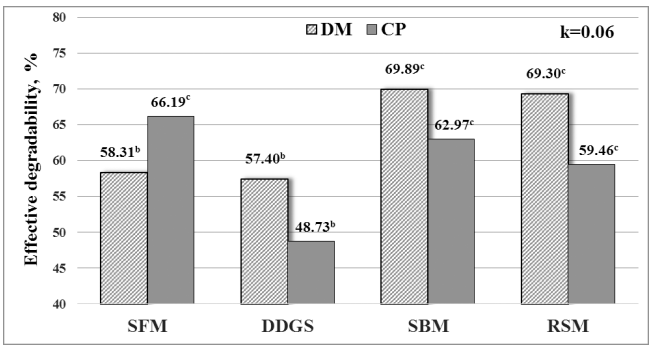


[a,b] Data for a given nutrient differ significantly at P < 0.05 if don't have same letter

Fig. 5. Data for rate of degradation (as decimals per hour) of dry matter (DM) and crude protein (CP) of the four tested feedstuffs (SFM – sunflower meal, DDGS – dry distillers grain with solubles, SBM – soybean meal, and RSM – rape seed meal)

Effective degradability independently of passage rate per hour of rumen content of dry matter in sunflower meal and dry distills grain with solubles were significantly lower, than for soybean meal and rapeseed meal. (Table 4 and Figure 6). The higher crude fiber content of sunflower meal is the reason for the difference with SBM and RSM. Lower values for DDGS are probably result of the lack of easy fermentable starch into rumen.

Effective degradability of crude protein of dry distillers grain with soluble was significantly lower (P < 0.05) than for the other three protein-rich feeds (Table 4 and Figure 6). Effective degradability was the highest for sunflower meal,



[b,c] Data for a given nutrient differ significantly at P < 0.05 if don't have same letter

Fig. 6. Comparison of data for effective degradability of dry matter and crude protein at 6%/h rumen passage rate (k = 0.06)

followed by soybean meal and rapeseed meal but the differences were not significant (P > 0.05). Therefore, the portion of feed protein reaching the small intestine was the biggest for DDGS, followed by other feeds. Our data are similar to reported by Wei Gao et al. (2015) for SFM and DDGS.

Estimated data for degradability of protein in dry distillers grain with solubles are slightly higher compared to average NRC (2001) data. However in a more resent Bulgarian study (Yosifov, 2014) degradability of DM and CP from RSM and DDGS was lower than our estimations.

Cherenková et al. (2010) found out approximately similar effective protein degradability (EPD) for DDGS, and SBM, but higher EPD for SFM and RSM.

Contrary to our data, at a rumen turn-over rate of 5% per hour Ha and Kennelly (1984) reported higher (65.5%) effective degradability of rapeseed meal protein than in soybean meal (53.6%). Kendall et al. (1991) observed 61.5%, effective degradability of rapeseed meal protein compared to 59.1% for soybean meal protein. According to Hvelplund and Madsen (1990) effective degradability of rapeseed meal protein was 68% vs. 60% for SBM at 8%/h delusion rate.

However, Woods et al. (2003) reported that the effective degradability of rapeseed meal protein was lower (66.8%) than for soybean meal (73.8%). Comparisons of data for rumen degradability of protein reach feeds, used as protein supplements in production experiments, with published data from other authors show some differences. When compared with average data from many trials summarized in NRC (2001) publication it appeared that degradation rate in SBM and RSM used in our experiments with dairy cows were low. We assumed that this difference was associated either with the variety of crops used for production of oil or with processing methods. There were only small differences in data for different protein fractions and rate of degradability for soybean meal tested by us and average literature data (NRC, 2001). The rates of degradation of soybean meal determined in our trial were very close to data estimated in Nordic countries (Valden, 2011). Richardson et al. (2003) reported lower degradability of RSM compared to our data.

Taking in consideration the big variation in degradability of tested feeds depending on the origin of primary seeds from which oil meal and DDGS were produced, and especially the substantial influences of processing methods (Yieldiz and Todorov, 2014) it is possible to assume that our data are within the framework of all observed variations.

Correlation of degradability of protein with true protein yield by milk of cows

There is relatively high correlation between protein digestible into intestine (PDI) (equal to metabolisible pro-

tein) calculated by published in literature average data for degradability summarized by Todorov et al. (2007) (r = 0.72) or by real degradability determined in this experiment (Table 4) (r = 0.75). Differences between two correlation coefficients are not significant.

Correlation of milk true protein yield with lysine digestible in small intestine, calculated by data of Todorov et al. (2007), or after taking into account real rumen degradability of supplementary protein was lower (r = 0.48 and 0.57), compared to correlation with PDI. Even correlation with lysine digestible in intestine calculated from real degradability data was lower than calculations on the basis of literature data. This was not expected because lysine is evidently the first limiting amino acid in experimental rations. We suppose that the reasons for observed discrepancy with expected results were connected first with the changes in microbial protein synthesized into rumen when different rations are fed to dairy cows. Moreover that microbial protein was a bigger source of high quality protein for dairy cows. In this experiment with dairy cows the share of supplementary protein which is examined for degradability was between 46 and 58% of total dietary crude protein (Yildiz et al., 2015). Undegradable protein supply by supplementary protein sources as percent of total protein in experimental rations for dairy cows varied from 15.9% for SFM, 17% for SBM, 20.3% for RSM to 29.7% for DDGS. The balance of protein into rumen was positive for SFM, SBM and RSM rations and slightly negative for DDGS which indicated that the supply of amino acids to the mammary glands by supplementary protein sources was slightly different from percentages above. In spite of that the share of protein with measured degradability in this experiment was below 30%. In this experiment with dairy cows only one supplementary protein in the ration was used for a more precise determination of effects of different protein sources. A practical ration usually contains more than one source of supplementary protein. Thus, the impact of small differences in rumen degradability of supplementary protein in the rations could be easily compensated. Therefore, increasing microbial protein contribution for supply of amino acids to ruminant animals has an even bigger importance than decreasing dietary protein degradability.

The second reason for the unexpected lower correlation of lysine digestible into intestine with true yield of protein with milk is connected with mammary extraction efficiency of amino acids which were available in small deficit or excess compared to requirements. There was especially a better use of lysine in the ration with DDGS, as a main dietary protein source. A similar situation is mentioned by Mjoun et al. (2010b). They reported increased lysine extraction efficiency by mammary gland for DDGS diet (76.1%) versus SBM diet (65.4%), which can help increasing milk yield when DDGS is fed to dairy cows. In the experiments of Mjoun et al. (2010a,b) despite the apparent deficiency of lysine, milk protein percentage was increased in cows fed DDGS diet. Even in the experiments of Pamp et al. (2006), the ration with distillers grain tended to give higher milk yield than the diet with SBM.

Conclusions

The rumen degradability of supplementary protein sources used in the present production experiment with dairy cows was variable but within the broad range of data obtained by other authors. The correlation coefficients between the quantity of protein digestible in intestine (PDI) and lysine digestible in intestine (LDI) in the ration of dairy cows and true protein yield with milk did not differ significantly when calculation of PDI and LDI were made from average literature data for protein degradability into rumen or from real degradability determined for supplementary protein sources used in the experiment with cows. It seems that changes in microbial protein synthesized into rumen and degree of mammalian utilization of amino acids (lysine) when cows received different rations obscured the effect of the relatively smaller part of amino acids supplied by undegradable into rumen feed protein.

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