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COMPARISON OF THE RUMEN DEGRADABILITY AND INTESTINAL DIGESTIBILITY OF DM AND CP OF DRIED DISTILLERS'S BY-PRODUCTS FROM BULGARIAN DISTILLERY COMPANIES

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Abstract

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The objective of this study was to compare the rumen degradation kinetics and intestinal digestibility of different dried distillers by-products produced in all Bulgarian ethanol factories. Three non-lactating Jersey cows with an average body weight of 436 ± 18 kg each fitted with a rumen and T-duodenal cannulas were used in the experiment. Six dried distillers by-products (three from wheat DDGSw1, DDGSw2, DDGSw3 and three from corn DDGSc1, DDGSc2 and DDGc4 - numbers indicate the individual factory) were collected from four of the biggest ethanol plants in Bulgaria. Tested feeds were incubated in the rumen of fistulated cows for 0, 2, 4, 8, 16, 24 and 48 in 6 replications. The effective degradability of DM was higher for both wheat and corn DDGS products from numbers 1 and 2 ethanol factories than other two sources ($P<0.05$). The range of CP fraction *a* in the by-products of Bulgarian ethanol producing plants, varied from 23.85% for DDGSw2 to 12.38% for DDGc4 with significantly higher values for wheat by-products than those from corn ($P<0.05$).The values obtained for effective degradability of CP at different outflow rates for wheat DDGSw1 and DDGSw2 were higher than those for dried distillers by-products from corn. It was observed a high correlation between all of the color parameters (*L*, *a* and *b*) of the wheat DDGS by-products and their effective degradability. Further investigations are necessary in order to check the possibilities of using those easy estimated parameters for a control of the protein nutritional value of DDGS. The intestinal digestibility of rumen degradable dry matter ranged from 51.95% for DDGSw3 to 38.26% for DDGc4 and had been significantly higher for wheat dried distillers by-products compared to DDGSc1 and DDGc4. The values for intestinal digestibility of proteins in both wheat and corn DDGS not differed significantly and it was observed a higher variation among the wheat by-products samples which were estimated to be 79.65% to 93.64%. Although, variation in nutrient contents due to the influence of different technological regimes and quality of raw materials, the results for the main parameters of the nutritional value of the feeds (PDI and PBR), obtained in the present experiment can be used about formulating rations for ruminants.

Key words: rumen degradability; intestinal digestibility; protein value; dried distillers's by-products
Abbreviations: CP – crude protein; DDG – dry distilled grains; DDGS – DDG with solubles; DM – dry matter; PBR – protein balance in the rumen; PDI – protein digestible in small intestine; RUP – *rumen undegradable protein*

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Introduction

Dried distillers grains with solubles (DDGS), generated by the ethanol industry in Bulgaria are economically valuable source of protein for cattle and sheep farming. For rational use of these feedstuffs it is important to know exactly, their nutritional value and its variations according to the quality of raw materials and the treatment applied to those feed sources. The rumen degradability and intestinal digestibility are the two most important parameters of the protein nutritional value. Data for rumen degradability and intestinal digestibility of dried distillers' by-products in Bulgaria are missing. It was tested only one sample of corn DDGS from the factory of Zaharni Zavodi Ltd., Gorna Oriachovitza (Todorov et al., 2016).

At Bulgarian factories for ethanol production, the corn is the predominant grain source and soft varieties of wheat are the second. Generally, all Bulgarian factories apply approximately the same processing technology of dry grinding, without kernel fractionation, resulting in lesser expenses (Rausch and Belyea, 2006). Despite the similar technology, there are a few differences in the technological regime and the amount of individual additives such as the proportion of chemicals, enzymes and yeast culture. There are also some differences in the amount of added condensed distillers' solubles fraction before drying of the grain residues which is known to influence ruminal DM and CP degradability and intestinal protein digestibility (Cao et al., 2009). Each factory applies its own know-how modifying one or many steps according to own conditions, experience, resulting in no completely standardized processing methods among factories (Kirsten, 2013).

In our country we use the data about the nutritional value of DDGS published abroad and taken from studies which tested by-products manufactured under the similar technology of dry grinding. The raw material is different because the varieties, soils, fertilization of corn and wheat and climatic conditions are different. There are also small technological differences which require studying the Bulgarian DDGS in order to find the real protein value of these feeds.

The objective of this study was to compare the rumen degradation kinetics, intestinal digestibility and protein feeding value of different dried distillers by-products produced in Bulgarian ethanol factories.

Materials and Methods

Animals and samples

The animal experiment was carried out with consistency of Bulgarian legislation in field of the animal welfare and with the respect of the Bulgarian Food Safety Agency (*License № 126 registered in BFSA*).

Three non-lactating Jersey cows with an average body weight of 436 ± 18 kg each fitted with a rumen and T-duodenal cannulas were used for evaluation of the rumen degradability and intestinal digestibility of DM and CP for various DDGS samples. During the adaptation and experimental periods cows were fed maintenance level ration of 80% roughages (63.6% alfalfa hay and 16.4% barley straw) and 20% concentrate (30.5% corn, 26.5% barley, 23.0% wheat bran, 17.0% sunflower meal and 3% minerals and vitamins supplements). Cows received their assigned ration at approximately 8.00 h and 16.00 h.

Six dried distillers by-products have been collected from four of the biggest ethanol factories (*numbers indicate the individual factory*) in Bulgaria as follows: Vinprom Peshtera Ltd., factory in village of Katunitza (DDGSw1 and DDGSc1), Zaharni Zavodi Ltd., Gorna Oriachovitza (DDGSw2 and DDGSc2), Vinrpom Karnobat Ltd. (DDGSw3) and Almagest Ltd., Ichtiman (DDGc4). The DDGS by-products from wheat are marked with letter *w*, and from corm – *c*.

In situ procedures

Samples of each feed (approximately 2.5 g) were placed in a polyester dacron bags which were approximately 5 cm ×10 cm with pore size 45 μm (SEFAR® PET 1500, 9410 Heiden, Switzerland). The bags were tied and attached by a polyester rope and put into the rumen at approximately 45 cm from the cannula with 150 g weight. Tested feeds were incubated in the rumen for 0, 2, 4, 8, 14, 24 and 48 in 6 replications. Immediately after removal from the rumen, the bags were carefully washed by hand under running tap water until the water remained clear. Then, the bags were dried at 65°C for 48 h and the DM content of the residue was determined at 105°C for 2 h.

Mobile bag technique

The polyester bags (4 x 8 cm with pore size 16 μm, SEFAR® PET 1500, 9410 Heiden, Switzerland) were filled with approximately 1 g of tested sample previously incubated for 16 h in the rumen as described above. Samples are soaked for 1 h in HCl solution (pH 2.4) and thereafter incubated in HCl–pepsin solution (pH 2.4) for 2 h at 40°C. The bags were inserted into the proximal duodenum by a T-shaped duodenal cannula almost 2 hours after morning feeding (14 bags per cow per day with 30 min. interval between the individual insertions). The bags were recovered in faeces on the next day by rinsing with cold water through a large sieve. Finally, the bags were washed, dried (65°C for 48 h and105°C for 2 h) and weighed for the DM determination in tested feeds.

Chemical analysis

Each feed sample was milled and passed through a 2-mm screen. The chemical composition of the tested feeds was determined according to the AOAC (2007) (Table 1). The nitrogen content of the residues incubated in the rumen and passed through the intestine were assessed using an automatic N analyzer (*Kjeltec™ 8400 Analyzer Unit, FOSS,* DK-3400 Hillerod, Denmark). The CP content of the DM was determined by multiplying the percentage of nitrogen with 6.25.

Colorimetric measurement

The colorimetric measurement of different dry distillers' by-products was performed using a Minolta colorimeter (Chroma meters CR-400). The colors were expressed in (L), (a) and (b) color scale. It is also known as Hunterlab color score L (Hunter Associates Laboratory, 2002), in which (L) indicates lightness and values (a) and (b) indicate redness and yellowness, respectively.

Calculations and Statistical analysis

The degradation kinetics of DM and CP for each feed were fitted to the equation described by Orskov & McDonald (1979), using the Marquardt algorithm for non-linear regression procedure (SPSS ver. 23, Chicago, USA):

$$d = a + b(1 - Exp(-^{ct}),$$

where d is the degradability at time, a is the water-soluble and rapidly degradable fraction, b is the potentially degradable fraction, c is the rate of degradation of potentially degradation fraction and t is the time of incubation (h).

The Effective degradability (ED) of tested feeds was calculated by the equation:

$$\text{ED} = a + (b \times c)/(c + k),$$

where k is the outflow rate, which was assumed to be 0.045, 0.06 and 0.08 h^{-1}.

The values for Protein truly digestible in small intestine (PDI) and Protein balance in the rumen (PBR) were calculated according to the Bulgarian protein system (Todorov et al., 2007) by the following equations:

$$\text{PDI} = 1.11*CP(1 - ED)*IDRUP + 0.093*FOM + 4$$
$$\text{PBR} = CP*(ED - 0.1) - 0.145*FOM$$

where *IDRUP* is the intestinal digestibility of rumen undegraded protein, *FOM* is the fermented organic matter and *ED* is effective degradability.

The data were subjected to the analysis of variance (ANOVA) using the SPSS Program (Version 23). The significance was declared at $P < 0.05$. The bivariate Pearson correlation was used for measuring the linear relationship between color parameters of wheat DDGS by-products and effective degradability at different outflow rates ($P = 0.01$).

Results and Discussion

Chemical composition of tested feeds

CP content for wheat DDGS products was higher than the corn DDGS and corn DDG products and varied slightly (Mean 32.723%, SD 0.346). The crude protein content of DDG without solubles was significantly lower compared to the DDGS produced in other factories. Wheat DDGS is usually higher in CP which is connected with the higher protein value of the wheat (Nuez Ortin and Yu, 2009). Corn DDGS products had higher ether extracts content compared to wheat DDGS. The chemical composition of the six dried distillers by-products (Table 1) were similar to the data in the handbook of animal nutrition (Todorov et al., 2007). The nutrient content of both wheat and corn DDGS pro-

Table 1. Chemical composition of dried distillers grains, (% of DM)

Feeds	Dry matter, %	Crude protein	Crude fiber	Ether extract	Nitrogen free extract	Crude ash	Ca	P
Wheat DDGS								
DDGSw1	90.41	32.98	8.25	7.56	46.54	4.67	0.25	0.93
DDGSw2	91.23	32.33	8.15	6.37	48.48	4.72	0.28	0.86
DDGSw3	93.79	32.86	7.32	8.93	46.24	4.65	0.26	0.87
Mean (SD)	91.810 (1.763)	32.723 (0.346)	7.907 (0.511)	7.620 (1.281)	47.087 (1.216)	4.680 (0.036)	0.263 (0.015)	0.887 (0.031)
Corn DDGS								
DDGSc1	91.3	26.59	9.42	13.95	45.94	4.1	0.30	0.83
DDGSc2	92.51	24.56	7.98	12.69	49.92	4.85	0.37	0.78
Mean (SD)	91.905 (0.856)	25.575 (1.435)	8.7 (1.018)	13.32 (0.891)	47.93 (1.990)	4.475 (0.530)	0.335 (0.049)	0.805 (0.035)
Corn DDG								
DDGc4	90.71	22.29	13.34	8.56	52.56	3.45	0.05	0.32

duced by Zaharni Zavodi, Ltd. are also in accordance with the data reported by Slavov et al. (2014). Though, there are a lot of data for a higher crude protein content in some dried distillers by-products, especially for those produced from corn – 30.7% (Woods et al., 2003), 30.6% to 33.5% (Kleinschmit et al., 2007), 33.3% (Lee at al., 2016). The reason is probably a lower nitrogen fertilization of corn in Bulgaria. It is well known that the nutrient content of corn and wheat DDGS can vary among different ethanol producers (Spiehs et al., 2002). The lower minerals content of DDG (without solubles) is due to the fact that upon centrifugation, more minerals go to the soluble than the solid fraction (Liu and Han, 2011).

Data for rumen disappearance of dry matter and crude protein

The results for *in situ* rumen disappearance of DM and CP in different dry distilllers by-products are presented in Table 2 and Table 3, respectively. The DM disappearance of DDG was much slower at each hour of rumen incubation (P<0.05). The DM degradability of DDGSc1 was slower compared to DDGSw1 and become significantly higher for 16th and 48th incubation hours, despite the fact that DDGSw1 and DDGSc1 were produced at the same factory, under the equal technological conditions. DM degradability of DDGSw3 produced by Karnobat factory became lower after the 8th hour of rumen incubation compared to by-products from other plants, probably because of the higher temperature of drying.

The CP disappearance varied greatly among different DDGS and also by different incubation hours. It was significantly higher in first two incubation hours (0 h, 2 h) for the wheat DDGS samples compared to the corn by-products (P<0.05). The CP degradability of DDGSw3 was lower in comparison with other samples of dry distillers by-products which could be attributed to the different types of grain and also to the different processing methods applied in each factory.

Table 2
Disappearance of dry matter after different time of rumen incubation, %

Feedstuffs	Rumen incubation, h						
	0	2	4	8	16	24	48
Wheat DDGS							
DDGSw1	36.93^{a}	43.68^{a}	46.58^{a}	56.02^{ab}	62.53^{b}	72.11^{ab}	83.28^{b}
DDGSw2	37.05^{a}	43.15^{ab}	47.59^{a}	54.18^{bc}	64.15^{b}	73.31^{a}	82.39^{b}
DDGSw3	38.67^{a}	43.58^{a}	46.71^{a}	51.6^{d}	57.02^{c}	61.1^{c}	76.55^{c}
Corn DDGS							
DDGSc1	29.22^{c}	39.77^{b}	44.76^{a}	57.05^{a}	67.16^{a}	73.21^{a}	88.10^{a}
DDGSc2	34.42^{b}	43.49^{ab}	46.94^{a}	53.61^{cd}	64.74^{ab}	69.91^{b}	81.22^{b}
Corn DDG							
DDGc4	6.81^{d}	9.47^{c}	15.01^{c}	30.07^{e}	39.13^{d}	50.27^{d}	73.63^{c}

$^{a-d}$ Means within a column lacking common superscript differ significantly at P < 0.05

Table 3
Disappearance of protein after different time of rumen incubation, %

Feedstuffs	Rumen incubation, h						
	0	2	4	8	16	24	48
Wheat DDGS							
DDGSw1	18.39^{b}	27.33^{a}	34.19^{ab}	48.20^{a}	56.23^{b}	69.62^{b}	85.33^{a}
DDGSw2	23.61^{a}	30.78^{a}	37.51^{a}	46.43^{ab}	60.40^{a}	75.40^{a}	86.81^{a}
DDGSw3	21.58^{a}	27.92^{a}	33.51^{b}	41.58^{c}	51.78^{c}	57.93^{d}	78.26^{b}
Corn DDGS							
DDGSc1	14.15^{c}	19.33^{b}	27.89^{c}	46.06^{ab}	59.79^{a}	69.23^{ab}	82.87^{a}
DDGSc2	14.91^{c}	26.49^{a}	34.05^{ab}	44.83^{b}	58.96^{ab}	67.55^{b}	80.49^{b}
Corn DDG							
DDGc4	11.92^{d}	15.57^{b}	27.71^{c}	38.27^{d}	50.68^{cd}	61.96^{c}	82.54^{a}

$^{a-d}$ Means within a column lacking common superscript differ significantly at P < 0.05

Degradation parameters of DM

The DM ruminal degradation parameters and effective degradability values at different outflow rates are presented in Table 4. The mean value for the rapidly degradable fraction *a* of DM was highest for the DDGSw3 (40.84%) and marked by lowest for DDG – 6.22% ($P < 0.05$). The main reason is the lack of condensed distillers' soluble fraction in DDG, which at this factory is unaccountably discarded. In general, the results for fraction *a* of the wheat DDGS products were higher than those for the corn dry distillers by-products, although DDGSc2 differ significantly only from DDGSw3. The results for potentially degradable fraction *b* ranged from 48.73 to 52.63% for the wheat DDGS samples, whereas the mean values for the corn by-products greatly varied between 47.11 (DDGSc2) to 57.79 (DDGSc1) The fraction *b* was significantly higher for DDGc4 without solubles 85.81%.

The mean values for DM *a* and *b* fractions of all wheat DDGS products are consistent with the results reported by Lee et al. (2016). On the other hand, those fractions for DM corn by-products greatly varied and only the results for DDGSc1 were similar to the values previously reported by Woods et al. (2003) (*a* – 31.7%; *b* – 59.12%). In a recent study, the degradability parameters of a different batches from the same corn by-product produced by Zaharni Zavodi Ltd., Gorna Oriachovitza (DDGSc2) were measured and the result obtained for fraction *a* was similar with a present data (36.55% vs 36.15%), but the potentially soluble fraction *b* was almost 50% higher – 47.11% vs 69.84% (Todorov et al., 2016). It is connected with the slower rate of degradation of potentially degradable fraction *b* at our previous trial (0.026 h^{-1}) than the rate for DDGSc2 at the present experiment (0.056 h^{-1}).

This could be mostly connected with the different nutrient composition of the raw material from which the DDGSc2 was produced and partially from the small differences in processing methods. Summarizing the sources of variation Kirsten (2013) indicated that variation of nutrients within the same grain is also expected mainly due to growing conditions, varieties, seasonal variation, environmental factors, fertilization and soil conditions. Furthermore, Belyea et al. (1998) found a higher variation in nutrient composition of condensed distillers soluble fraction among different batches which the authors explained as a result of the amount and quality of steep water added to fermenters and the addition of different chemicals.

The rate of degradation of the DM fraction *b* was higher for corn by-products (except DDGc4 without solubles) than for wheat DDGSw1 and DDGSw3 ($P<0.05$), which is in contrast with the results reported by Lee et al. (2016).

The effective degradability of DM at $k = 0.045$, 0.06 and 0.08/h was higher for both wheat and corn DDGS products from ethanol plants at village of Katuniza (DDGSw1; DDGSc1) and Gorna Oriahovitza (DDGSw2; DDGSc2) than other two sources. Expectedly, the lowest mean values of DM effective degradability were observed at dried distillers grains without solubles ($P < 0.05$). It was found that the ED of dry matter of some of the corn by-products used, was relatively higher than the results reported by Woods et al. (2003) and Lee et al. (2016), who found that effective degradability of DM at outflow rate $k = 0.06$ was 49.78% and 52.8%, respectively. However, Maxin et al. (2013) reported a value of 53.2% for the DM effective degradability of wheat DDGS at outflow rate $k = 0.074$ which is consistent with our results for DDGSw3 (52.57%) at a little bit higher outflow rate $k = 0.08$.

Table 4
Dry matter degradation parameters and effective degradability of dry matter of wheat and corn dry distillers by-products

Feedstuffs	a, %	b, %	c (h^{-1})	Effective degradability of DM at different outflow rates, %, h^{-1}		
				$k = 0.045$	$k = 0.06$	$k = 0.08$
Wheat DDGS						
DDGSw1	38.25^{b}	50.06^{cd}	0.047^{b}	63.74^{ab}	60.16^{a}	56.70^{a}
DDGw2	38.02^{b}	48.73^{cd}	0.051^{ab}	63.88^{ab}	60.36^{a}	56.93^{a}
DDGSw3	40.84	52.63^{bc}	0.023^{c}	58.61	55.39	52.57
Corn DDGS						
DDGSc1	31.63	57.79^{b}	0.062^{a}	65.10^{a}	60.98^{a}	56.84^{a}
DDGSc2	36.55^{b}	47.11^{d}	0.056^{a}	62.74^{b}	59.37^{a}	56.02^{a}
Corn DDG						
DDGc4	6.22	85.81^{a}	0.031^{c}	41.54	35.75	30.46

$^{a-d}$ Means within a column lacking common superscript differ significantly at $P < 0.05$

Degradation parameters of CP

The range of CP fraction *a* in the by-products of Bulgarian ethanol producing plants presented in Table 5, varied from 23.85% for DDGSw2 to 12.38% for DDGc4 with significantly higher values for wheat by-products than those from corn ($P < 0.05$). A little bit higher values of the CP fraction *a* for wheat DDGS by-products were obtained by Maxin et al. (2013) (27.6%) and Lee et al. (2016) (26.5%). The result for rapidly degradable fraction *a* in DDGSc2 (17.03%) agreed with values reported by Kleinschmith et al.(2007) for various sources of DDGS from corn (15.9 to 19.7%), although in a previous study we've obtained a much higher result (28.71%) for a sample from the same ethanol plant (Todorov et al., 2016). The data for other corn products (DDGSc1 and DDGc) were lower than the values previously reported from several authors (NRC, 2001; Kleinschmith et al., 2007; Mjoun et al., 2010; Gao et al., 2015; Lee et al., 2016). Cao et al. (2009) found a linear increase in fraction *a* when the amount of condensed distillers solubles is added to DDG during the processing cycle. This allegation could explain the lower result of fraction *a* for DDGs without solubles, while the lower value for DDGSc1 could be partially connected with the amount of solubles added to DDG. Moreover, DDGSw1 was produced under the same technological conditions and it also has a lower water soluble fraction *a* compared to other wheat DDGS by-products ($P < 0.005$). At this factory owned by Vinprom Peshtera Ltd., the amount of condensed distillers' solubles fraction that is added back to the solid fraction, because of the pH correction was declared to be approximately 20%. Mjoun et al. (2010) also reported a lower values (11.1%) of CP fraction *a* for high-protein DDGS. Kirsten (2013) indicated that fraction *a* of eight different samples of corn DDGS ranged from 10.0% to 21.1%.

The potentially degradable CP fraction *b* was bigger for DDGc4 (79.29%) compared to wheat DDGS by-products and DDGSc1 and 2 ($P<0.05$). The range of CP fraction *b* of wheat DDGS was within values reported by Lee et al. (2016). The values for CP fraction *b* of corn DDGS products showed a bigger variation but the results that have been already published also greatly varied and ranged from 50.6% to 83.23% in different distillers by-products from corn (Woods et al., 2003a; Kleinschmith et al., 2007; Mjoun et al., 2010; Gao et al., 2015; Todorov et al., 2016). Consistent with the results of this experiment, Li et al. (2010) found that the rate of degradation of the CP fraction *b* was lower for wheat DDGS than for corn DDGS and only DDGSc2 made an exception. Generally, the $c(h^{1})$ for the CP of both corn and wheat DDGS products was within previously reported ranges (Woods et al., 2003; Maxin et al., 2013).

The values obtained for effective degradability of CP at different outflow rates for wheat DDGSw1 and 2 were higher than those for dried distillers' by-products from corn ($P < 0.05$). Many authors also reported that wheat DDGS had higher *in situ* degradability than corn DDGS (Nuez Ortin and Yu, 2009; Chrenkova et al., 2012; Lee et al., 2016). Although, the values of CP effective degradability obtained for corn distillers by-products were much higher than previously reported by several authors (NRC, 2001; Woods et al., 2003; Kleinschmith et al., 2007; Kelzer et al., 2010; Mjoun et al., 2010). The content of rumen undegradable protein in CP of DDGS was 67.1% much higher than the values of DDGS produced with a traditional dry milling process.

The lower rate of degradation of potentially degradable fraction *b* of DDGSw3 is the main reason for a lower CP degradability compared to both wheat and corn dried distillers with solubles by-products. Another reason is associated with

Table 5
Crude protein degradation parameters and effective degradability of crude protein of wheat and corn dry distillers' by-products

Feed stuffs	a, %	b, %	c (h^{-1})	Effective degradability of CP at different outflow rates, %, h^{-1}		
				$k = 0.045$	$k = 0.06$	$k = 0.08$
Wheat DDGS						
DDGSw1	20.29^{b}	70.07^{b}	0.052^{b}	57.77^{b}	52.74^{b}	47.82^{b}
DDGSw2	23.85^{a}	69.79^{b}	0.051^{bc}	60.75^{a}	55.75^{a}	50.86^{a}
DDGSw3	23.62^{a}	67.35^{bc}	0.033^{d}	52.28^{d}	47.68^{d}	43.43^{d}
Corn DDGS						
DDGSc1	12.57^{e}	72.77^{b}	0.066^{a}	55.94^{bc}	50.79^{c}	45.57^{cd}
DDGSc2	17.03^{c}	65.11^{c}	0.068^{a}	56.00^{c}	51.41^{bc}	46.76^{bc}
Corn DDG						
DDGc4	12.38^{d}	79.29^{a}	0.043^{c}	51.29^{d}	45.65^{e}	40.26^{c}

$^{a-e}$ Means within a column lacking common superscript differ significantly at $P < 0.05$

huge variations of processing methods of different ethanol factories, especially the drying temperature which influences the solubility of CP in the rumen (Li et al., 2012; Nuez Ortin and Yu, 2012; Lee et al., 2016).

Furthermore, the wheat DDGSw1, which was produced at the same ethanol factory from where the sample of DDGSc1 was taken, had the lowest CP effective degradability values among other wheat DDGS sources. At this factory both wheat and corn DDGS are dried at 104°C in a tumble dryer. The fermentation batches are the more important source of variation of nutrient composition of DDGS than different ethanol factories (Belyea et al., 2010). Kirsten (2013) concluded that the proportion of condensed distillers' solubles added back to the solid fraction, the drying temperature and all other sources of variation can be present as single factors or there could be interactions, making the identification and control of variation in processing steps and finally the composition of DDGS more difficult.

The Hunter Lab color score for the tested feeds is presented in Table 6. Some authors have made a linear correlation between the color score and CP quality expressed by the amount of ADiCP (Acid detergent insoluble protein), which is considered to be totally undegraded. Cromwell et al. (1993) found high correlation between the color score and ADiCP concentrations in DDGS (r = 0.79), while Harty et al. (1998) and Kleinschmit et al. (2007) reported this relationship to be poorly correlated.

Table 6
Color score of dried distillers by-products produced by Bulgarian ethanol industry

Feedstufs	L	a	b
Wheat DDGS			
DDGSw1	29	6.82	11.87
DDGSw2	33.51	6.8	14.8
DDGSw3	25.04	5.96	8.25
Corn DDGS			
DDGSc1	39.99	8.75	20.4
DDGSc2	36.67	8.59	19.33
Corn DDG			
DDGc4	49.38	4.75	21.84

It was observed a high correlation between all of the color parameters (*L*, *a* and *b*) of the wheat DDGS by-products and their CP effective degradability (Table 7).

However, the effective degradability values of corn DDGS were very similar and there were only two samples, wherefore such a correlation was not found. But the color parameters are usually easily tested, so it is recommended to test more dried distillers' by-products for such a correlation in order to use the color as an indicator of DDGS quality.

Table 7
Correlations (r) between color parameters and wheat DDGS's effective degradability of CP at different outflow rates

Color score	$k = 0.045$	$k = 0.06$	$k = 0.08$
Wheat DDGS			
L	0.815**	0.823**	0.819**
a	0.949**	0.950**	0.938**
b	0.859**	0.866**	0.860**

** Correlation is significant at the < 0.01 level

Intestinal digestibility of DM and CP

The mean values of intestinal digestibility as a percentage of rumen undegraded DM and CP are presented in Table 8.

Table 8
Dry matter and crude protein intestinal digestibility of the residue after 16 h of rumen incubation

Feedstuffs	Intestinal digestibility of rumen undegraded DM, %	Intestinal digestibility of RUP, %
Wheat DDGS		
DDGSw1	47.94[ab]	93.64[a]
DDGSw2	51.95[a]	88.52[b]
DDGSw3	45.21[b]	79.65[c]
Average	48.37	87.27
Corn DDGS		
DDGSc1	43.71[b]	86.76[b]
DDGSc2	46.1[b]	85.39[b]
Average	44.91	86.19
Corn DDG		
DDGc4	38.26[c]	79.01[c]

[a–d] Means within a column lacking common superscript differ significantly at P<0.05

The intestinal digestibility of rumen degradable DM ranged from 51.95% for DDGSw2 to 38.26% for DDGc4 and was significantly higher for wheat dried distillers by-products compared to DDGSc1 and DDGc4 ($P < 0.05$). Gao et al. (2015) obtained a higher value (62.2%) for intestinal digestibility of rumen undegradable DM of corn DDGS, but the intestinal digestibility at their experiment was determined by *in vitro* method.

The result for the intestinal digestibility of CP for wheat DDGS was the highest for DDGSw1, while for DDGSw2 was intermediate and for DDGSw3 was the lowest ($P<0.05$). Perhaps, the lower results of DDGSw3 are partly connected with its darkest color which could be due to a higher drying temperature. However, it is only assumption since we didn't know the exact drying temperature at the factory where

DDGSw3 was produced. The intestinal digestibility among corn DDGS is almost equal and relatively higher than dried distillers grains without solubles (DDGc4) (P < 0.05). The values for intestinal digestibility of proteins in wheat DDGS were estimated to be 79.65% to 93.64% and they are comparable to the values (79.6 – 92.2%) reported by Chrenkova et al. (2012). In accordance with our results, the same authors found that the intestinal digestibility of the rumen undegraded CP of corn DDGS samples were more stable than the values obtained for wheat by-products, which greatly varied in their study. However, Chrenkova et al. (2012) found slightly higher values for the intestinal digestibility of corn DDGS compared to our average results (94.3% vs. 86.19%). Kelzer et al. (2010) also estimated a higher value for DDGS produced with a traditional dry milling process estimated with a mobile nylon bag technique. However, Woods et al. (2003b) reported that intestinal digestibility of crude protein of corn DDGS also varied from 75.0% to 93.5% among different samples. Likewise, several authors have also reported that the digestibility of rumen undegraded protein estimated for DDGS vary greatly, ranging from 61.6% to 93.5% (Kleinschmit et al., 2007; Mjoun et al., 2010; Wei Gao et al., 2015) but at their experiments the intestinal digestibility was determined by *in vitro* three-step procedure.

Calculation of protein digestible in intestine (PDI) and protein balance in the rumen (PBR) values

The PDI and PBR, the main indicators of a protein nutritional value of the feeds adopted in the last 20 years in our country (Table 9) differed significantly among individual samples (P < 0.05). Overall, the PDI values of DDGS produced from wheat were significantly higher compared to corn dried distillers by-products (P < 0.05).

The data of PDI values, calculated using the results for the effective degradability and intestinal digestibility obtained in the present experiment, are significantly higher than those reported by Todorov et al. (2007), which are 148 g/kg and 155 g/kg at k=0.06 for wheat and corn DDGS , respectively.

It was mentioned above that in our country we use a literature data published abroad (Todorov et al., 2007), so the observed differences could be connected with both variations in raw materials and the processing technologies. The average PBR values for wheat DDGS (61) are equal with the data published by Todorov et al. (2007) but the average result of PBR (25) for corn by-products differs from the result obtained in our experiment. The protein balance values in the rumen were higher and differed significantly as well as within individual wheat DDGS as in comparison with corn by-products (P < 0.05).

Conclusions

No significant differences were observed in the present study in rumen degradability between wheat and corn DDGS. The variations are likely to be attributed to the difference of the processing methods in an individual factory and to the nutrient composition of grains used to produce ethanol. The intestinal digestibility of rumen undegredable protein varied among the wheat DDGS samples while for dried distillers' by-products from corn the values were more stable.

It was observed a high correlation between all of the color parameters (*L*, *a* and *b*) of the wheat DDGS by-products and their effective degradability of crude protein. Further investigations are necessary in order to check the possibilities of using those easily estimated parameters for a control of the protein nutritional value of DDGS.

The protein nutritional values of DDGS currently used in Bulgarian handbooks are significantly lower compared to the results calculated at the present study. The protein digestible in intestine (PDI) and protein balance in rumen (RBR) are

Table 9
PDI and PBR values of 1 kg dry matter of dried distillers products calculated at different outflow rates*

Feedstuffs	PDI			PBR		
Outflow rates	$k = 0.045$*	$k = 0.06$	$k = 0.08$	$k = 0.045$	$k = 0.06$	$k = 0.08$
DDGSw1	198[a]	215[a]	232[a]	81[b]	65[b]	48[b]
DDGSw2	178[c]	193[c]	209[c]	88[a]	71[a]	56[a]
DDGSw3	192[b]	205[b]	217[b]	63[c]	47[c]	33[c]
Average	189	204	219	77	61	46
DDGSc1	168[d]	181[d]	195[d]	42[d]	28[d]	15[d]
DDGSc2	158[e]	168[e]	179[e]	33[e]	22[e]	11[d]
Average	163	175	187	39	25	13
DDGc4	150[f]	161[f]	172[f]	12[f]	- 0.29[f]	- 12[e]

*PDI and PBR values were calculated using the data for FOM (Fermented organic matter) published by Todorov et al. (2007)
[a – f] Means within a column lacking common superscript differ significantly at P < 0.05

significantly higher for wheat DDGS than for DDGS from corn. The average values for different DDG presented in Table 9 could be used in formulating rations for ruminants when the origin of a DDG is not known. When the level of DM intake and expected outflow rate is different from 0.06 h^{-1} it is advisable to use PDI and PBR depending on the outflow rate.

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