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POSSIBILITIES OF DRIED DISTILLERS' GRAINS WITH SOLUBLES APPLICATION IN ANIMAL FEED

MOGUĆNOSTI PRIMENE SUVE DESTILERIJSKE DŽIBRE U SMEŠAMA ZA ISHRANU ŽIVOTINJA

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ABSTRACT

The possibility of utilization of dried distillers' grains with solubles (DDGS) in animal feed was investigated. Samples of maize hybrids ZP 548 and ZP 655b, and DDGS obtained from hybrids ZP 548 and ZP 548c were used as components of the feed. The total protein content ranged from 12.42% to 31.18%, moisture from 5.49% to 9.55, and ash content from 1.85% to 4.37%. The contents of NDF, ADF, ADL, hemicellulose and cellulose fibers ranged from: 13.90% to 48.13%; 2.9% to 20.69%; 0.27% to 2.44%; 10.69% to 30.17%, 2.62% to 18.32%, respectively. In vitro, dry matter digestibility ranged from 55.20% to 89.76%. It was concluded that the samples of DDG obtained from red and yellow maize hybrids kernel are very suitable as components for the preparation of animal feed.

Keywords*: maize, animal feed, bioethanol, dried distillers' grains with solubles (DDGS).*

REZIME

Ispitivana je mogućnost primene suve kukuruzne džibre u smešama za ishranu domaćih životinja. Kao komponente korišćeni su uzorci cele biljke i zrna hibrida ZP 548 i ZP 655b i uzorci džibre dobijeni od hibrida ZP 548 i ZP 548c (hibrid crvenog zrna). Uzorci kukuruzne džibre, sporednog produkta iz proizvodnje bioetanola, dobijeni su postupkom odvojene dvostepene hidrolize i fermentacije skroba iz kukuruznog brašna. Metoda se zasniva na korišćenju enzimskih preparata u fazama hidrolize i primeni kvasca S. cerevisiae var. ellipsoideus tokom fermentacije. Uzorci džibre sušeni su u ventilacionoj sušnici na temperaturi 60˚C u trajanju od 48h, a potom samleveni u laboratorijskom mlinu. Sadržaj proteina kretao se od 12,42% do 31,18%, vlage od 5,49% do 9,55%, a pepela od 1,85% do 4,37%. Sadržaj NDF-a kretao se od 13,90% (zrno ZP 548 + 15% džibre ZP 548) do 48,13% (cela biljka ZP 655b + 20% džibre ZP 548c); ADF-a od 2,96% (zrno ZP 548 +15% džibre ZP 548c) do 20,69% (cela biljka ZP 655b + 10% džibre ZP 548c); ADL-a od 0,27% do 2,44f%: hemiceluloze od 10,69% do 30,17% i celuloze od 2,62% do 18,32%. In vitro svarljivost suve materije određena enzimskom pepsin-celulaznom metodom kretala se u rasponu od 55,20% do 89,76%. Na osnovu dobijenih rezultata ustanovljeno je da su uzorci suve kukuruzne džibre dobijeni od zrna žutog i crvenog hibrida kukuruza pogodni kao hranivo za pripremu smeša za ishranu domaćih životinja.

Ključne reči: *kukuruz, hrana za životinje, bioetanol, suva kukuruzna džibra.*

INTRODUCTION

Cultivated on around one million hectares annually, maize (*Zea mays* L.) represents the most important field crop in the Republic of Serbia. Serbia produces around 8 million metric tons yearly and takes 16th place in world maize production. According to the United States Department of Agriculture (USDA), the total estimated 2021 world maize production was 1.13 billion metric tons (*World Agricultural Production.com, 2021*).

Bioethanol is one of the major alternative fuels worldwide that can be produced by fermentation of sugars from the biomass and used as a fuel or as a gasoline enhancer *(Mojović et al., 2012)*. The total world bioethanol production has reached 109.9 billion liters in 2019 with the United States as the largest bioethanol producer, however, this industry has suffered some setbacks during the past two years due to the COVID-19 pandemic (*Nikolić et al., 2020; RFA, 2020*).

Dry distillers' grains with solubles (DDGS) are a valuable and affordable by-product of the bioethanol industry available in large quantities that can be used as a replacement for traditional feedstuff (*Lei et al., 2017; Semenčenko et al., 2014*). During the bioethanol production process, a total of 229 kg of DDGS with 90% dry matter content can be obtained, while producing 293 liters of bioethanol per 1000 kg of maize grain (12% moisture and 65% starch, calculated on dry matter) (*Chatzifragkou et al., 2015; Liu, 2011; Mojović et al., 2007*). Unlike fresh stillage that can be used only for animal feed on farms located close to the bioethanol plants, due to susceptibility to spoilage, the dried product is quite stable and can be utilized as a component of animal feed during the whole year (*Djukić-Vuković et al., 2015; Mojović et al., 2014*).

This by-product of the bioethanol industry is an excellent source of protein and energy for animals and is therefore most commonly used as a component of feed for farm animals. DDGS represents a good source of water-soluble vitamins and minerals, as well as a range of complex organic macromolecules, predominantly polysaccharides. Maize DDGS contains yeast residues (β-glucans) and chemical compounds such as minor fractions of conjugated linoleic acid and xanthophyll that can be beneficial for the immune system of the animals, reduce intestinal inflammation without affecting the performance and carcass properties (*Jiang et al., 2014; Ruan et al., 2017*). On the other hand, some characteristics such as the content of crude fiber, mostly the insoluble fraction, the lack of some amino acids such as lysine, the high fluctuations in gross energy and oil content, as well as the proportion and quality of crude protein of maize DDGS represent obstacles to the addition of a higher percentage of DDGS in diets for some groups of animals (*Rochell et al., 2011; Meloche et al.,2013*). Some authors reported that the processing parameters during bioethanol and DDGS production (temperature, the type of process, duration of separate phases of the process) can decrease the bioavailability and the digestibility of crude protein and amino acids, mainly lysine (*Dalólio et al., 2020*).

Studies have shown that DDGS can contribute significantly to the overall economy of the maize dry-mill processes, along with carbon dioxide and ethanol (*Ferreira et al., 2018*). The use of DDGS as a component of feed could generate income in the amount of 14% of the total revenues from potential bioethanol production in Serbia. DDGS as a by-product of the cereal-based bioethanol industry could have a positive impact on the development of bioethanol production by being used in the nutrition of farm animals as a component of feed in a percentage higher than previously practiced in the world and in our country (*Semenčenko, 2013*).

Previous studies conducted at the Maize Research Institute determined that the high content of both digestible and metabolic energies indicated that DDGS samples obtained after bioethanol production from maize hybrids grain can be classified as both protein and high-energy feeds and can be used for the preparation of feed for different types and categories of farm animals (*Nikolić et al., 2020a; Semenčenko et al., 2015; Semenčenko et al., 2014; Semenčenko, 2013*).

The aim of this study was to investigate the potentials of adding different proportions of maize DDGS as a component to the maize-based feed for ruminants. The quality parameters of the feed were assessed through analyses of chemical composition and *in vitro* dry matter digestibility.

MATERIAL AND METHODS

Maize hybrids: ZP 548 (yellow dent), ZP 548c (red dent) and ZP 655b (white dent) were sown in the experimental field of the Maize Research Institute, Zemun Polje, Belgrade, Serbia. The field trial was set up according to the randomized complete block design with two replicates. The area of the elementary plot amounted to 21 m^2 (6 rows with 4.7 m length) and the sowing density was 60000 plants per hectare. Five average maize plants from each replication were cut off from two inner rows in the maturity stage optimal for silage production when the dry matter content of the whole plant ranged between 30 and 35%, i.e. between one-quarter and one-half milk-line kernel stages of maturity. The whole maize plant samples were first chopped and later dried at 60˚C for 48 h in a forced-air drying oven until constant moisture content was reached, and ground afterward in the laboratory mill with 1-mm diameter mesh sieves. Ground whole maize plants and grains of maize hybrids, as well as DDGS samples, were used as components of the analyzed feed (Table 1).

DDG samples were obtained by the process of separate two-stage hydrolysis and fermentation of starch from wholegrain flour samples using commercial enzyme preparations Termamyl SC and SAN Extra L (Novozymes, Denmark) and yeast *S. cerevisiae* var. *ellipsoideus* during hydrolyzate fermentation after the procedure previously described by *Semenčenko et al.* (*2013*). DDGS samples were dried in a forced-air drying oven at a temperature of 60˚C for 48 h. The dry samples were first crushed in a mortar and then ground in a water-cooled laboratory sample mill with a stainless steel rotating blade.

The moisture content of the samples was determined after standard drying method at 105 ˚C to constant mass. The Kjeldahl method was applied to determine the crude protein content as the total nitrogen multiplied by 6.25 (*AOAC, 1990*). The ash content was analyzed by the slow combustion of the samples at 650 ˚C (*AOAC, 1990*). The lignocellulosic fibers: hemicellulose, cellulose, neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using the detergent method by *Van Soest* (*1980*), with some modification (*Mertens, 1992*). This method is based on the differences in the solubility of individual lignocellulosic fibers in neutral, acidic and alkaline reagents. The content of hemicellulose was calculated as a difference between NDF and ADF contents, whereas the cellulose content was calculated as the difference between ADF and lignin contents. The obtained results are shown as the percentage per dry matter (d.m.). *In vitro* dry matter digestibility (IVDMD) of the investigated samples was analyzed by the enzymatic pepsin-cellulase method according to *Aufrere* (*2006*).

Table 1. Analyzed maize and DDGS feed samples.

Sample number	Composition
1	Grain ZP 548 + 15% DDGS ZP 548
2	Grain ZP 548 + 30% DDGS ZP 548
3	Grain ZP 655b + 15% DDGS ZP 548
4	Grain ZP 655b + 30% DDGS ZP 548
5	Whole plant ZP 548 + 10% DDGS ZP 548
6	Whole plant ZP 548 + 20% DDGS ZP 548
7	Whole plant ZP 655b + 10% DDGS ZP 548
8	Whole plant ZP 655b + 20%DDGS ZP 548
9	DDGS ZP 548
10	Grain ZP 548 + 15% DDGS ZP 548c
11	Grain ZP 548 +30% DDGS ZP 548c
12	Grain ZP 655b + 15% DDGS ZP 548c
13	Grain ZP 655b + 30% DDGS ZP 548c
14	Whole plant ZP 548 + 10% DDGS ZP 548c
15	Whole plant ZP 548 + 20% DDGS ZP 548c
16	Whole plant ZP 655b + 10% DDGS ZP 548c
17	Whole plant ZP 655b + 20% DDGS ZP 548c
18	DDGS ZP 548c

Minitab19 Statistical Software was used for data analysis by applying the one-way ANOVA test of variance with Tukey's LSD (Least Significance Difference) procedure. Differences with probability $p<0.05$ between the means were accepted as statistically significant, while the confidence level was set at 95%.

RESULTS AND DISCUSSION

According to the results shown in Table 2, moisture content of the analyzed feed samples varied between 5.49 and 9.55%, meaning that in all samples it was below 13%, which is the maximum value according to Serbian Regulation on Quality of Feedstuff (*Pravilnik o kvalitetu hrane za životinje, 2017*). The protein content in the samples of feed with distillers' dried grains varied from 12.42% (sample 3) to 32.20% (sample 18), which is within the recommended values for some categories of animals (*Pravilnik o kvalitetu hrane za životinje, 2017*).

Table 2. Chemical composition (%)

Sample number	Moisture content	Crude protein	Ash
1	9.45±0.08[abc]	12.71±0.30[g]	1.85±0.01[g]
2	8.84±0.23[de]	16.10±0.11[ef]	2.23±0.00[e]
3	9.45±0.04[abc]	12.42±0.39[g]	1.92±0.06[fg]
4	8.77±0.06[de]	15.80±0.12[f]	2.28±0.01[e]
5	9.47±0.13[ab]	12.95±0.40[g]	3.25±0.01[b]
6	8.56±0.02[efg]	16.73±0.22[cde]	3.40±0.03[b]
7	8.94±0.18[de]	12.44±0.30[g]	3.17±0.01[bcd]
8	8.16±0.04[g]	16.39±0.08[def]	3.28±0.06[b]
9	5.49±0.18[h]	31.18±0.02[b]	4.24±0.09[a]
10	9.55±0.04[a]	13.13±0.07[g]	1.88±0.13[g]
11	9.01±0.08[cd]	16.21±0.19[ef]	2.19±0.01[ef]
12	9.44±0.15[abc]	12.44±0.05[g]	1.94±0.16[fg]
13	8.81±0.13[de]	16.03±0.07[ef]	2.24±0.06[e]
14	9.49±0.06[a]	13.11±0.10[g]	2.95±0.10[cd]
15	8.63±0.14[def]	17.21±0.12[cd]	3.20±0.10[bc]
16	9.05±0.01[bcd]	12.85±0.07[g]	2.92±0.05[d]
17	8.22±0.01[fg]	17.51±0.38[c]	3.18±0.04[bcd]
18	5.72±0.04[h]	32.20±0.33[a]	4.37±0.06[a]
Minimum	5.49	12.42	1.85
Maximum	9.55	32.20	4.37
Average	8.61	16.52	2.81
SD	1.18	5.83	0.78

Results are given as mean ± standard deviation. Means that do not share a letter are significantly different

For example, according to the regulations, ruminant animals, such as cattle (dairy cows, calves, etc.) require lower dietary protein intake, from 12 to 16% in the complete feed, while poultry animals need more protein in their diet, from 15 to 22% depending on the category. DDGS is considered a mid-protein feed that is used in the industry of cattle and poultry as a source of protein and energy in feed rations. DDGS can provide the same or higher energy as maize but contains less protein than soybean meal. Nutrients from DDGS can be more readily used by ruminants, such as beef and dairy cattle, than monogastric animals, such as hogs and poultry. Compared with maize, DDGS have higher calcium, phosphorus, and sulfur content so that, depending on the percentage of DDGS addition to an animal's diet it may decrease the need for phosphorus supplementation. (*Abudabos et al., 2021; Hoffman and Baker, 2011*). Recent studies have shown that DDGS can usually be used at low concentrations (10 or 15%) as a feed ingredient in laying hen diets without negative effects on laying performance, feed utilization, nutrient digestibility, egg quality parameters and economics (*El-Hack et al., 2015*). A study by *Świątkiewicz et al.* (*2014*) showed that a high dietary level of DDGS (20%) had no negative effect on the bone quality of laying hens. The maximum level of ash in the feed must not exceed 8% for the majority of animal categories, hence all the investigated feed samples met this criterion (Table 2). The contents of lignocellulosic fibers are among the most important parameters of the nutritional value and technological quality of maize biomass used for ruminant nutrition (*Terzić et al., 2020, Milašinović-Šeremešić et al., 2017*). In general, the NDF fraction constituents are cell wall materials including cellulose, hemicellulose, lignin and silica. From a nutritional point of view, lignin is important because it is completely indigestible and its presence lowers the availability of the cellulose and hemicellulose contents of the feed. Nonetheless, even though it can be a negative indicator of feed quality, NDF is required by ruminants. Acid detergent fiber (ADF) primarily consists of cellulose, indigestible lignin and inorganic silica and is negatively correlated with the digestibility of forages. Animals tend to consume less forage as the content of NDF increases (*Bittman, 2004; Nikolić et al., 2020a*).

Table 3. Lignocellulosic fibers content (%)

Sample number	NDF	ADF	ADL	Hemicellulose	Cellulose
1	13.90±0.15[h]	3.21±0.10[gh]	0.27±0.02[f]	10.69±0.05[g]	2.95±0.12[hi]
2	15.68±0.13[fgh]	4.08±0.18[fg]	0.33±0.05[f]	11.60±0.05[fg]	3.78±0.13[g]
3	16.72±0.91[fgh]	3.67±0.06[fgh]	0.30±0.07[f]	13.05±0.97[fg]	3.37±0.13[gh]
4	17.69±0.93[fg]	4.17±0.06[f]	0.29±0.19[f]	13.53±0.87[fg]	3.88±0.13[g]
5	41.41±0.70[bcd]	17.90±0.04[b]	1.33±0.11[cd]	23.51±0.74[de]	16.58±0.06[b]
6	39.26±1.27[de]	15.74±0.26[cd]	1.20±0.00[de]	23.52±1.00[de]	14.53±0.26[d]
7	44.55±0.17[ab]	19.82±0.18[a]	1.74±0.11[bcd]	24.74±0.35[cde]	18.08±0.06[a]
8	39.64±1.42[cd]	16.51±0.10[c]	1.39±0.07[cd]	23.14±1.32[e]	15.12±0.17[cd]
9	35.79±0.11[e]	8.74±0.10[e]	0.70±0.16[ef]	27.05±0.01[abc]	8.05±0.06[f]
10	14.08±0.58[gh]	2.96±0.02[h]	0.34±0.03[f]	11.13±0.60[fg]	2.62±0.01[i]
11	18.24±0.69[f]	3.96±0.01[fg]	0.51±0.03[f]	14.28±0.68[f]	3.45±0.04[gh]
12	15.76±0.21[fgh]	3.37±0.16[fgh]	0.41±0.01[f]	12.39±0.06[fg]	2.97±0.16[hi]
13	17.51±0.54[fgh]	3.89±0.04[fg]	0.39±0.03[f]	13.62±0.58[fg]	3.50±0.35[gh]
14	43.22±1.51[bc]	17.51±0.70[b]	2.10±0.35[ab]	25.72±0.81[bcde]	15.41±0.35[c]
15	40.69±1.02[cd]	15.37±0.11[d]	1.80±0.01[bc]	25.32±0.92[cde]	13.57±0.09[e]
16	47.29±0.68[a]	20.69±0.28[a]	2.37±0.04[a]	26.61±0.96[bcd]	18.32±0.31[a]
17	48.13±1.65[a]	17.96±0.10[b]	2.44±0.33[a]	30.17±1.65[a]	15.52±0.33[c]
18	37.87±1.27[de]	9.19±0.33[e]	1.61±0.06[bcd]	28.68±0.95[ab]	7.59±0.26[f]
Minimum	13.90	2.96	0.27	10.69	2.62
Maximum	48.13	20.69	2.44	30.17	18.32
Average	30.41	10.49	1.09	19.93	9.40
SD	13.44	6.93	0.78	7.06	6.23

Results are given as mean ± standard deviation. Means that do not share a letter are significantly different

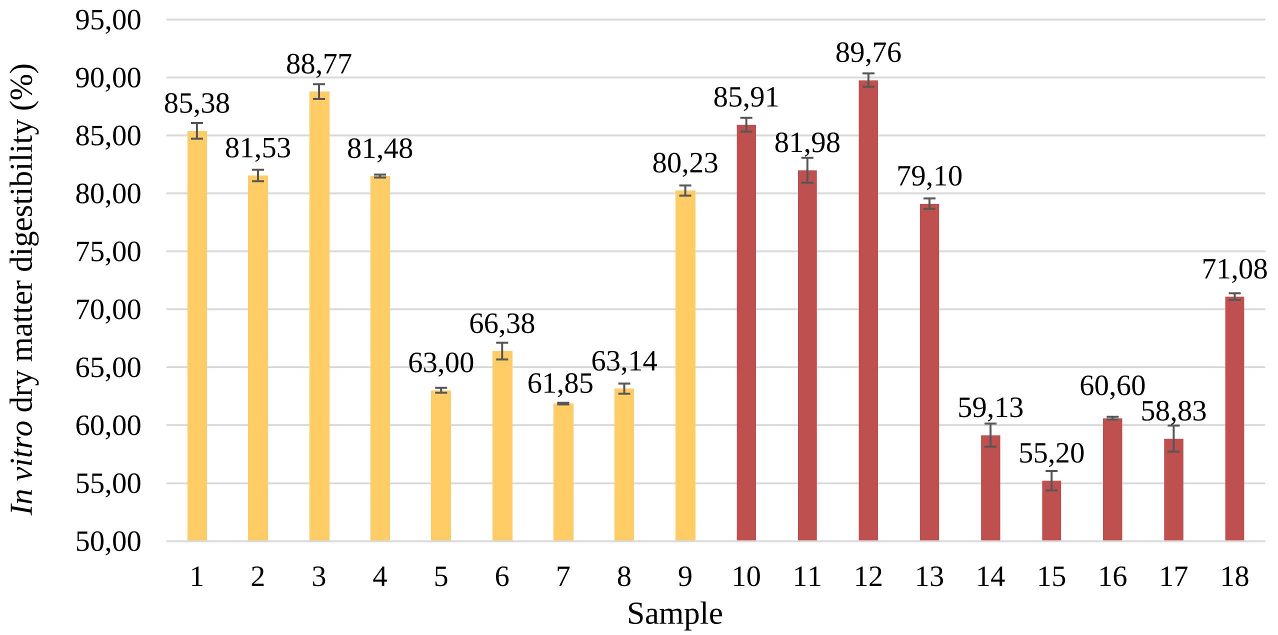

Fig. 1. In vitro dry matter digestibility of the feed

Samples of both DDGS obtained from yellow kernel maize ZP 548 and red kernel maize ZP 548c, as well as all the feed made with whole-plant samples, and DDGS had a significantly higher content of all the lignocellulosic components than the feed with maize grain (Table 3). The highest NDF was detected in sample 17 (48.13%), and the lowest in sample 1 (13.90%).

Sample of DDGS obtained from red kernel hybrid ZP 548c wholegrain flour had lower *in vitro* dry matter digestibility (71.08%) than the one made from the standard – yellow kernel hybrid ZP 548 (80.23%) (Figure 1). This can be explained by a slightly higher content of NDF, ADF, and ADL in the red ZP 548c DDGS (Table 3) The highest enzymatic degradation was observed in feed 3 and feed 12 that both contained 15% of DDGS and white dent grain ZP 655b. Feed prepared with grain had higher overall *in vitro* dry matter digestibility than those prepared with whole maize plant samples. *Liu et al. (2011)* reported that an increase in digestibility of dry matter used in diets for broilers with maize DDGS inclusion up to 20% was associated with xylanase supplementation. A study by *Applegate et al.* (*2009*) showed that the addition of a higher percentage of maize DDGS can reduce the digestibility of dry matter and crude protein, as well as lower apparent metabolizable energy and amino acids content.

CONCLUSION

The results of this study showed that all investigated feed, as well as DDGS samples, had high *in vitro* dry matter digestibility as well as crude protein levels appropriate for dietary needs of certain animal categories. Moisture and ash contents were also in accordance with referenced regulations on the quality of feedstuffs. Hence, it was concluded that DDGS obtained from ZP maize hybrids can be used as nutrients for the preparation of complete and complementary feed. However, further research needs to be conducted in order to determine the more detailed chemical composition and properties of the feed that would be optimal for particular categories and groups of animals.

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