

Model prediction of ruminal dry matter digestibility of serbian maize genotypes

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Abstract

The focus of this study is on the physical quality traits, the carbohydrates and in vitro dry matter digestibility (IVDMD) of various maize kernel genotypes produced in Serbia. Furthermore, the aim was to determine the relationship among these quality traits, as well as, their effects on the IVDMD.

Ten maize genotypes with different endosperm type and kernel color have been studied (2018 growing season). All kernel traits significantly varied among selected maize genotypes. IVDMD ranged from 83.1-91.2%. *In vitro* regression model of IVDMD in ruminants was obtained. The physical quality traits such as test weight (TWt), 1000-kernel weight (KWt), density (Den) and hard endosperm portion (HE) had significant role in predicting the digestibility of maize kernel. The IVDMD was mostly affected by test weight and density in the FOP model ($p < 0.01$ level). The determined in vitro digestibility model can serve for screening various maize kernel genotypes due to estimate their utility value for feed industry.

Abbreviations

IVDMD: *In Vitro* Dry Matter Digestibility

TWt: Test Weight

KWt: 1000-Kernel Weight

Den: Density

HE: Hard Endosperm Portion

FI: Flotation Index

FOP: First Order Polynomial

NDF: Neutral Detergent Fibre

ADF: Acid Detergent Fibre

ADL: Acid Detergent Lignin

HC: Hemicellulose

CEL: Cellulose

Introduction

Maize (*Zea mays* L.), is considered as the most widely grown cereal crop in the world, with multipurpose utilization and a key role in animal and human nutrition. Therefore, maize represents one of the most important but also highly productive cereals which will become the most significant crop in the world by 2025, while maize cultivated area in developing countries will double by 2050 (Sherman *et al.*, 2015). With advancement in maize breeding and prosperous biomass production, a range of hybrids of standard chemical composition and hybrids with specific grain properties (specialty maize) were developed with a significant potential for increased yield and quality. In the Republic of Serbia, maize is the cereal of prime importance due to optimal climate and soil conditions for high-yield. For a long time Serbia is perceived as a leader in terms of maize production as its export in Europe is ranked among the top ten exporters in the world with annual production

of 6.5 million tons (2015-2020). Most of the produced maize is used for feed, while the leftovers are used for the human consumption and starch industry (FAOSTAT, 2022).

Depending of various estimable and inestimable factors, maize kernels can vary in chemical composition and consist mainly of starch (61% to 78%, dry basis, db), non-starch polysaccharides (about 10%, db), protein (6% to 12%, db), and lipids (3% to 6%, db) as the major components (Ai & Jane, 2016). Listed carbohydrates which are major component in whole grain are grouped as follows: (i) structural carbohydrates (carbohydrates of cell walls), with NDF (neutral detergent fibers - hemicellulose+cellulose+lignin), ADF (acid detergent fibers - cellulose+lignin), and ADL (lignin) and (ii) nonstructural carbohydrates that include NFC (carbohydrates present in the grain cell content) made of starch, sugars and pectin (Radosavljević *et al.*, 2012).

With appropriate ratio of digestible fibers (hemicellulose and cellulose) in animal feed acetic acid is produced in the rumen that result with an increase the growth hormone level in blood, and potentially higher increased milk production (Nafikov & Beitz, 2007). This implies that fiber or cell wall present in maize kernel has to be analyzed because it accounts for large amounts of this cereal crop diet. Alongside, fiber fraction in ruminant nutrition is of major concern because its affects both feed intake and animal performance. Given that the plant genetics can affect the quality and digestibility of this maize it is meaningful to study hybrid with different genetic background in order to improve the animal diet (Barrière *et al.*, 2003).

Maize genotypes exhibit variations in kernel hardness, and therefore may contribute in the manifestation of nutritional values and technological traits (millability, fermentability etc) (Milašinović *et al.*, 2007; Semenčenko *et al.*, 2013). Hard maize kernel was found to be responsible in dry matter digestibility and lower N excretion, which contributed to the higher production in 42-day-old broilers (Benedetti *et al.*, 2011).

Very few papers related to the prediction model of maize kernel *in vitro* digestibility were published. Using near-infrared reflectance spectroscopy Choi *et al.* (2014) confirmed that the IVDMD of maize kernels can be predicted (the coefficient of determination, $R^2_{val}=0.68$; the root mean squared error of prediction, $RMSEP=1.69$). Given that specific laboratory analyses and procedures require substantial time and high cost the model prediction of the IVDMD can be of great practical importance due to screen utility value of various maize kernel genotypes for feed industry.

The focus of this study is on the maize physical traits, carbohydrates (starch and lignocellulose structure) and the *in vitro* dry matter digestibility of maize kernel genotypes commonly produced in Serbia under the semi-arid condition. In addition, the objectives of the study were to determine the relationship of quality parameters, as well as, their effects on the IVDMD and to assess a rapid, simple, inexpensive method (model) to predict the IVDMD of maize kernel.

Materials and methods

Materials

The study is focused on maize genotypes with different endosperm type (dent, semi-dent and flint) and kernel color (yellow, white and red) developed at the Maize Research Institute "Zemun Polje" (Serbia). Ten samples of maize kernels were collected by hand at full waxy maturity stage from plants grown in a field-trial at the Maize Research Institute „Zemun Polje“. All the genotypes received the same management practices

under the semi-arid condition in 2018 growing season. The experiment was set up by a random block design (RCB) with three replications. The experimental plot size amounted to 21m², while sowing density was 60,000 plants per hectare. Plants of each replicate were harvested at the full waxy maturity stage from the area of 7m² (two inner rows).

Physical traits

Test weight (TWt) was evaluated by the AACC method 55–10.01 (AACC International, 2017). The 1000-kernel weight was estimated by counting and weighting of 4×250 of unbroken maize kernels.

Kernel density (Den) was measured by weighting of approximately 33 g (± 0.001 g) of whole kernels. Volume determinations were done using a Beckman model 930 air-comparison pycnometer (Ignjatovic-Micic *et al.*, 2015). The analyses was performed in three replicates.

The kernel hardness was assessed according to the Stenvert-Pomeranz test by milling a 20 g of maize kernels in micro hammer-mill at 3600 rpm and 2-mm sieve (Pomeranz *et al.*, 1985). Results were calculated and showed as hard endosperm portion (%). The analysis was performed in three replicates.

Flotation index (FI) was estimated as follows. One hundred kernels were placed on a beaker with 300 mL of NaNO₃ solution (1.250 g/mL); they were mixed and then left standing for 1 min. The number of floating kernels is counted; when 0–12 kernels floated they are characterized as very hard, if FI is 13–37 kernels are hard, FI of 38–62 matches with intermediate hardness, while a FI of 63–87 and 88–100 indicates soft and very soft kernels (Preciado-Ortiz *et al.*, 2018).

The structural composition (proportions of endosperm, pericarp and germ) was determined by hand-dissection in 25 grains.

Chemical composition and *in vitro* digestibility

The starch content was determined by Ewers polarimetric method (ISO 10520:1997). Dry matter content in the maize flour was determined by the standard drying method in an oven at 105°C to constant mass.

The fiber (NDF, ADF, ADL, cellulose, hemicellulose) determination procedure was performed by Van Soest detergent method (Van Soest, 1963). *In vitro* dry matter digestibility (IVDMD) of the whole maize kernel was done by the Aufréré method (Aufréré, 2006). This analysis is based on the protein hydrolysis of the whole maize kernel in the pepsin acid solution (Merck 2000 FIP u/g Art 7190) at 40°C for 24 h, in addition on the hydrolysis of carbohydrates in the cellulase solution (cellulose Onozuka R10) in duration of 24 h

Statistical analysis

All chemical analyses were performed in two replicates, and the results were statistically analyzed. A factorial analysis of variance (ANOVA) for trials was conducted using a randomized complete block (RCB) design at the significant level of $p < 0.05$. Treatment means were assessed using post hoc Tukey HSD to perform a pairwise comparison of the means for significant differences among investigated traits. The data were statistically processed by using the program STATISTICA series 12.6. Pearson's correlation coefficient was used for determining correlations between the estimated traits.

Results and Discussion

Kernel physical traits and *in vitro* dry matter digestibility

Maize kernel properties such as physical traits and chemical composition are crucial for various utilisations; animal feed, food, industrial and ethanol production. Depending on the genetic background and various environmental factors maize kernel can differ in structure, composition and digestibility. Taking into account that the digestibility does not determined by the energy concentration, the estimation of kernel differences in physical traits and chemical composition is of great importance. In this respect, among the genotypes, there were significant variations in the all selected traits.

The results of the physical quality traits and the IVDMD of the selected maize samples with different genetics are shown in Table 1. Between the all samples, there were significant variations in the all physical traits and the IVDMD. Considering the basic anatomical parts of the maize kernels, the largest kernel part was made of endosperm (81.24% on average), followed by the germ (11.59% on average) and the pericarp (7.16% on average). The larger germ value was observed for two

specialty genotypes, ZP 775b and ZP 366c, while the endosperm and FI values were increased for ZP 333c, ZP 333 and ZP 606 samples

Maize kernels with KWt greater than 320 g and FI less than 20% are preferable in food industry (Serna-Saldívar, 2010). The results of TWt and 1000-kernel weight for ten maize genotypes ranged from 782.69 to 907.39 kgm^{-3} and from 128.40 to 376.50 g, respectively. The kernel density, as major physical trait, ranged from 1.27 to 1.40 $\text{g}\cdot\text{cm}^{-3}$. The data determined by the Stenvert hardness test had considerable variability among the selected genotypes. The percentage of the hard endosperm fraction in the total milled sample varied from 53.29 (in a dent genotype) to 76.28% (in a popcorn genotype). The highest TWt, HE, pericarp and Den values were observed for ZP 614k and ZP 611k. The results confirm previous research with the same quality parameters by Radosavljevic *et al.* (2000) who obtained relatively smaller share of hard endosperm fraction in flint maize versus dent maize kernels.

Considerable variability was estimated for the flotation index (FI) among the selected materials. The trait is an indicator of kernel hardness and its value was below 10% in seven maize samples and below 20% in another two samples (the both are yellow dent kernel, ZP 333 and ZP 606). In red dent maize kernels (ZP 333c) FI was 42.71% .

Kernel chemical composition

Comparing with other cereals maize (*Zea mays* L.) has been recognized as an essential energy feed ingredient providing the highest conversion of dry matter into products such as meat, milk and eggs. Starch is the predominant chemical constituent (approximately 70%) in maize grain. Due to its availability starch is the main energy component used in ruminant feeds.

The results of the starch content (Table 2) of various

Table 1 - Kernel physical quality traits and IVDMD of various ZP maize genotypes

Genotypes	Pericarp (%)	Germ (%)	Endosperm(%)	TWt ($\text{kg}\cdot\text{m}^{-3}$)	KWt (g)	Den ($\text{g}\cdot\text{cm}^{-3}$)	FI (%)	HE (%)	IVDMD (%)
ZP 333	5.9 ^a	11.37 ^b	82.73 ^b	788.85 ^a	345.45 ^c	1.28 ^a	18.98 ^d	53.29 ^a	87.50 ^b
ZP 333c	6.15 ^a	11.32 ^b	82.53 ^b	782.69 ^a	359.49 ^c	1.27 ^a	42.71 ^f	53.34 ^a	87.33 ^b
ZP 366	7.07 ^b	11.86 ^b	81.07 ^a	854.86 ^c	351.52 ^c	1.33 ^a	0.87 ^{ab}	61.51 ^b	89.68 ^c
ZP 366c	7.63 ^b	12.06 ^{bc}	80.31 ^a	803.74 ^{ab}	374.28 ^d	1.31 ^c	3.4 ^{bc}	57.62 ^b	83.07 ^a
ZP 553b	6.57 ^{ab}	13.02 ^c	80.41 ^a	814.53 ^b	283.27 ^b	1.31 ^c	9.51 ^e	61.03 ^b	86.79 ^b
ZP 555	6.59 ^{ab}	12.03 ^{bc}	81.38 ^a	820.92 ^b	333.94 ^c	1.31 ^c	3.45 ^{bc}	58.53 ^b	87.75 ^b
ZP 606	5.94 ^a	11.66 ^b	82.4 ^b	796.44 ^a	376.5 ^d	1.29 ^b	18.82 ^d	59 ^b	86.95 ^b
ZP 611k	9.58 ^c	9.54 ^a	80.88 ^b	900.21 ^d	133.74 ^a	1.4 ^d	0 ^a	75.55 ^c	88.54 ^c
ZP 614k	9.32 ^c	9.58 ^a	81.1 ^a	907.39 ^d	128.4 ^a	1.39 ^d	0 ^a	76.28 ^c	91.18 ^d
ZP 775b	6.88 ^b	13.49 ^c	79.63 ^a	809.16 ^{ab}	307.72 ^{bc}	1.32 ^c	3.9 ^c	59.93 ^b	84.85 ^a

TWt = test weight (kgm^{-3}), KWt = 1000-kernel weight (g), Den = density (gcm^{-3}), FI = flotation index (%), HE = hard endosperm (%), IVDMD = *in vitro* dry matter digestibility (%). Different letters in subscript in columns indicate that there is significant difference at $p < 0.05$, according to Tukey's HSD test.

Table 2 - Carbohydrates in maize kernel of various ZP maize genotypes

Genotypes	Starch (%)	NDF (%)	ADF (%)	ADL (%)	HC (%)	CEL (%)
ZP 333	68.82 ^{cd}	13.87 ^b	2.57 ^a	0.32 ^{bcd}	11.3 ^c	2.25 ^{ab}
ZP 333c	67.97 ^c	11.73 ^a	2.46 ^a	0.32 ^{bcd}	9.27 ^b	2.14 ^a
ZP 366	68.96 ^d	14.39 ^{bc}	3.07 ^b	0.29 ^{bc}	11.32 ^c	2.78 ^d
ZP 366c	65.54 ^{ab}	11.57 ^a	2.65 ^{ab}	0.28 ^{ab}	8.92 ^a	2.37 ^b
ZP 553b	68.11 ^c	11.39 ^a	2.51 ^a	0.37 ^{cd}	8.88 ^a	2.14 ^a
ZP 555	68.25 ^c	15.27 ^c	2.59 ^a	0.24 ^a	12.68 ^d	2.35 ^b
ZP 606	66.26 ^b	11.96 ^a	2.4 ^a	0.25 ^{ab}	9.56 ^b	2.15 ^a
ZP 611k	64.55 ^a	11.47 ^a	2.88 ^{ab}	0.3 ^{bc}	8.59 ^a	2.58 ^c
ZP 614k	64.69 ^a	15.04 ^{bc}	3.06 ^b	0.33 ^{bcd}	11.98 ^{cd}	2.73 ^d
ZP 775b	65.12 ^a	17.03 ^d	2.8 ^{ab}	0.39 ^d	14.23 ^e	2.41 ^b

NDF = neutral detergent fibre (%); ADF = acid detergent fibre (%); ADL = acid detergent lignin (%); HC = hemicellulose (%); CEL = cellulose (%). Different letters in subscript in columns indicate that there is significant difference at $p < 0.05$, according to Tukey's HSD test.

genotypes showed significant differences between the selected samples. The starch content of the maize kernels ranged from 64.6-69.0%. The lowest contents of starch were recorded in the specialty genotypes (popcorn ZP 611k and ZP 614k; white and red colored kernels ZP 775b and ZP 366c), while the highest content were in yellow dent and semi-dent kernels ZP 333, ZP 555 and ZP 366.

Concerning the lignocellulose fibers, the greatest variability was estimated in two components, NDF (11.39-17.03%) and hemicellulose (8.59-14.23%). ADF, ADL and cellulose ranged from 2.40-3.07%, 0.24-0.39% and 2.14-2.78%, respectively. The highest CEL and ADF, as well as digestibility values were observed for ZP 614k. The larger NDF and HC values were observed for ZP 775b, while the starch values were increased for ZP 555 and ZP 333 samples. Further, the results of the chemical composition are in an agreement with our previous research (Radosavljević *et al.*, 2012; Zilic *et al.*, 2011).

Although with the highest percentage of pericarp, the popcorn showed the highest IVDMD. This could be attributed to specific morphogenesis in the maize kernels with different endosperm structures (Cui *et al.*, 2014).

A recent study demonstrated that kernel vitreousness determines more by starch-protein interactions, starch granule size and shape than by the molecular characteristics of starch (Kljak *et al.*, 2018). The mentioned traits reflecting these interactions should be considered together in order to precisely explain for the IVDMD. Trínáctý *et al.* (2016) found that small variations of kernel texture could significantly affect on ruminal *in situ* degradability and total tract *in situ-in vitro* dry matter and starch digestibility.

On the basis of data analysis and obtained correlation coefficients very high dependences were observed between proportion of pericarp and several physical traits – TWt, KWt, Den and HE (0.912⁺, -0.874⁺, 0.957⁺, 0.925⁺) as well as proportion of germ and the traits - TWt, KWt, Den, HE and IVDMD (-0.687*, 0.676*, -0.620**, -0.669*, -0.650*). High dependences are observed between IVDMD and TWt, KWt and HE (0.671*, -0.561**, 0.562**) indicating a strong relationship between the digestibility and the physical quality traits (table 3). The carbohydrates (starch, NDF, ADL, HC, CEL) did not show high dependence to the digestibility (Table 4). According to the obtained relationships it can be concluded that several quality traits such as test

Table 3 - Correlation matrix for kernel physical traits and *in vitro* dry matter digestibility (IVDMD) for 10 maize kernel samples

	Germ	Endosperm	TWt	KWt	Den	FI	HE	IVDMD
Pericarp	-0.683*	-0.436	0.912+	-0.874+	0.957+	-0.610**	0.925+	0.371
Germ		-0.360	-0.687*	0.676*	-0.620**	0.063	-0.669*	-0.650*
Endosperm			-0.318	0.284	0.702*	-0.357	0.327	
TWt				-0.882+	0.971+	-0.654*	0.960+	0.671*
KWt					-0.908+	0.459	-0.936+	-0.561**
Den						-0.711*	0.976+	0.495
FI							-0.611**	-0.119
HE								0.562**

+Correlation statistically significant at $p < 0.01$ level; *Correlation statistically significant at $p < 0.05$ level;

**Correlation statistically significant at $p < 0.10$ level

Table 4 - Correlation matrix for kernel carbohydrates and *in vitro* dry matter digestibility (IVDMD) for 10 maize samples

	NDF	ADF	ADL	HC	CEL	IVDMD
Starch	-0.029	-0.328	-0.155	0.011	-0.302	0.117
NDF		0.460	0.231	0.994*	0.420	0.184
ADF			0.175	0.358	0.980*	0.522
ADL				0.221	-0.024	-0.092
HC					0.319	0.128
CEL						0.549

*Correlation statistically significant at $p < 0.05$ level

weight, 1000-kernel weight, hard endosperm portion and germ portion had the highest effect on the *in vitro* digestibility of the selected maize kernels.

Prediction of the *in vitro* dry matter digestibility

Table 5 - ANOVA table of the digestibility evaluation (sum of squares)

Term	df	IVDMD
TWt	1	26.518+
KWt	1	1.865
Den	1	17.781+
HE	1	0.041
Error	5	2.319
r ²		0.950

+Significant at $p < 0.01$ level, error terms have been found statistically insignificant, df - degrees of freedom

ANOVA of FOP models shows the effects of the independent variables, those are TWt, KWt, HE and Den, on the IVDMD. The analysis detected that the linear term of the IVDMD model Eq. (1) was found statistically significant for most cases. The ANOVA test demonstrates the significant effects of the independent variables to the responses (Table 1.). The IVDMD was mostly affected by two parameters, TWt and Den, in the FOP model ($p < 0.01$ level).

All the mentioned models represented the data satisfactorily which can be observed from the all FOP models with an insignificant lack of fit tests. A high coefficient of determination (r^2) is indicative that the variation was recorded and that the data fitted satisfactorily to the proposed regression model. The r^2 values for observed responses were found very satisfactory and showed the good fit of the model to experimental results.

The obtained regression model for the IVDMD evaluation was as follows:

$$IVDMD(\%) = 186.68 + 0.17 \times TWt - 0.01 \times KWt - 179.58 \times Den + 0.05 \times HE \dots \text{Eq. (1)}$$

Conclusions

This research indicates the importance of estimating physical quality traits of maize kernels in terms of screening and assessing the utility value of maize kernel for various uses. The physical traits such as test weight, 1000-kernel weight, density and hard endosperm portion had a very important role in determining and/or predicting the digestibility.

Yellow semi-dent genotype, ZP 366, had higher IVDMD (89.68%) and is characterized as very suitable for feed production. Specialty genotypes (red and white kernels) had significantly lower IVDMD and they are rated as suitable for human nutrition. A large number of factors influenced the IVDMD of maize kernel. The most reliable regression model for predicting the IVDMD is estimated using the physical traits which are simply measurable, economical, and not time-consuming laboratory tests. In order to attain greater reliability of the statistical models, it is necessary to keep on with the research taking a larger number of samples into consideration.

The information gathered in this paper will be useful for the selection and utilization of different maize genotypes and the development of maize-based components to prepare nutritious feed as well as to help the local feed industries to choose the most suitable maize genotypes for specific purpose and the development of innovative maize based products.

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