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CHEMICAL COMPOSITION, MINERAL CONTENT, *IN VITRO* GAS PRODUCTION AND RELATIVE FEED VALUE OF *STEVIA REBAUDIANA* BERTONI

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Abstract

GERDZHIKOVA, M., D. PAVLOV, N. GROZEVA, M. TZANOVA, D. DIMANOV, S. TERZIEVA and JIVKO KRASTANOV, 2018. Chemical composition, mineral content, *in vitro* gas production and relative feed value of *Stevia rebaudiana* Bertoni. *Bulg. J. Agric. Sci.*, 24 (Suppl. 1): 40–46

The aim of present study was to determine the chemical composition, digestibility, gas production, energy nutrition, relative feed value, and the use of *S. rebaudiana* as a forage crop. The chemical composition and mineral content of biomass from *Stevia rebaudiana* Bertoni were determined. The average crude protein content in the biomass was 86.33 g/kg of dry matter (DM); crude fat – 11.79 g/kg DM; crude fibre – 284.68 g/kg DM; ash – 96.56 g/kg DM and nitrogen free extracts (NFE) – 520.64 g/kg DM. The mineral content in the biomass of *S. rebaudiana* was similar to that of meadow grasses. Structural fibre components were on average for neutral detergent fibre (NDF) 35.52% and acid detergent fibre (ADF) 31.18% which are close to that of alfalfa and legume grasses. New data were obtained concerning *in vitro* gas production of *S. rebaudiana* biomass at 24 hour period – it was 225.83 dm/ml average, and at 48 hour period – 246.70 dm/ml (CO_2 and CH_4), which is close to the group of legume and cereal meadow grasses. The relative feed value (RFV) of *S. rebaudiana* biomass is close to that of perennial legumes. Regression equations were developed for advanced determination of: the quantity of metabolizable energy (ME), through the gas production at 24 hour period; and the relative feed value (RFV) and acid detergent fibre through the neutral detergent fibre.

Key words: Stevia rebaudiana; chemical composition; mineral content; detergent fibre; *in vitro* gas production; relative feed value

Introduction

Stevia rebaudiana Bertoni is a plant that grows successfully in many areas of the world. The interest in it is growing on a global and national scale. In the general practice, *S. rebaudiana* is grown mainly because it is used as a natural sweetener instead of sugar. The high content of flavonoids, polyphenols, antioxidants and anti-allergens (Grozeva et al., 2015) is also important.

Data on growing stevia as a cultivated plant in different parts of the world are published by Sumida (1968), Lewis (1992), Goettemoeller and Lucke (2006), Acharya (2008), Hossain (2008) and Lankes and Pude (2008). In Bulgaria Varbanov et al. (1996) developed nutrient media for accelerated rooting and *in vitro* multiplication of various *S. rebaudiana* origins in order to create new selected lines. Studies on different Stevia origins are conducted by Uchkunova and Uchkunov (2012), Uchkunova et al. (2012; 2013), Mehmed (2016), Denev et al. (2017). The morphological performance and productivity were studied by Nikolova (2013).

In recent years, *S. rebaudiana* is also of interest as a forage plant. Its favorable chemical composition and nutrient content are primary for feeding in various animal species (Wood et al., 1996; Atteh et al., 2008; Awney et al., 2011).

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The purpose of this study was to determine the chemical composition, digestibility, gas production, energy nutrition, relative feed value, and the use of *S. rebaudiana* as a forage crop.

Material and methods

The surveys were conducted in the region of Stara Zagora – at Malka Vereya village with a crop of *Stevia rebaudiana*, originating from Germany. The sowing was made by planting rhizomes. *S. rebaudiana* was cultivated without fertilization, with a planting density of 80 000 plants/ha (50/25 cm). Crude protein, crude fat, crude fiber, ash, and nitrogen free extracts (NFE) contents were determined by the Weende method (AOAC, 1984). Mineral content was determined by AAS ANALYST 800 AA, Perkin Elmer.

Potassium and calcium content was determined spectrophotometrically. Sodium, magnesium, iron, manganese, zinc and copper content were determined by atomic absorption spectrophotometer. The amount of gas generated was determined by the ANCOM RF Gas Production System. The content of acid detergent fibers (ADF) and neutral detergent fibers (NDF) was established with the appropriate buffer solutions. The digestibility was determined *in vitro* with rumen content.

Metabolizable energy was determined by the methodology of ANKOM through stomach – intestinal gas production system.

The relative feed value (RFV) was defined by Undersander and Moore (2002), Jeranyama and Garcia (2004), Stallings (2006), Boga et al. (2014) by the formulas:

DMI (dry mater intake) = 120 / (% NDF).

DDM (digestible dry mater) = 88.9 - (0.779 * % ADF).

RFV (relative feed value) = (DMI* % DDM) / 1.29.

Results and discussion

Chemical composition in the above-ground biomass of *S. rebaudiana*

The crude protein content in the dry matter of *S. rebaudiana* above-ground biomass is on average 86.33 g/kg DM (Table 1). The protein content is different in leaves and in stems. The leaves have higher protein content – 100.22 g/kg. The stem protein is 72.45 g/kg – 27.7% less than the protein in the leaves.

Average crude fat content in the dry matter of above-ground biomass of *S. rebaudiana* is 11.79 g/kg DM. The amount of fat in the leaves is 20.91 g/kg – 7.8 times more than in the stems.

Crude fiber content in the above-ground biomass of *S. rebaudiana* is on average 284.68 g/kg DM. The leaves have lower fiber content – 168.86 g/kg. Stems contain 2.4 times more fiber than leaves – 400.49 g/kg.

Mineral substances (ash) in the biomass of *S. rebaudiana* averaged 96.56 g/kg DM. The quantity of mineral substances in leaves and stems is almost the same, 97.20 and 95.92 g/kg respectively.

Nitrogen free extracts (NFE) occupy the highest amount from all substances in the biomass of *S. rebaudiana* – 520.64 g/kg DM. Their content in the leaves – 612.82 g/kg is significantly higher than in the stems – 428.46 g/kg. The higher content of NFE in the leaves is due to the lower crude fiber content in them.

According to Gasmalla et al. (2014) the chemical composition in the leaves of *S. rebaudiana* is influenced by the drying method. The results obtained in this study for the protein content in the leaves are the closest to those of Goyal and Goyal (2010), Serio (2010), Abou-Arab et al. (2010) – 11.2-11.4% (112-114 g/kg). In terms of fat, they are closest to Goyal and Goyal (2010) and Atteh et al. (2011) – 1.9-2.6% (19-26 g/kg); of fibers – to Serio (2010), Goyal and Goyal (2010) and Abou-Arab et al. (2010) – 15-15.5 % (150-155 g/kg); of ash – to Abou-Arab et al. (2010) and Tadhani and Subhash (2006) – 7.41-13.1% (74.1-131 g/kg); of NFE – to Abou-Arab et al. (2010) – 61.9% (619 g/kg). The data are summarized by Marcinek and Krejpcio (2015). Atteh et al. (2011) established protein and fiber content in *S. rebaudiana* stems at 6.7 and 45.1% (67 and 451 g/kg) respectively – very similar to the results obtained in this survey.

Table 1
Chemical composition in the above-ground biomass of *S. rebaudiana*, g/kg DM

Parts of plants	Crude protein, g/kg	Crude fat, g/kg	Crude fiber, g/kg	Ash, g/kg	NFE, g/kg
Leaves	100.22	20.91	168.86	97.20	612.82
Stems	72.45	2.68	400.49	95.92	428.46
Average	86.33	11.79	284.68	96.56	520.64

Mineral content in the above-ground biomass of *S. rebaudiana*

As the content of organic substances, the mineral content is also essential for the use of plants as animal feed. They perform an important role in the metabolism of the animal organism.

Nitrogen is one of the most important nutrients involved in the synthesis of amino acids, proteins, enzymes and other compounds. The nitrogen content in the above-ground biomass of *S. rebaudiana* averaged 13.81 g/kg DM (Table 2).

In the leaves the amount of nitrogen is higher and reaches 16.03 g/kg. The nitrogen content in the stems is 11.59 g/kg.

The potassium content is on average 16.79 g/kg DM. The potassium content in the stems – 18.11 g/kg, is slightly higher than in the leaves 15.46 g/kg.

The calcium content averaged 7.98 g/kg DM. It is higher in the leaves. The role of calcium for metabolic processes in the animal organism is associated with its hepatoprotective action and the development of a healthy bone system.

The above-ground biomass of *S. rebaudiana* contains phosphorus in a small amount - around 2 g/kg DM. Its amount in leaves and stems is the same.

S. rebaudiana contains an average of 1.86 g/kg DM magnesium. Its content in the leaves is twice as high as in the stems. Magnesium participates in the formation of chlorophyll and is mainly in the leaves.

Trace elements involve in metabolic processes as catalysts for many reactions and are important for normal metabolism.

The sodium content in *S. rebaudiana* is on average 128.31 mg/kg DM (Table 3). Its amount is slightly larger in the stems and less in the leaves.

The biomass of *S. rebaudiana* contains on average 216.25 mg/kg DM of iron. Leaves are richer in iron, involved in transferrin formation, and have a significant impact on oxidant reductive processes and blood hemoglobin.

The amount of manganese is on average 96.12 mg/kg DM. Leaves are significantly richer in manganese compared to stems. The difference is over 3.5 times. Manganese is also important for metabolic processes in organisms.

The zinc content is on average 35.27 mg/kg DM. The zinc content in the leaves is about twice more than the average.

S. rebaudiana contains on average 10.29 mg/kg DM of copper. Contrary to the other trace elements, which are at higher concentrations in the leaves, the copper content in the leaves is about two times less than in the stems.

The mineral content in the leaves of *S. rebaudiana* is studied by Mishra et al. (2010), Goyal and Goyal (2010), Abou-Arab et al. (2010), Tadhani and Subhash (2006) and Atteh et al. (2011), and in the stems – by Atteh et al. (2011).

The high content of Ca, Mg, Fe, Zn and Mn in the leaves and of Na and K in the stems of *S. rebaudiana* established in this study coincides with the results obtained by Atteh et al. (2011).

Fibre composition, digestibility, *in vitro* gas production, energy value and relative feed value

The analysis of the above-ground biomass of *S. rebaudiana* shows that in dried state it has color and scent simi-

Table 2
Mineral content in the above-ground biomass of *S. rebaudiana*, g/kg DM

Parts of plants	N, g/kg	K, g/kg	Ca, g/kg	P, g/kg	Mg, g/kg
Leaves	16.03	15.46	11.03	2.2	2.52
Stems	11.59	18.11	4.94	2.0	1.20
Average	13.81	16.79	7.98	2.1	1.86

Table 3
Mineral content in the above-ground biomass of *S. rebaudiana* – trace elements, mg/kg DM

Parts of plants	Na, mg/kg	Fe, mg/kg	Mn, mg/kg	Zn, mg/kg	Cu, mg/kg
Leaves	120.30	297.76	151.34	45.30	7.89
Stems	136.32	134.74	40.89	25.23	12.68
Average	128.31	216.25	96.12	35.27	10.29

lar to all meadow plants. Stevia leaves have almost the same amount of neutral detergent and acid detergent fibers, 25.199 and 25.578%, respectively (Table 4). Dry matter digestibility is 78.893%.

The amount of gas formed in 24 hours is 306.36 dm/ml and in 48 hours - 331.34 dm/ml. The rate of decomposition and the gas production at 24 and 48 hour periods is 108.15%, indicating an increased intensity of decomposition. Due to the high digestibility and low content of detergent fibers, the energy nutrition of the leaves expressed in metabolizable energy is high - 12.12 MJ/kg DM.

The relative feed value - RFV - 291.24 indicates that the leaves have very good fodder qualities.

In the stems the content of acid detergent fibers and neutral detergent fibers is significantly higher than in the leaves. Stems have twice more detergent fibers. The amount of acid detergent fibers - 62.513% is more than the neutral detergent ones - 49.034% (Table 4).

Digestibility of stems - 48.269% is much lower compared to the digestibility of leaves. The main reason for this is the high content of acid detergent fibers the digestibility of which is very low. Due to this reason, the amount of gas formed in 24 and 48 hours is significantly less in stems than in leaves. The low digestibility and the high content of detergent, difficult to digest fibers form the significantly lower energy nutritional value - 8.6 MJ/kg.

In inflorescences, the content of neutral detergent and acid detergent fibers is the lowest - 18,849 - 18,936% (Table 4). Dry matter digestibility is very high - 82.214%. This implies low degree of gas production and low energy nutrition. The relative feed value of inflorescences is significantly higher than that of the leaves and stems.

The total above-ground biomass of *S. rebaudiana* contains 35.52% neutral detergent fibers, 31.18% acid detergent fibers (Table 4). Digestibility is high - 69.79%. The amount of gas formed - 225.83 - 246.7 dm/ml is medium and the rate of decomposition is medium between concentrated forages, where it is very high, and roughage (straw) where it is very low. Energy nutrition expressed as metabolizable energy - 9.77 MJ/kg DM is medium. The relative feed value of 256.26 is at very good rate. Digestibility and energy nutrition characterize *S. rebaudiana* as high-quality, bulky feed for ruminants.

The comparison between the data obtained on fiber components, digestibility and metabolizable energy of *S. rebaudiana* and on other herbage fodder shows that by content of NDF and ADF, the biomass of *S. rebaudiana* is close to that of the perennial cereal and legume herbage fodder (Todorov et al., 2007).

Naydenova et al. (2005, 2014) obtained similar results about fiber components in new varieties of perennial cereal herbage grown separately and in mixture: NDF - 54.5%; ADF - 29.97% and digestibility - 71.88%. Higher digestibility - 80.12% was found in new varieties of spring forage peas (Naydenova and Todorova, 2009).

The values for NDF, ADF and digestibility of *S. rebaudiana* obtained in this study differ from the ones by Kafilzadeh and Maleki (2012) for straw of four varieties of chickpeas (*Cicer arietinum* L.). Structural fiber components have lower values in *S. rebaudiana* biomass and its digestibility is higher.

Table 4
Content of fibre components, digestibility, *in vitro* gas production, energy value and relative feed value of *S. rebaudiana*

Parameters	Leaves	Stems	Blossom	Average
NDF, %	25.199	62.513	18.849	35.52
ADF, %	25.578	49.034	18.936	31.18
Digestible dry mater, %	78.893	48.269	82.214	69.79
Gas production, 24 h, dm/ml	306.36	186.03	185.11	225.83
Gas production, 48 h, dm/ml	331.34	195.91	212.85	246.70
Speed of degradation and gas formation (Gas production 48 h/Gas production 24 h), %	108.15	105.31	114.99	109.24
ME, MJ/kg DM	12.12	8.60	8.58	9.77
RFV, Standard *Medicago sativa* in bloom - 100 units	291.24	71.80	405.74	256.26

Comparison with results for *in vitro* gas production in perennial meadow grasses and alfalfa, obtained by Yancheva et al. (1997a, 1997b) shows that by the amount of gas formed, *S. rebaudiana* biomass approaches meadow hay and perennial legume herbage. This distinguishes *S. rebaudiana* from rough fodder plants and places it along with the valuable fodder plants such as alfalfa and perennial cereals.

The relatively small difference in the amount of gas formed in 48 hours compared to in 24 hours - 109.24%, confirms that *S. rebaudiana* biomass has good nutritional value and is decomposed in the same way as high quality feed. Unlike *S. rebaudiana,* in roughages this indicator is high - over 140%. This indicates that they decompose slowly at the beginning and faster towards the 48th hour. The difficult initial decomposition of NDF and ADF is due to their higher content in these forages.

The correlation analysis of the relationship between the chemical composition, detergent fiber content, digestibility, gas formation, energy nutrition, and relative feed value parameters shows that there is very high correlation between the amount of gas generated in 24 hours and the exchange energy - r = 0.999 (Table 5). There is also a high correlation between the amount of gas generated in 48 hours and the exchange energy - r = 0.993. There is high positive correla-

Table 5
Correlations among the parameters

Parameters	NDF, %	ADF, %	DDM, %	Gas prod 24 h, dm/ml	Gas prod 48 h, dm/ml	Gas prod 48 h/ Gas prod 24 h, %	ME, MJ/kg	RFV
NDF, %	1.000	0.997	-0.612	-0.373	-0.483	0.824	-0.374	0.999
ADF, %		1.000	-0.671	-0.301	-0.414	0.865	-0.302	0.993
DDM, %			1.000	-0.505	-0.397	-0.952	-0.504	-0.582
Gas production 24 h, dm/ml				1.000	0.993	0.218	0.999	-0.408
Gas production 48 h, dm/ml					1.000	0.098	0.993	-0.516
Gas production 48 h/ Gas production 24 h, %						1.000	0.217	0.802
ME, MJ/kg							1.000	-0.409
RFV								1.000

Table 6
Regression equations

Parameters	R^2	SEE	F	P<
Metabolizable energy, MJ/kg DM				
Y = 3.167+0.0292*G, 24 h	0.999	0.0115	350380.9	0.001075
Relative feed value				
Y = -684.2113+48.847*NDF	0.999	75.3000	685.5954	0.024302
Acid detergent fibre, %				
Y = 7.444+0.668*NDF	0.997	2.0700	169.7371	0.048769

R^2 – coefficient of determination; SEE – standard error; F – ratio, P < – statistical significance;
G, 24 h – gas production in 24 h; NDF – neutral detergent fibre, %

tion between the neutral detergent fibers and the relative feed value r = 0.999, between the neutral detergent fibers and acid detergent fibers - r = 0.997.

On the basis of correlation dependencies, regression equations have been developed for indicative estimation of some parameters related to exchange energy, relative feed value and acid detergent fibers. Exchange energy can be determined with good accuracy by the amount of gas generated in 24 hours. The determination coefficient is $R^2 = 0.999$ (Table 6).

The relative feed value can be determined by neutral detergent fibers. The determination coefficient is $R^2 = 0.999$. Acid detergent fiber content can also be indicatively determined by the amount of neutral detergent fibers. The determination coefficient is $R^2 = 0.997$. All equations are linear and are statistically proven at $P<0.05$-0.001.

Conclusions

Dry matter of *S. rebaudiana* contains an average of: crude protein - 86.33 g/kg; crude fat - 11.79 g/kg; crude fiber - 284.68 g/kg; ash - 96.56 g/kg; and NFE - 520.64. The leaves contain more protein, fats, ash and NFE and less fiber than the stems.

The high content of nitrogen, calcium, magnesium, iron, manganese and zinc in the leaf biomass of *S. rebaudiana* is a prerequisite for the beneficial effect of these mineral substances on metabolism and the health effects on animals.

The content of structural fiber components is higher in stems of *S. rebaudiana* and lower in leaves, and the amount of gas formed in the rumen of ruminants is lower in stems.

Digestibility, energy nutrition and relative feed value are higher for leaves of *S. rebaudiana* compared to stems. These indicators characterize *S. rebaudiana* as high-quality bulky feed for ruminants.

Between the relative feed value and the neutral detergent fiber content there is very high correlation - r = 0.999. This allows the relative feed value to be determined indicatively by regression equations through neutral detergent fibers.

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