SOME BIOLOGICAL FEATURES AND THE BIOCHEMICAL COMPOSITION OF *POLYGONUM SACHALINENSE* IN MOLDOVA

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Abstract: *The mobilization and acclimatization of new herbaceous plant species, plant breeding and development of technological elements in order to obtain the maximum biomass yield for further processing into various types of products, are important research activities. We investigated some biological features and the biochemical composition of the biomass of a non traditional crop, giant knotweed – Polygonum sachalinense cv. "Gigant", and evaluated the green mass nutritional value for animals and biomethane productivity for renewable energy. It has been determined that, in the 3rd year, the cv. "Gigant" was characterised by high growth and development rates; the green mass yield reached 12.3 kg/m^2; the calculated fodder productivity – 22 t/ha nutritive units and 3700 kg/ha digestible protein; it can be a good source of essential amino acids in the livestock feed, exceeding substantially traditional crops, such as alfalfa – Medicago sativa. The biochemical methane production potential 249-253* L/kg VS.

Keywords: amino acids, biological features, biomethane productivity, nutritional value, *Polygonum sachalinense*.

INTRODUCTION

The rapid rates of population growth, the reduction of agricultural lands and the socio-economic development require identification of new sources to overcome global problems caused by climate change, desertification, soil salinization, environmental pollution and food and energy shortages.

The scientific research conducted in the National Botanical Garden (Institute) of Moldova, during the last decades was focused on the mobilization, selection and implementation of non-traditional plant species with multiple uses. An important role has been played by perennial herbaceous plants that can be cultivated in marginal, polluted and degraded soils, providing vegetal fodder for animals and, besides, can become a stable source of biomass for different industries [TELEUŢĂ AND ŢÎŢEI, 2016].

The species, giant knotweed or Sakhalin knotweed, *Polygonum sachalinense* (sin. *Fallopia sachalinensis* Ronse Decr., *Pleuropterus sachalinensis* Moldenke, *Reynoutria sachalinensis* Nakai, *Tiniaria sachalinensis* Janch.), fam. *Polygonaceae* Juss., is native to eastern Russia and northern Japan, herbaceous perennial plant growing up to 2–4 m tall, with strong, extensively spreading rhizomes forming large clonal colonies, was introduced in Europe in 1860 in the Botanical Garden of St. Petersburg, by the botanist Friedrich Karl Schmidt, being implemented as a crop during the 20th century due to its tolerance to the soil climatic factors and stable productivity, serving as fodder from early spring until late autumn. This species is a natural source of secondary metabolite compounds, which possesses biological activity [IVANOVA AND TITEI, 2014]. From different plants organs, there have been isolated the compounds of stilbene group (resveratrol and its glycoside derivatives) and flavonoids, phenylpropanoids and its glycoside derivatives [ZHANG ET AL., 2005; KOVÁŘOVÁ ET AL, 2011]. Currently, the species *Polygonum sachalinense* is studied in different universities as crops with multiple use [LEHTOMÄKI, 2006; KARKUSOVA, 2014; STOLARSKI ET AL., 2014)

The objective of this research was to evaluate some biological features and the biochemical composition of the biomass of *Polygonum sachalinense* grown under the conditions of Moldova as fodder for animals and substrate for biomethane production.

MATERIALS AND METHODS

The cultivar "*Gigant*" of giant knotweed, *Polygonum sachalinense*, created in the National Botanical Garden (Institut), registered in 2012in the Catalogue of Plant Varieties** and patented in 2016, by the State Agency on Intellectual Property (AGEPI) of the Republic of Moldova patent nr. 205/31.05.2016*, served as research subject. The experiments were carried out on the experimental plot of the Botanical Garden. The *Polygonum sachalinense* plantlets were planted in spring. The scientific research on plant growth, development and productivity was performed according to methodological indications [NOVOSELOV ET AL., 1983]. The green mass was harvested manually. The 3-year-old *Polygonum sachalinense* plants were mowed for the 1st cut at the end of May, but the control, alfalfa *Medicago sativa*, in the fourth year of growing – in the same period, end of May. Green mass productivity was determined by weighing. At the Institute of Biotechnology in Animal Husbandry and Veterinary Medicine, the dry matter content was detected by drying samples up to constant weight at 105 °C; crude protein – by Kjeldahl method; crude fat – by Soxhlet method; crude cellulose – by Van Soest method; ash – in muffle furnace at 550 °C; nitrogen-free extract (NFE) was mathematically appreciated, as the difference between organic matter values and analytically assessed organic compounds; organic dry matter, or volatile solids (VS), was calculated through differentiation, the crude ash being subtracted from dry matter, the nutritional value was calculated according to the methodical indications [PETUKHOV ET AL., 1989]. Amino acid analysis was performed after hydrolysis 6M HCl with a T 339 Amino Acid Analyzer. The method of Van Soest was used for fibre fraction content analysis, at the University of Agricultural Sciences and Veterinary Medicine, Iaşi, the samples were analysed to determine the cell wall content applying the standard methods and estimating relative feed value. The carbon content of the substrates was obtained from data on volatile solids, using an empirical equation reported by BADGER ET AL.,2014, the gas forming potential of nutrients was determined according to BASERGA, 1998, corrected by the nutrient digestibility.

The biochemical biogas potential (Yb) and methane potential (Ym) were calculated according to the equations of DANDIKAS ET AL., 2014, based on the chemical compounds – acid detergent lignin (ADL) and hemicellulose (HC) values:

biogas potential Yb=727+0.25 HC-3.93 ADL

methane potential Ym=371+0.13HC-2.00ADL

RESULTS AND DISCUSSIONS

As a result of the investigated biological features of *Polygonum sachalinense*, it was found that, in the first 28-34 days after planting, the plantlets of both species grew slowly, but the root system developed more intensively. Later, the growth rate of *Polygonum sachalinense* increased; an erect stem developed and reached a height of 60 cm at the end of July, having 6-7 internodes with dark green leaves, which grew 18-25 cm long and 10-12 cm wide. In the following 2 months, the central stem branched, producing first, second and third degree branched shoots, on which smaller leaves developed, some shoots gave rise to flower buds. By the end of the growing season, the plants grew 155-170 cm tall, the first internodes were lignified and the taproot reached 2 m, on the underground side of the stem, there were dormant buds, which would contribute to the development of new shoots and the formation of the bush in the next year. The green mass yield of giant knotweed may be 20-25 t/ha in the first year.

The resumption of growth of giant knotweed, in the 3rd year, started 5-7 days earlier and the development of shoots began 7-9 days later in comparison with alfalfa. The growth rate of the shoots of giant knotweed was very high, reaching up to 9 cm/day. Thus, by the end of May, the erect, tubular stems with 20-22 leaves reached over 300 cm in height, their branching began, and 2-3 leaves from the bottom part of the plant entered the senility stage and fell, reducing the foliage to 38 %. The green mass yield obtained in this period constituted 7.0 kg/m^2 or 1.49 kg/m^2 dry matter. Giant knotweed recovered quickly after being cut and, until the end of the growing season, it was mowed in July and at the end of September. The harvested green mass obtained after the cuts contained over 47-57 % leaves and 23-27 % dry matter. The annual productivity was 12.3 kg/m^2 green mass or 2.52 kg/m^2 dry matter.

It is well known that domestic animals, in order to maintain their vital functions and to provide different animal products, are in constant need of an exogenous supply of nutrients that they receive from fodder. The results of the study on the biochemical composition of the dry matter from the harvested green mass are presented in Table 1. Among the nutrients present in fodder, the most important ones are the proteins, which cannot be replaced by any other nutrients. The amount of crude protein contained in giant knotweed was very high (21.88 %), as compared with alfalfa (15.08 %). The same tendency applied to the content of fats. The biomass of *Polygonum sachalinense* was characterised by optimal content of crude cellulose (27.66 %), low content of nitrogen free extract (39.01 %) and mineral substances (7.53 %), as compared with the control.

Table 1

The biochemical composition and the nutritional value of cv. "*Gigant*" of *Polygonum sachalinense*, 1first harvest

Indices	*Medicago sativa* (control)	*Polygonum sachalinense*
crude protein, %	15.08	21.88
crude fats, %.	1.76	3.92
crude cellulose, %.	32.43	27.66
nitrogen free extractive substances, %	41.39	39.01
mineral substances, %	9.34	7.53
1 kg forage contains:		
dry matter, g	209.5	216.70
nutritive units	0.17	0.19
metabolizable energy for cattle, MJ/kg	1.84	2.00
digestible protein, g	23.69	28.91
digestible protein, g/nutritive unit	137.0	150.7

In Russia, numerous studies on giant knotweed have been conducted. According to FILATOVA ET AL., 2005, the biochemical composition of dry matter of *Polygonum sachalinense* in Perm region, mowed in June was, in leaves, 22.00 % protein, 13.41 % sugars, 3.8 % pectin, and 322 %/mg Vitamin C, but in shoots – 18.50 %, 9.75 %, 8.29 % and 74 %/mg, respectively. KARKUSOVA, 2014, remarked that the biomass of giant knotweed, in the conditions of Alania, Russia, in the shoot development stage, contained 9.58 % sugars, 24.40 % protein, 3.60 % fats, 19.20 % cellulose and 4.9 % ash, but in the flowering stage – 16.84 %, 3.78 %, 1.60 %, 38.31 % and 6.07 %, respectively.

The production of animal protein has an essential function in livestock farming. The quality of protein supply is determined by its potential to cover the physiological requirements in terms of amino acids, for maintenance and performance (growth, reproduction, production of milk and meat). The determination of the amino acid composition of proteins in forage is of great importance, the amino acid level is one of the indicators of the nutritional value of fodder. Sixteen amino acids were found in the examined samples (Table 2), including all the essential amino acids: lysine, isoleucine, leucine, methionine, phenylalanine, valine, threonine, except for tryptophan, which was decomposed during the acidic hydrolysis of fodder samples. Cysteine was found in very low concentration and there were only traces in the studied samples. The protein quality is determined by the ratio of certain amino acids, which provide the biological value of the feed. The efficiency of using vegetal protein in animal feed production strongly depends on the content of essential amino acids in various crops and the composition of compound feedstuffs. By analyzing the amino acid content, we found that the forage obtained from *Polygonum sachalinense* contained a high amount of essential amino acids (48.18 g/kg), as compared with *Medicago sativa* (31.71 g/kg), being in direct correlation with crude protein content. We would like to mention that, among nonessential amino acids, glutamine and asparagine were found in the highest concentration. It was found that, in the dry matter of *Polygonum sachalinense*, there was a very high concentration of glutamine and a slightly lower content of asparagine, in comparison with *Medicago sativa*. Besides, a low amount of histidine was observed in giant knotweed forage.

The content of organic substances and their biochemical composition influence the nutritional and energy value of fodder. Thus, 100 kg of giant knotweed natural fodder contains 19.2 nutritive units, 199 MJ/kg metabolizable energy, 1044 g essential amino acids, including 35 g methionine and 163 g lysine, but alfalfa – 17.3 nutritive units, 184 MJ/kg metabolizable energy, 664 g essential amino acids, 23 g methionine and 107 g lysine, respectively. The digestible protein content of a nutritive unit meets the zootechnical standards - 150.7 g.

Table 2

The content of amino acids in fodder of cv. "*Gigant*" of *Polygonum sachalinense* (g/kg dry matter)

Amino acids	*Medicago sativa* (control)	*Polygonum sachalinense*
Asparagine	21.05	12.32
Threonine	5.00	7.18
Serine	6.70	5.76
Glutamine	11.12	21.75
Proline	6.35	7.04
Glycine	4.95	9.86
Alanine	5.67	8.97
Valine	5.14	8.17
Methionine	1.10	1.62
Isoleucine	3.43	6.03
Leucine	6.90	11.34
Tyrosine	3.78	3.48
Phenylalanine	5.01	6.32
Histidine	4.84	3.93
Lysine	5.13	7.52
Arginine	3.24	5.13
The sum of essential amino acids	31.71	48.18

The knowledge of the distribution patterns of carbohydrates in herbage biomass could support harvest management. The analyses concerning the assessment of the cell wall composition allowed the calculation of some parameters for the estimation of the feed quality and the results are presented in Table 3. The obtained data showed that the concentrations of carbohydrates and their compositional content in natural fodder differed significantly, depending on the species. It was found that, in giant knotweed fodder, there were optimal concentrations of acid detergent (332.9 g/kg) and neutral detergent fibres (405.6 g/kg), but lower in comparison with *Medicago sativa*, which influenced positively the digestible dry matter content and the relative feed value.

Table 3

The cell wall composition and the relative feed value of cv. "*Gigant*" of *Polygonum sachalinense*

Indices	*Medicago sativa* (control)	*Polygonum sachalinense*
Acid detergent fibre (ADF), %	41.17	33.29
Neutral detergent fibre (NDF), %	53.81	40.56
Digestible dry matter	56.83	62.97
Dry matter intake	2.23	2.96
Relative feed value (RFV)	98	144

The giant knotweed natural fodder obtained from the first harvest, with calculated relative feed value 144, can be classified as class 1, but alfalfa, in the fourth year of growth, with relative feed value 98 – as class 3, according to the criteria of the American Quality standards of grasses, legumes and grasses-legumes mixtures.

The annual productivity of giant knotweed can reach 22 t/ha nutritive units and 3700 kg/ha digestible protein.

Table 4

The gas forming potential of nutrients and the biochemical methane potential of *Polygonum sachalinense*

Indices	*Medicago sativa* (control)	*Polygonum sachalinense*
Carbon nitrogen ratio (C/N)	21	15
Digestible protein, g/kg	113.40	133.40
Digestible fats, g/kg	8.10	21.2
Digestible carbohydrates, g/kg	454.50	430.30
Gas forming potential of nutrients, l/kg VS	449	459
Methane content, %	53.9	55.2
Acid detergent lignin (ADL),%	6.85	6.60
Cellulose, %	34.32	26.69
Hemicellulose, %	12.64	7.27
Biogas potential, L/kg VS	489	486
Biomethane potential, L/kg VS	250	249

The use of phytomass to produce biogas through anaerobic digestion is a successful method applied in many countries around the world, being considered one of the most attractive solutions for the production of renewable energy, and the digestate can be used as a fertilizer for soil and plants, according to the principles of organic farming. It is well known that the quality of the substrate for the production of biogas by anaerobic digestion is closely related to the organic matter content, the carbon and nitrogen ratio and the degree of digestion nutrient by methanogenic bacteria. It was determined (Table 4) that the biomass of giant knotweed, at the first harvest, contained a moderate amount of digestible nutrients (584.9 g/kg) and acid detergent lignin (66.0 g/kg), but there was lower carbon and nitrogen ratio (15/1) and hemicellulose content (72.7 g/kg), in comparison with alfalfa. The gas forming potential of nutrients from giant knotweed biomass reached 459 l/kg VS or 253 l/kg methane. The biochemical methane production potential based on the chemical compounds – acid detergent lignin and hemicellulose of *Polygonum sachalinense* reached 249 l/kg, at the same level as *Medicago sativa.* The obtained values are in good accordance to LEHTOMÄKI, 2006 who reported, 15t/ha annual dry matter yield, 3800 m^3/ha methane and gross energy potential 36 MWh/ ha of giant knotweed.

CONCLUSIONS

The cv. "*Gigant*" of giant knotweed, *Polygonum sachalinense*, is a promising fodder and energy crop, which develops well under the climatic conditions of the Republic of Moldova.

The green mass yield of cv. "*Gigant*", at the first harvest (end of May), was 7.0 kg/m^2, the plants recovered quickly after being cut, they could be mowed 2-3 times per year, and the maximum yield reached 12.3 kg/m^2.

100 kg of natural fodder contained 19.2 nutritive units and 199 MJ/kg metabolizable energy for cattle; 1044 g essential amino acids, including 35 g methionine and 163 g lysine. The relative feed value of giant knotweed was 144 and it was classified as class 1; a nutritive unit contained 150.7 g digestible protein with high concentrations of limiting amino acids, thus it met the zootechnical standards.

The gas forming potential of digestible nutrients reached 459 L/kg with 55.2 % methane, but the biogas yields based on the concentration of acid detergent lignin and hemicellulose – 486 L/kg with 51.2 % methane.

Thanks to the high biomass productivity, cv. "*Gigant*" of giant knotweed can reach a potential of 22 t/ha nutritive units and 3700 kg/ha.

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