AGROECONOMIC VALUE OF FINE-LEAF VETCH, *VICIA TENUIFOLIA*, UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

Fabaceae species play a key role in ecosystem functioning through their capacity to fix atmospheric nitrogen and mobilize phosphorus, produce food rich in nitrogenous compounds, improve forage quality and performance in animal production, besides, they have valuable medicinal properties and can be used for multiple purposes in biorefineries. The local ecotype of fine-leaf vetch, *Vicia tenuifolia*, maintained in monoculture, served as object of study. The 3-year-old *Vicia tenuifolia* started vegetating in the second half of March; the plants had high growth and development rates. In the middle of May, fine-leaf vetch plants bloomed and the shoots were up to 100 cm long, the fresh mass yield reached 4.18 kg/m^2, with higher proportion of leaves (58 %) in comparison with alfalfa, *Medicago sativa.* The results of the study on the biochemical composition of the dry matter in the harvested fresh mass and hay prepared from *Vicia tenuifolia*: 23.50 % and 21.94 % raw protein, 3.65 % and 1.49 % raw fats, 29.08% and 31.78 % raw cellulose, 37.56 % and 36.71 % nitrogen-free extract, 8.10 % and 8.08 % ash. The nutritional and energy value of *Vicia tenuifolia* forage: 100 kg fresh mass reached 17.93 nutritive units, 3.45 kg digestible protein and 189.51 MJ metabolizable energy, but 100 kg of hay had 75.9 nutritive units, 14.32 kg digestible protein and 827 MJ metabolizable energy, respectively. The gas-producing potential of fermentable organic matter of *Vicia tenuifolia* reached 503 L/kg or 284 L/kg methane yields in green mass substrate and 422 L/kg or 235 L/kg methane yields in hay substrate, exceeding *Medicago sativa* substrates.

Keywords: biological peculiarities, biochemical composition, biomethane, forage value, *Vicia tenuifolia*

Population growth makes it necessary to increase food and energy supplies. The more efficient use of natural resources, such as soil, light, water, nutrients and energy, is and will be one of the great challenges of the modern agriculture.

Over the last decades, global industrialization has caused a considerable increase in energy consumption, with the Earth's fossil energy resources being exploited to maximum in this demand, with negative impact on climate changes.

World plant biodiversity, its conservation and prospects for practical use are becoming increasingly pressing problems in the 21st century. There are about 50 000 edible spontaneous plant species, but a small part of these species have been domesticated and cultivated.

Reducing the use of chemical fertilizers and their economic and ecological costs is one of the greatest agronomic and environmental challenges.

Fabaceae species play a key role in ecosystem functioning through their capacity to fix atmospheric nitrogen via their symbiosis with *Rhizobium* bacteria. These plants are also able to acidify their rhizosphere and thus, mobilize phosphorus, increase the humus and nutrient content of the soil, improve land desalinization, soil structure and its biological activity. They are pioneer plants helping to rehabilitate damaged lands, and their deep roots mine minerals, which enrich and stabilize soils. Many legumes are used as cheap sources of food rich in nitrogenous compounds, partially replacing meat and dairy products in the human diet. They also improve forage quality and performance in animal production, have valuable medicinal properties, are used for various purposes in biorefineries and, besides, they are excellent honey plants. Domestication and development of new or alternative legume crops could increase crop diversity and reduce human reliance on only a few major food crops, and if done thoughtfully, it could improve the resilience and sustainability of food production. Perennial *Fabaceae* species may have distinct advantages over annuals (Stoddard F.L., 2013; Stinner P.W., 2015).

The genus *Vicia* L. belongs to the tribe *Vicieae* of the *Fabaceae* family, includes about 230 species, which are distributed throughout the temperate regions of Europe, Asia, North America

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and South America (Kupicha F. K., 1976; Abozeid A., *et al*, 2017). *Vicia* species have different characteristics in terms of growth habit, life cycle and climatic adaptation. About 40 species have economic importance, some of them are food crops, but more than a dozen are forage and cover crops.

In the spontaneous flora of the Republic of Moldova, there are 18 species of genus *Vicia*, including 6 perennial species (Negru A., 2007).

The most promising perennial forage plant of the genus *Vicia* for cultivation is fine-leaf or bramble vetch, *Vicia tenuifolia* Roth., due to its high productivity, palatability and upright growth habit. It can by used as perennial component for the long-term establishment of artificial grasslands. *Vicia tenuifolia* Roth. (syn. *V. antiqua* Grossh., *V. boissieri* Freyn, *V. brachytropis* Kar. & Kir. *V. cracca* subsp. *tenuifolia* (Roth) Gaudin, *V. tenuifolia* subsp. *boissieri* (Freyn) Radzhi, *V. variabilis* (Freyn & Sint.) Dinsm., *Cracca tenuifolia* (Roth) Gren. & Godr. *Ervum tenuifolium* (Roth) Trautv.) is a perennial herb with well-branched, numerous, rigid, slightly pubescent, almost upright stems, 50-90 cm tall. Its leaves are compound pinnate, with 8-12 pairs of opposite leaflets, 2.5-3.0 cm long and 2-3 mm wide, lanceolate, covered by hairs on both sides, greenish-gray. The racemes are laxiflorous, with 15-20 flowers, pale blue or violet, 14-15 mm long. It produces elongated pods, 20-30 mm long and 5-7 mm wide, with 3-5 brown seeds. It is cross-pollinated mainly by bees, blossoms in June-July; the seeds ripen in July-August. It has a chromosome number of 2n=24. This species occurs on open dry slopes, in meadows, in shrublands, along wood edges, rivers and roads (especially railway roads), in mountains up to the middle zone. In terms of xerophilicity, it is not inferior to sainfoin. It does not tolerate flooding and excess moisture in the soil (Dzyubenko N. I. & Dzyubenko E. A., 2008).

This research was aimed at evaluating some biological peculiarities, the biochemical composition of the local ecotype of fine-leaf vetch, *Vicia tenuifolia*, and the possibility of using phytomass as fodder for animals and as feedstock for biogas production.

MATERIALS AND METHODS

The local ecotype of fine-leaf vetch, *Vicia tenuifolia*, grown in monoculture on experimental land in "Alexandru Ciubotaru" National Botanical Garden (Institute), latitude 46°58′25.7″ and longitude N28°52′57.8″E, served as subject of the research, and the traditional leguminous fodder crop – alfalfa, *Medicago sativa*, was used as control. The growth and development of plants as well as their productivity were assessed according to methodical indications (Novoselov Y. K. *et al, 1983*). The green mass was harvested manually; the first cut was done in the budding-flowering stage. The green mass productivity was determined by weighing the yield obtained from a harvested area of 10 m^2, which was afterwards transformed per hectare. The leaves/stems ratio was determined by separating the leaves, buds and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The analyses were performed in the Laboratory of Nutrition and Feed Technology of 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 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, according to standard laboratory procedures for forage quality analysis (Petukhov E.A. *et al, 1989*).

The carbon content of the substrates was determined from data on volatile solids, using an empirical equation reported by Badger C.M. *et. al., 1979*.

The biogas and biomethane, litre per kg of volatile solids (L/kg VS), were calculated using the gas forming potential of nutrients corrected with its digestible index according to Baserga U**.**, 1998.

RESULTS AND DISCUSSIONS

We could mention, as a result of the phenological observations, that the studied perennial forage legumes are characterised by different growth and development rates, in the first growing season. Thus, it was determined that the plantlets of *Vicia tenuifolia* emerged non uniformly at the soil surface, 23 days after sowing, 16 days later as compared with the control, *Medicago sativa*. It can be noted that the root emerged first, then the shoot with leaves initially pointing downwards, the cotyledons remained below ground, at the first node, the first leaf fully unfolded with one pair leaflets was formed on the 4th-6th day after seeding. In *Vicia tenuifolia*, along with the prominent main taproot, rhizomes also developed well, so the system can be called taproot-rhizome system. The main root, at the time of the appearance of the first leaf, reached 12-14 cm. Below the root collar (1-2 cm), the main root of most plants was abundantly branched. With the appearance of the first 2-3 leaves, on the main root, nodules with nitrogen-fixing bacteria appeared in large numbers, which subsequently developed on the entire root system.

From the embryonic bud, a shoot grew and, emerging at the surface of the soil, formed the first stem. The buds at the root collar developed aboveground (stems) and underground (rhizomes) shoots. The root collar of perennial plants is the part of the base of the stem from the cotyledons to the soil surface. Usually, the root collar consisted of 3-4 nodes. Due to the intensive diminution of the root collar, the internodes almost merged in the first year of life. Because of this, the top node, being initially at the surface, by the end of the year, was in the soil at a depth of 1.5-2.5 cm. In the first year of life, during the second half of the summer, rhizomes grew from the buds of the root collar and extended to 10-42 cm in the soil. Reaching the soil surface, they generated green shoots. At the same time, wintering buds developed on the root collar of the main root and on the base of the stems grown from rhizomes.

In the first year of life, the main root grew rapidly into the soil, extended lower into the subsoil, reaching 100 cm.

After the appearance of 2-3 leaves, the stem was formed and, after 25-33 days, it branched abundantly from the base. The plants of *Vicia tenuifolia* are characterized by very slow growth and development of the aerial part, so, they produced only vegetative shoots, which reached up to 30 cm, but the tap root, if not inhibited by subsoil constraints, reached to 100 cm.

In the second year, *Vicia tenuifolia* developed 3-5 aerial shoots and new rhizomes from wintering buds. Each branching node of the rhizomes produced its tap root. Thus, a multi-node root system with several root collars and their tap roots was formed. The number of stems increased to 12-17, during the growing season, thanks to the newly formed nodes of the rhizomes. The length of shoots reached 70-83 cm in the full flowering phase. In the second year of life, *Vicia tenuifolia* reached the fruiting phase and the seeds ripened in July.

In the following years, the leguminous species, studied by us, resumed its growth and development in spring, in the second half of March, when temperatures above 5-10 °C were established. Multiple lateral branches of *Vicia tenuifolia* developed from buds on the basal part of previous year's shoots. Fine-leaf vetch is a slender, initially erect plant that has thin stems and trailing habit when in advanced growth stages, distinguished by faster grow and development rates. Thus, by the end of April (Tab. 1), the 3-year-old plants of *Vicia tenuifolia* reached 50 cm in height, while the control – about 31 cm. The budding stage of *Vicia tenuifolia* began 11 days earlier in comparison with *Medicago sativa*, the flowering stage – 15 days earlier.

In the middle of May, fine-leaf vetch plants bloomed and the shoots were up to 100 cm long, the green mass was harvested. The local ecotype of *Vicia tenuifolia*, was characterized by higher fresh mass productivity (4.18 kg/m^2), but lower content of dry matter in the harvested mass (18.3 %), as compared with *Medicago sativa*.

The leaves contained larger proportions of non-lignified tissues and had faster rates of cell wall digestion than stems. Legume leaves are much more quickly digested than grass leaves because of the differences in tissue lignifications of these forages. Generally, leaf proportion has a great impact on forage quality and hence should be considered when selecting the forage crop. The highest proportion of leaves was in the forage obtained from *Vicia tenuifolia*, leaves accounted for 58 % of the total plant mass. In other studies on the productivity of *Vicia tenuifolia*, it was mentioned that under the climatic conditions of Lower Volga region, Russia, the green mass varied from 12-22 t/ha, on non irrigated land, up to 55 t/ha, on irrigated land (Maevsky V.V. *et al, 2003*) but, in Armenia, the subspecies *Vicia variabilis*, under the conditions of dry mountain plain, reached up to 65-70 cm in height and the hay yield varied from 3.5 to 4.0 t/ha (Gonean G.G., 1961).

Table 1

Some agrobiological peculiarities of *Vicia tenuifolia*

Indicators	*Medicago sativa*	*Vicia tenuifolia*
Resumed vegetation up to:		
- budding, days	74	63
- flowering, days	87	72
Plant height, cm		
- at the end of April	30.8	50.4
- at the flowering stage	89.3	105.3
The yield (1st harvest):		
- fresh mass, kg/m^2	3.44	4.18
- dry matter, kg/m^2	0.68	0.76
The leaf content, %	42	58

One of the basic needs in the mobilisation and use of new and non-traditional plants and achieving optimum performance of livestock is meeting the nutritional needs of livestock in terms of energy, protein, minerals and vitamins. This is only possible when the quality of vegetal forage, in terms of chemical composition, is known for each region. The results of the study on the biochemical composition of the dry matter from the harvested green mass and prepared hay are presented in *table 2*. It is well known that the crude protein is used during growth, in the production of new tissues and in the repair of damaged ones. Its nitrogen enters into the fibrin of blood, the albumen of muscle, the gelatin of bones and tendon, the casein of milk, and to a certain extent into the surplus fat. Hence, the digestible crude protein in the fodder is its most important constituent. The natural fodder of *Vicia tenuifolia*, is characterised by very high content of raw protein, reaching 23.50 % of dry matter, in comparison with 16.50 % in *Medicago sativa.* The same trend applies to hay.

Fats are the main source of energy for animals because they are necessary for the organism in order to ensure the normal development of vital processes and transportation of soluble vitamins in fatty acids and it also contributes to the accumulation of fat in milk. The natural fodder of *Vicia tenuifolia* contains a high amount of raw fats (3.65 %), greatly exceeding *Medicago sativa.* It was found that during the preparation of hay, the raw fat content decreased. It decreased significantly in the hay of *Vicia tenuifolia* (1.49%) in comparison with *Medicago sativa* and, as a result, there was about the same amount of raw fats in the hay of both species.

Following the process of digestion of food by herbivorous animals, the carbohydrates become a source of heat and energy, and any surplus that remains above the immediate needs is stored up in the animal's body as fat. The content of raw cellulose is quite low in the dry matter from the green fodder and in the hay of *Vicia tenuifolia* (29.08-31.78 %). We also mention that the optimal cellulose content has a beneficial effect on the synthesis of protein substances in the rumen of animals and on the reduction of the nitrate content. The amount of nitrogen-free extract in the fodder of fine-leaf vetch is at the same level in alfalfa.

The concentration of minerals in forage varies due to factors like botanical species, plant developmental stage, morphological fractions, climatic conditions, soil characteristics and fertilization regime. Minerals are required in reproductive process because of their role in maintenance, metabolism and growth. Mineral deficiencies are likely to lead to depressed feed intake, forage utilization and subsequently poor animal performance. Optimal ash content was found in the leguminous species studied by us. The average ash content was lower in *Vicia tenuifolia* hay, but in green fodder – at the same level as in *Medicago sativa.*

Table 2

Biochemical composition and nutritional value of *Vicia tenuifolia* fodder

Indices	*Green fodder*		*Hay*	
	Medicago sativa	*Vicia tenuifolia*	*Medicago sativa*	*Vicia tenuifolia*
Biochemical composition:				
- raw protein, % d.m.	16.50	23.50	15.10	21.94
- raw fat, % d.m.	2.59	3.65	1.57	1.49
- raw cellulose, % d.m.	34.48	29.08	36.26	31.78
- nitrogen free extracts, % d.m.	37.95	37.53	37.77	36.71
- minerals, % d.m.	8.48	8.10	9.30	8.08
1 kg of forage contains:				
- dry matter, g	198.3	183.3	875.1	867.4
- nutritive units	0.17	0.18	0.73	0.76
- metabolizable energy, MJ	1.75	1.90	7.94	8.27
Digestible protein g/ nut. unit	148	192	153	189

The nutritional and energy value is determined by the biochemical composition and the digestibility of the organic substances from the fodder, which influence the health and the productivity of animals. We can mention that the nutritional and energy value of 100 kg fresh mass of *Vicia tenuifolia* fodder was 17.93 nutritive units, 3.45 kg digestible protein and 189.51 MJ metabolizable energy, but 100 kg hay had 75.9 nutritive units, 14.32 kg digestible protein and 827 MJ metabolizable energy, respectively.

Some authors mentioned various findings about the quality of fine-leaf vetch fodder. Larin, 1951, reported that the nutritive values of *Vicia tenuifolia* ranged from 18.7 to 22.3 % crude protein, 2.4 to 4.2 % fats, 24.2 to 32.6 % cellulose, 35.5 to 42.9 % nitrogen-free extract and 6.9 to 9.1 % ash. Gonean G.G., 1961, remarked that, under the climatic conditions of Armenia, *Vicia variabilis* hay contained 18.26-24.72 % raw protein, 1.95-2.40 % fats, 33.23-35.41 % cellulose, 30.71- 37.31 % nitrogen-free extract, 6.49-8.16 % ash, including 1.75-2.5 % calcium, 0.43-0.70 % phosphorus and 1.63-2.80 % potassium. According to Hadjipanayiotou M. et al, 1983, in Cyprus, the chemical composition of *Vicia tenuifolia* was 9.7 % protein, 2.1 % fat, 40.7 % cellulose, neutral detergent fibre 62 %, acid detergent fibre 48 %, 11.7 % lignin, 7.4 % ash and the digestible energy content was 8.2 MJ/kg dry matter. Maevsky V.V. *et al*, 2011, reported that *Vicia tenuifolia* fodder harvested in small pod stage, in Saratov region, Russia, contained 20.97 % protein, 1.48 % fat, 31.19 % fibre, 39.50 % nitrogen-free extract, 6.86 % ash and 51.83 mg/kg carotene.

The substitution of natural gas by a renewable equivalent is an interesting option to reduce the use of fossil fuels and the accompanying greenhouse gas emissions, as well as from the point of view of security of supply. Biomass gasification may offer a large potential in producing sustainable transportation fuels. The anaerobic digestion process is dependent on the growth of microorganisms. Thus, there is a necessity to supply nutrients in sufficient amounts and at right proportions to sustain an optimal growth of the bacteria and archaea to obtain an efficient biogas production from a given substrate. The fats and carbohydrates of an organic substrate mostly provides carbon, oxygen and hydrogen, while nitrogen and sulfur are supplied via proteins and phosphorus from e.g. nucleic acids phospholipids. The nutrient balance will govern the degree of degradation of the substrate and therefore the biogas production efficiency at a given hydraulic retention time and organic loading rate.

The characteristics of the digestible organic matter and biomethane production potential of *Vicia tenuifolia* biomass are presented in Table 3. The total digestible nutrients contents in substrate depicted major differences between the studied fodder legumes species. Higher values were determined for *Vicia tenuifolia* in green mass substrate (627.9 g/kg dry matter). Substrates with high protein and fat contents are desirable substrates for biogas production as they have high methane potentials. In our research, the highest concentration of digestible proteins were present in *Vicia tenuifolia* substrates, 188.0 g/kg dry matter in green mass and 143.2.0 g/kg dry matter in hay. The highest concentration of fats was found in *Vicia tenuifolia* green mass 27.0 g/kg dry matter, and the lowest in *Medicago sativa* hay 6.1 g/kg dry matter. The similar levels of digestible carbohydrates in green mass substrates of the studied species. The content of digestible carbohydrates in *Vicia tenuifolia* hay was lowest (379.2 g/kg dry matter).

At the beginning of the anaerobic digestion bacteria need nitrogen to start the process, but in later stages nitrogen could act as an inhibitor. Most research results suggest that biogas production is mostly influenced by the carbon to nitrogen (C:N) ratio. In *Vicia tenuifolia* substrates the carbon to nitrogen ratio were lower in comparation with *Medicago sativa* substrates due to the higher nitrogen concentration in the biomass.

The optimal C/N ratio is expected to be in the range 15-25, when the anaerobic digestion process is carried out in a single stage, and for the situation when the process develops in two steps, the optimal C/N ratio will range: for step I: 10-45; for step II: 20-30 (Dobre *et al*, 2014).

The gas-producing potential of fermentable organic matter of *Vicia tenuifolia* reached 542 L/kg or 284 L/kg methane yields in green mass substrate and 526 L/kg or 276 L/kg methane yields in hay substrate, exceeding *Medicago sativa* substrates.

The obtained values of *Vicia tenuifolia* substrates are in good accordance to Ahlberg I. and Nilsson T., 2006, who reported in *Vicia villosa* biomass very high protein content (25.6%) and methane yield (305-343 L/kg). According to Oleskowicz-Popiel P., 2010, practical methane yield of dried vetch *Vicia villosa* was *273* L/kg.

Table 3

The biogas and biomethane production potential of *Vicia tenuifolia* biomass

Indices	*Green mass*		*Hay*	
	Medicago sativa	*Vicia tenuifolia*	*Medicago sativa*	*Vicia tenuifolia*
Organic dry matter, g/kg	915.2	918.0	907.0	919.2
Digestible nutrients, g/kg	574.8	627.9	517.0	531.3
Digestible proteins, g/kg	123.8	188.0	111.7	143.2
Digestible fats, g/kg	11.9	27.0	6.1	8.9
Digestible carbohydrates, g/kg	439.1	437.9	399.2	379.2
Biogas, l/kg ODM	448	503	401	422
Biomethane, l/kg ODM	244	284	217	235
Methane, %	54.5	56.5	54.3	55.7
Ratio carbon/nitrogen	19	14	21	15

CONCLUSIONS

In the first growing season, the plants of *Vicia tenuifolia* are characterized by very slow growth and development of the aerial part, so, they produced only vegetative shoots, which reached up to 30 cm, but the tap root, if not inhibited by subsoil constraints, reached to 100 cm.

In the second year, *Vicia tenuifolia* developed 3-5 aerial shoots and new rhizomes from wintering buds, in the flowering period the shoots reached 70-83 cm.

The 3-year-old *Vicia tenuifolia* plants have accelerated growth and development rates that allow mowing them at the middle of May. The green mass yield reaches 4.18 kg/m^2, but the content of dry matter is low (18.3 %), as compared with *Medicago sativa*.

The dry matter from natural fodder *Vicia tenuifolia* is characterized by very high content of raw protein (23.50 %), and high amount of fats (3.65 %) and optimal cellulose content (29.08%).

The prepared hay was distinguished by homogeneous green colour and pleasant smell, high content of raw protein and lower ash.

The nutritional and energy value of *Vicia tenuifolia* forage: 100 kg fresh mass reached 17.93 nutritive units, 3.45 kg digestible protein and 189.51 MJ metabolizable energy, but 100 kg of hay had 75.9 nutritive units, 14.32 kg digestible protein and 827 MJ metabolizable energy, respectively.

The gas-producing potential of fermentable organic matter of *Vicia tenuifolia* reached in green mass substrate 503 L/kg biogas and 284 L/kg methane, but in hay substrate 422 L/kg biogas and 235 L/kg methane, exceeding *Medicago sativa* substrates.

The local ecotype of *Vicia tenuifolia* could be used as subjects of the research to create new varieties, seed production and restoration of degraded, polluted and eroded land, besides, they can be used for fodder and energy biomass.

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