

SOME BIOLOGICAL FEATURES AND BIOCHEMICAL COMPOSITION OF CROWN VETCH (*CORONILLA VARIA* L.) IN MOLDOVA

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Abstract

Forage legumes are an essential component of agricultural systems in temperate regions of the world, providing high quality animal feed, suitable ground cover, and a valuable source of nitrogen. The crown vetch, *Coronilla varia*, maintained in monoculture, on non irrigated experimental land in the Botanical Garden (I) of the Academy of Sciences of Moldova, served as object of study, the traditional forage legumes: alfalfa, *Medicago sativa* and common sainfoin, *Onobrychis viciifolia* were used as control variants. It has been established that *Coronilla varia* germinates very slowly, in the first year, grows and develops slower than common sainfoin and alfalfa, but in the following years, it starts vegetating 2-3 days earlier. The 3-year-old *Coronilla varia* plants have moderate growth and development rates that allow mowing them at the end of May the green mass yield reaches 3.92 kg/ m², at the same level as sainfoin and by 25% more than alfalfa, but the forage is characterised by a high content of leaves (63-68%) and a low content of dry matter, in comparison with the traditional crops. The chemical composition of *Coronilla varia* dry matter: 14.72% raw protein, 2.81% raw fat, 35.46% raw cellulose, 39.74% nitrogen-free extractive substances and 7.27% minerals. *Coronilla varia* green mass is characterized by high level of potassium and iron, but lower – of magnesium and sodium. The forage value of 1 kg natural forage accounts 0.20 nutritive units, 2.22 Mj metabolizable energy and digestible protein content – 132.10 g /nutritive unit. The calculated methane yield *Coronilla varia* green mass at the first mowing may reach 2311 m³/ha, exceeding *Medicago sativa*. The local ecotype of species *Coronilla varia* could be used for restoring degraded, polluted and eroded land, and also for reseeding and increasing economic value of grasslands.

Key words: biochemical composition, biological features, *Coronilla varia*, forage value, methane yield

Legumes play a crucial role in natural ecosystems, agriculture, and agroforestry, where their ability to fix N in symbiosis makes them excellent colonizers of low-N environments, and also economic and environmentally friendly crops, pasture and tree species. Over 1500 species of legumes (from about a total of 19 400 *Fabaceae* species worldwide) can be used as feed for livestock, although only about 60 species have been developed and widely used as cultivated forages. Legumes from pastures and meadows contribute N to a complex dynamic recycling system, organic matter containing legume proteins may be mineralized in soil, liberating N as nitrates (NO₃) and NH₄ that may be used by grass. They are important in livestock feeding systems because they have the potential to extend the grazing season, increase the quantity of grazed forage and hay, and reduce the amount of N fertilizer needed. Legume feed not only improves forage quality but also increases the intake of the ration, hence, gives better performance in terms of livestock production. Many legume forage crops are also excellent honey plants (Frame J., 2005; Luscher A. et al., 2013; Stoddard F.L., 2013).

The genus *Coronilla* L. contains about 20 species native to Europe and North Africa (Sokoloff D. D. 2003). In the spontaneous flora of the Republic of Moldova, there are 2 species: *Coronilla elegans* Pancic (synonym *C. latifolia* (Hazsl.) Jav.) and *Coronilla varia* L. (synonym *Securigera varia* (L.) Lassen), from these species *Coronilla varia* is the most widespread.

Coronilla varia L. commonly known as crown vetch or purple crown vetch is a herbaceous perennial plant, has spreading to diffuse stems that can measure up 0.3 to 1.8 meters long, deeply tap-rooted with numerous lateral roots, spreading via rhizomes which penetrate the soil to 2 m deep. Leaves are deep green, alternate compound pinnate with 9 to 25 oblong to elliptic leaflets. Leaves are 5-10 cm long and leaflets are 1 cm-2 cm long, have smooth margins with small points at the tips. Racemes are umbrella-shaped, 15-20-floral, located on flower stalks exceeding leaves, variegated in color from white and purple. Seed pods are indehiscent, may reach 2-6 cm long, with 3-7 cylindrical segments each containing one ovate-oblong, yellow-orange to reddish-brown rod-shaped seed, 3-3.5 mm in length and

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1-1.5 mm in width. It has a karyotype $2n = 24$ and a high proportion of seeds is hard. *Coronilla varia* can grow in soils of low fertility, it is tolerant to low temperatures (down to -33°C) and can withstand periods of drought as well as heavy precipitation (up to 165 cm annual precipitation), but is shade-intolerant. In the United States and Canada, *Coronilla varia* has been extensively planted for erosion control along many roads, highways, and disturbed areas. It has also been widely planted for ground cover, mine reclamation, and as a cover crop as it provides nitrogen to soil through its association with cyanobacteria. Crown vetch is toxic to horses and other non-ruminants because of the presence of nitroglycosides. Recently it has been deemed as good forage when fed as hay to or grazed by ruminant animals such as cattle, goats, sheep, elk and deer. These aliphatic nitro compounds are degraded in ruminant digestion and do not affect the animals. *Coronilla varia* produces little or no bloat hazard in grazing (Acar Z. et al, 2001, Frame J., 2005; Mueller-Harvey I., 2006; Luneva N.N., 2008; Gucker C. L., 2009).

Coronilla varia is a source of phytochemicals with cardiac, diuretic, purgative, antibacterial and anticancer activity (Zackova P. et al, 1986; Dehpour A. A., 2016).

This research was aimed at evaluating the biological peculiarities, the biochemical composition of the local ecotype of crown vetch and the possibility to use it as fodder for ruminant animals or as biogas substrate.

MATERIAL AND METHOD

The local ecotype of crownvetch, *Coronilla varia* maintained in monoculture, served as object of study. The traditional leguminous fodder crops: alfalfa, *Medicago sativa*, and common sainfoin, *Onobrychis viciifolia*, were used as control variants. The experiments were performed on non irrigated experimental land in the Botanical Garden (Institute) of the Academy of Sciences of Moldova (ASM) in spring, when the soil had reached the physical readiness. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2 . The seeds were sown at a depth of 2.0-3.0 cm with soil compaction before and after sowing. The plant growth, development and productivity were assessed according to methodical indications (Novoselov Y. K. et al, 1983). The green mass was harvested in the flowering period (the end of May). The green mass yield was measured by weighing. The dry matter content, or total solids (TS), 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 . Organic dry matter, or volatile solids (VS), was calculated through differentiation, the crude ash being subtracted from dry matter. Nitrogen-free

extractive substance (NFE) was mathematically appreciated, as the difference between organic matter values and analytically assessed organic compounds. The mineral content was investigated by the standard spectrometric methods (Petukhov E.A. et al, 1989).

The carbon content of the substrates was obtained from volatile solids (organic dry matter) data using an empirical equation reported by (Badger C.M. et al., 1979).

The biogas production potential and specific methane yields were evaluated by the parameter "content of fermentable organic matter", according to (Weissbach F., 2008).

RESULTS AND DISCUSSIONS

We observed that, after sowing, the emergence of *Coronilla varia* plantlets was uneven and required a 10-17 day longer period as compared with traditional forage crops, probably due to the fact that the seeds of this species were characterized by a denser coat, but water availability and temperature could also influence germination. Over a period of 4-6 weeks, the plants developed fine roots, which grew 15-20 cm long and ensured the necessary water and nutrients for growth and development. We found that during the first month after the emergence of seedlings, the growth and development rate of the aerial part of the plant was very slow, the rosette formed the next month, then the growth rate accelerated and allowed the development of an erect stem, which in August, the flowering season, reached 37-41 cm. In the first year, until the end of the growing season, the plants didn't produce any seeds. Underground, they developed an extensive tap root system and strong fleshy rhizomes, extending down to 70-80 cm. The traditional leguminous forage crops, alfalfa and sainfoin, went through all ontogenetic phases. Alfalfa was harvested twice, common sainfoin and crown vetch – once. The fresh mass yield of crown vetch was 1.59 kg/m^2 with high content of leaves (68%), suitable for hay making or grazing.

Coronilla varia was characterized by slow germination, seedling emergence and development compared with red clover, birdsfoot trefoil or alfalfa (Peiffer R. A., 1972).

The next year, in spring, when temperatures above 5°C established, the growing period of the *Coronilla varia* started 2 days later in comparison with *Onobrychis viciifolia*, but 6 days earlier than *Medicago sativa*. Shoots developed from large buds, which were located just above the collar. The plants were tap-rooted and rhizomatous with overwintered rhizomes emerging in spring to initiate new shoots, which would eventually take root and become independent plants. The flowering stage started at the end of May and the seed ripening period after 60-70 days (end of July- first half of August) because of the indeterminate flowering habit of

crown vetch. The seed yield was 19 g/m² and the average 1000-seed weight – 3.64 g.

Analyzing the results from the 3-year-old, we noted that the *Coronilla varia* plants had resumed vegetation 2-3 days earlier in comparison with *Medicago sativa* and *Onobrychis viciifolia*, and observed that stems had been produced in abundance from rhizomes, being characterized by a faster growth rate than in previous years. Thus, at the end of April, decumbent to ascending shoots of *Coronilla varia* were up to 47.2 cm long, exceeding alfalfa with 8.1 cm and sainfoin with 13.3 cm, this tendency was maintained during the flowering period, when crown vetch plants reached 122.10 cm, while the traditional leguminous forage crops achieved 83.20-85.50 cm (table1).

In other studies, it was mentioned that *Coronilla varia* plants may reach 6 feet or 2 m long (Gucker C. L., 2009).

We observed, in the 3-year-old, budding phase of *Coronilla varia* started at the same time as *Medicago*

sativa and 5 days earlier than *Onobrychis viciifolia*. We could also mention that during the next period *Coronilla varia* had a more rapid development rate and was distinguished by a short budding-flowering period (7 days) comparative with *Onobrychis viciifolia* (24 days). *Coronilla varia* produced considerable foliage (63%) and the fresh mass yield at the first mowing reached 3.92 kg/m², at the same level as sainfoin and by 25% more than alfalfa, but it had a lower content of dry matter in the harvested mass (22.4%).

In some papers it was mentioned that the productivity of *Coronilla varia* under the climatic conditions of Russia reached 65 t/ha green mass (Dronova T.N. et al., 2009); in South Africa crown vetch yielded 10.6 t/ha of dry matter, but alfalfa - 7.1 t/ha (LeRoux C.J.G. et.al., 1988).

Table 1

Agro biological peculiarities of the studied species of the family Fabaceae

Indicators	<i>Medicago sativa</i>	<i>Onobrychis viciifolia</i>	<i>Coronilla varia</i>
Resumed vegetation up to:			
- budding, days	70	75	70
- flowering, days	82	99	77
- seed ripening, days	143	133	141
Plant height, cm			
- at the end of April	39.10	35.90	47.20
- at flowering	83.20	85.50	122.10
The yield in the first mowing:			
- fresh mass, kg/m ²	3.11	3.95	3.92
- dry matter, kg/m ²	0.82	1.03	0.87
The leaf share of the fodder, %	44	39	63

Table 2

Biochemical composition and nutritional value of the studied species of the family Fabaceae

Indicators	<i>Medicago sativa</i>	<i>Onobrychis viciifolia</i>	<i>Coronilla varia</i>
Raw protein, % dry matter	17.03	17.44	14.72
Raw fats, % dry matter	2.30	3.39	2.81
Raw cellulose, % dry matter	33.31	33.50	35.46
Nitrogen free extract, % dry matter	39.41	39.43	39.74
Minerals, % dry matter	8.01	6.24	7.27
1 kg of natural fodder contains:			
nutritive units	0.21	0.23	0.20
metabolizable energy, Mj	2.28	2.86	2.22
dry matter, g	263.70	274.00	224.20
raw protein, g	46.10	47.80	33.03
digestible protein, g	34.50	35.87	26.42
raw fats, g	6.20	9.30	6.34
raw cellulose, g	80.30	86.30	79.43
nitrogen free extract, g	99.30	113.50	89.10
minerals, g	21.70	17.10	16.30
digestible protein, g/ nutritive unit	164.29	156.00	132.10

Forages are a major source of nutrients for herbivores around the world. Sometimes the balance of nutrients or the presence of some constituent in the forage will have positive or negative effects on animal health and productivity (McDonald P. et al, 2010). It was found that dry

matter *Coronilla varia* was characterized by optimal protein content (14.42%), which was lower in comparison with traditional forage leguminous crops (17.03-17.44%), about the same nitrogen free extractive substances (39.74%) and high level of raw cellulose (35.46%). In comparison with

traditional forage leguminous crops, the dry matter of *Coronilla varia* was characterised by lower fat content (2.80%) in comparison with *Onobrychis viciifolia*, but advanced – in comparison with *Medicago sativa*; and minerals – inversely proportional (table 2).

Some authors mention varied findings about the quality of *Coronilla varia* fodder. Acar Z. et al, 2001 remarked than *Coronilla varia* spp. *varia* in Pakistan contain 14.86% protein and 9.99% ash. According to Dronova T.N. et al., 2009, the chemical composition of dry matter crown vetch was: 25.2% protein, 3.3% fat, 25.5% cellulose, 34.3% nitrogen-free extractive, but alfalfa – 21.8%, 2.3%, 22.0% and 35.0%, respectively. Reynolds P.J. et al, 1967, reported than crown vetch forage contain 21.7% protein and 22.2% fibre, involving the digestibility in sheep was 65.6% protein and 46.2% fibre, but slightly less than the digestibility of alfalfa forage.

An essential component of protein characteristics is its amino acid composition, which is its main structural characteristic, irrespective of the kind, origin and physiological function. Determining the amino acid composition of green fodder of different species and the proportions of the respective amino acids facilitates the evaluation of its potential nutritional value, especially creating the possibility of not applying synthetic amino acids to enhance the nutritive value of animal feed mixtures.

Analyzing the results on the amino acid content in the fodder (table 3), it was found that the species *Coronilla varia* was distinguished by an optimal content of both essential and nonessential amino acids. Comparing each amino acid separately, we could mention that the content varied in comparison with traditional forage crops.

Table 3

The content of amino acids (g/100 g dry matter) of the studied species of the family Fabaceae

Amino acids	<i>Medicago sativa</i>	<i>Onobrychis viciifolia</i>	<i>Coronilla varia</i>
asparagine	1.711	1.751	1.857
threonine	0.564	0.565	0.551
serine	0.687	0.685	0.678
glutamine	1.360	1.398	1.418
proline	0.922	1.154	1.480
glycine	0.550	0.557	0.852
alanine	0.674	0.672	0.712
valine	0.559	0.654	0.459
methionine	0.139	0.091	0.101
isoleucine	0.459	0.459	0.344
leucine	0.913	0.920	0.898
tyrosine	0.458	0.491	0.442
phenylalanine	0.850	0.937	0.647
histidine	0.326	0.371	0.239
lysine	0.619	0.706	0.524
arginine	0.655	0.587	0.517

The role of the first deficient essential amino acid, limiting the nutritive value of protein, was played by methionine. We could mention that the methionine content of the species *Coronilla varia* reached 0.101 mg/100 mg dry matter, thus, it was higher than in *Onobrychis viciifolia*, but lower as compared with *Medicago sativa*. The second limiting amino acid for protein biosynthesis was lysine; the content of this essential amino acid was much lower as compared with *Onobrychis viciifolia* and *Medicago sativa*. We found that *Coronilla varia* fodder was very rich in proline and glycine, rich in asparagine, glutamine, glycine and alanine, but had lower content of phenylalanine, arginine, valine, isoleucine, leucine, histidine and tyrosine in comparison with traditional forage crops.

The presence of minerals in animal nutrition is indispensable for their growth and health, because they are essential components of all tissues and organs that maintain osmotic pressure at a constant level, participate in the regulation of acid-base balance, activate a number of enzymes, moderate the neuromuscular activity, prevent the emergence and development of diseases in animals (McDonald et al., 2010). The content of mineral elements in fodder is variable, depending on species. It was established that *Coronilla varia* was characterised by a high content of macro elements (table.4).

Table 4

The content of minerals per kg dry matter of the studied species of the family Fabaceae

Minerals	<i>Medicago sativa</i>	<i>Onobrychis viciifolia</i>	<i>Coronilla varia</i>
calcium, g	16.94	11.20	12.90
phosphorus, g	4.42	7.53	5.67
magnesium, g	2.71	3.28	2.31
potassium, g	15.38	15.17	21.54
sodium, mg	349.50	366.20	52.85
iron, mg	250.83	343.20	389.80
manganese, mg	50.90	91.55	63.69
zink, mg	22.37	26.15	26.96
copper, mg	7.00	6.75	5.55
strontium, mg	49.77	34.53	35.42

Comparing each macro element separately with traditional fodder leguminous crops, we could mention that the content varies from species to species. The species *Coronilla varia* in comparison with *Medicago sativa* is distinguished by low content of calcium (12.90g / kg) and higher content of phosphorus (5.67 g/kg), but inversely proportional in *Onobrychis viciifolia* fodder. *Coronilla varia* fodder is characterized by high level of potassium (21.54 g/kg), but lower – of magnesium (2.31 g/kg) and sodium (52.85 mg/kg). It was determined the content of trace elements in

dry matter of *Coronilla varia*, so, it included: 5.55 mg/kg copper, 26.96 mg/kg zinc, 63.69 mg/kg manganese, 389.80 mg/kg iron, and 35.42 mg/kg strontium. *Coronilla varia* fodder contained large amounts of iron and poor – of copper. In Canada it was determined that *Coronilla varia* fodder contains: 17.9-18.4 g/kg calcium, 2.2-2.8 g/kg phosphorus, 27.2-31.1 g/kg potassium, 1.7-1.6 g/kg magnesium, 0.15-0.16 g/kg sodium, 8.0-9.3 mg/kg copper, 34-40 mg/kg zinc, 36-40 mg/kg manganese, 169-179 mg/kg iron (Gervais P., 2000); in Russia crown vetch forage harvested in budding period contains 40 g/kg calcium, 8 g/kg phosphorus, 0.6 g/kg magnesium, 2.48 mg/kg copper, 14 mg/kg zinc, 49 mg/kg manganese, 105 mg/kg iron (Kshnikatkina A.N. et al, 2005); in Pakistan 22.2 g/kg calcium, 22.4 g/kg potassium, 1.88 g/kg magnesium, 14.46 mg/kg copper, 67.35 mg/kg zinc, 40.37 mg/kg manganese, 482.6 mg/kg iron (Acar Z. et al, 2001)

The organic matter content, its biochemical composition and digestibility of the green mass determine the nutritional value and methane yield.

Coronilla varia green mass contained: 0.20 nutritive units/ kg, 2.22 Mj/kg metabolizable energy and digestible protein – 132.10 g/nutritive unit. According to the mentioned indices, the natural fodder of *Coronilla varia* had lower nutritional value in comparison with traditional crops, because of the low content of dry matter and raw protein. Thus, if we compare the nutritional value of dry matter, 100 kg of dry matter of *Coronilla varia* contain 89 nutritive units, 990 MJ/kg metabolizable energy and 11.78 kg digestible protein, while *Medicago sativa* contains 80 nutritive units, 865 Mj/kg metabolizable energy and 13.81 kg digestible protein, *Onobrychis viciifolia* – 84 nutritive units, 1050 MJ/kg metabolizable energy and 13.09 kg digestible protein, respectively.

Table 5

Gas forming potential of the fermentable organic matter from the studied species of the family Fabaceae

Indicators	<i>Medicago sativa</i>	<i>Onobrychis viciifolia</i>	<i>Coronilla varia</i>
Ratio of content of carbon and nitrogen (C/N)	19	19	22
Fermentable organic matter, g/kg VS	642	658	626
Biogas, liter /kg VS	514	526	501
Methane, liter /kg VS	270	276	263
Methane productivity, m ³ /ha	2214	2843	2311

Grasslands provide a high biomass potential. They are often inefficiently used, so a new utilisation concept based on the biorefining approach can increase the added value from grasslands. Biorefining offers a way for combining feed and bioenergy production. Energy crops are of considerable importance for biogas production. The use of forage legumes as biogas substrate contributing to increase potential for bioenergy and reducing greenhouse gas emissions, through symbiotic nitrogen fixation and compensates inorganic N fertilizer in conventional farms, if the digestate is applied as a fertilizer to the non-legume crops (Stoddard F.L., 2013; Stinner P. W., 2015). The content of organic matter, biochemical composition, biodegradability and ratio of carbon and nitrogen (C/N) of the raw material are essential in the production of biogas. The C/N ratio of the studied species varied from 19 in biomass *Medicago sativa* and *Onobrychis viciifolia* to 22 in biomass *Coronilla varia* (tab. 5). 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 P. et al, 2014).

Fermentable organic matter represents the proportion of organic matter which can be biologically degraded under anaerobic conditions and, thus, can be potentially utilized in biogas facilities (Weissbach F.,

2008). The calculated gas forming potential of the fermentable organic matter of *Coronilla varia* reached 501 litre/kg VS, being lower than in the control species (514-526 liter/kg VS), but it had similar content of methane (52.5%).

The methane yield per ha of studied species of the family *Fabaceae* (first mowing) ranged from 2214 to 2843 m³/ha, *Coronilla varia* exceeding *Medicago sativa*.

CONCLUSIONS

Coronilla varia seeds germinate very slowly, need scarification and soil best temperature in comparison with sainfoin and alfalfa.

The 3-year-old *Coronilla varia* plants have moderate growth and development rates that allow mowing them at the end of May the green mass yield reaches 3.92 kg/ m², at the same level as sainfoin and by 25% more than alfalfa, but low content of dry matter, in comparison with the traditional crops.

The chemical composition of *Coronilla varia* dry matter: 14.72% raw protein, 2.81% raw fat, 35.46% raw cellulose, 39.74% nitrogen-free extractive substances and 7.27% minerals (high level of potassium and iron, but lower – of magnesium and sodium).

The forage value of 1 kg natural forage accounts 0.20 nutritive units, 2.22 Mj metabolizable energy; digestible protein - 132.10 g/nutritive unit.

The gas forming potential of *Coronilla varia* reached 501 litre/kg VS with 52.5% methane. The calculated methane yield *Coronilla varia* green mass at the first mowing may reach 2311 m³/ha, exceeding *Medicago sativa*.

The local ecotype of the species *Coronilla varia* could be used for restoring degraded, polluted and eroded land, and also for reseeding and increasing economic value of grasslands.

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