THE BIOCHEMICAL COMPOSITION OF SOME ANNUAL *FABACEAE* SPECIES AND THEIR POTENTIAL APPLICATION IN MOLDOVA

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Abstract

The research and exploitation of the agrobiological potential of the local Fabaceae species that are adapted to the soil and climatic conditions becomes more and more relevant. The annual leguminous species *Lathyrus sativus*, *Pisum sativum arvense*, *Trigonella foenum-graecum*, *Vicia hirsuta* and *Vicia sativa* grown in monoculture in an experimental sector in the NBGI Chişinău, served as objects of study. The research results revealed that the dry matter of the harvested whole plants contained 142-251 g/kg CP, 251-377 g/kg CF, 89-124 g/kg ash, 254-390 g/kg ADF, 416-598 g/kg NDF, 34-65 g/kg ADL, 220-327 g/kg Cel, 155-206 g/kg HC, 64-160 g/kg TSS with nutritive and energy value 62.2-85.1% DMD, 59.3-80.1% DOM, RFV=91-151, 9.51-11.09 MJ/kg ME and 5.53-7.10 MJ/kg NEl. The biochemical composition, nutritive and energy value of the prepared hay: 131-164 g/kg CP, 244-418 g/kg CF, 50-135 g/kg ash, 294-436 g/kg ADF, 475-645 g/kg NDF, 36-76 g/kg ADL, 258-360 g/kg Cel, 165-209 g/kg HC, 6-70 g/kg TSS, 53.1-74.5% DMD, 51.6-68.4% DOM, RFV=85-129, 9.00-10.63 MJ/kg ME and 5.00-6.65 MJ/kg NEl. The green mass substrates for anaerobic digestion have C/N= 11.8-22.3, with biochemical methane potential 305-378 l/kg. The biomass harvested from the studied annual leguminous species can be used as fodder for animals or as co-substrates in biogas plants for the production of biomethane as a renewable energy source.

Key words: annual leguminous species, biochemical composition, biochemical methane potential, forage quality

Plant biodiversity, particularly its conservation and prospects for practical use, has become an increasingly pressing problem worldwide, in the 21st century. Plant genetic resources are essential for avoiding a potential food crisis in Europe and enabling an innovative bioeconomy. There are about 50 000 edible spontaneous plant species, but a small number of these species have been domesticated and cultivated (ECPGR, 2021).

The Fabaceae species are important in global bioeconomy, providing biologically fixed nitrogen and improving the physical properties of soil, breaking cereal disease cycles and providing raw material for food and feed, including forage. The interest in systems based on legumes has increased significantly over the recent years due to their importance for sustainable and organic farming. In the European Union, the interest in forage legumes has increased for several economic and environmental reasons (European Parliament resolution 2011, 2018; ECPGR, 2021).

The use of legume substrates as feedstock for biomethane production increases the potential of renewable energy and contributes to the reduction of greenhouse gas emissions, and by symbiotic nitrogen fixation these species can compensate the use of inorganic nitrogen fertilizers in conventional farms, if the digestate is applied as a fertilizer to the non-legume crops (Stoddard F.L., 2013; Stinner P. W. *et al*, 2018).

The diversification of legume forage production has to be achieved by mobilization, acclimatization and implementation of new species and non-traditional crops from local flora and other floristic regions. Considering the limited natural and technical resources in the Republic of Moldova, the efficient use of the biological potential of the Fabaceae plants that are adapted to the local climatic conditions becomes more and more relevant.

In the spontaneous flora of Bessarabia, the family Fabaceae is represented by 146 species of 35 genera, including 15 species of the genus *Lathyrus* L., 3 species on the genus *Pisum* L., 3 species on the genus *Trigonella* L. and 19 species of the genus *Vicia* L. (Izverscaia T., 2020).

The main objectives of this study were to evaluate the biochemical composition of green mass and hay from annual leguminous species *Lathyrus sativus* L., *Pisum sativum subsp. arvense* (L.) Asch. & Graebn., *Trigonella foenum-graecum* L., *Vicia hirsuta* (L.) Gray, *Vicia sativa* L. and the

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prospects of their use as fodder for farm animals or as substrates for biomethane production.

MATERIALS AND METHODS

The local ecotype of the annual leguminous species *Lathyrus sativus*, *Pisum sativum arvense, Trigonella foenum-graecum, Vicia hirsuta* and *Vicia sativa*, grown in monoculture in the experimental sector of the National Botanical Garden (Institute) Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″ longitude, served as research subjects. The experimental design was a randomized complete block design with four replications, and the experimental plots measured 50 m^2. The plant growth, development and productivity were assessed according to accepted methodical indications in NBGI.

The green mass samples were collected in the flowering stage. The dry matter content was detected by drying samples up to constant weight at 105°C. The prepared hay was dried directly in the field. The fresh mass samples were dehydrated in an oven with forced ventilation at a temperature of 60°C. At the end of the fixation, the biological material was finely ground in a laboratory ball mill. The quality of the biomass was evaluated by analyzing such indices as: crude protein (CP), crude fibre (CF), minerals, total soluble sugars (TSS), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), dry matter digestibility (DMD), organic matter digestibility (DMD), which have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200 of the Research and Development Institute for Grassland Braşov, Romania. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), the metabolizable energy (ME), the net energy for lactation (NEl) and the relative feed value (RFV) were calculated according to standard procedures. The carbon content of the substrates was determined using an empirical equation according to Badger C.M. *et al,* (1979). The biochemical methane potential (Ym) were calculated according to Dandikas V. *et al* (2015).

RESULTS AND DISCUSSIONS

The morphological structure of the whole plant has significant impact on the nutrient content of the green mass. Analysing the results of the biochemical composition of dry matter, *Table 1*, we found that the harvested annual leguminous whole plants contained 142-251 g/kg CP, 251-377 g/kg CF, 89-124 g/kg ash, 254-390 g/kg ADF, 416-598 g/kg NDF, 34-65 g/kg ADL, 220-327 g/kg Cel, 155-206 g/kg HC, 64-160 g/kg TSS with nutritive and energy value 62.2-85.1% DMD, 59.3-80.1% DOM, RFV=91-151, 9.51-11.09 MJ/kg ME and 5.53-7.10 MJ/kg NEl. We found that *Lathyrus sativus* and *Trigonella foenum-graecum* green fodder was characterised by higher content of crude protein and low concentration of cell wall fractions (NDF, ADF, ADL) which had a positive effect on the digestibility, nutritional value and energy supply of the feed. *Vicia hirsuta* fodder contained higher amounts of crude protein and minerals and lower amount of soluble sugars than *Vicia sativa.* The lowest level of crude protein, total soluble sugars and higher concentration of cell wall fractions was found in *Pisum sativum arvense* fodder. The relative feed value and energy concentrations were optimal in *Vicia sativa* fodder, but reduced as compared with *Lathyrus sativus* and *Trigonella foenum-graecum* green fodder.

The results of the estimation of quality of green mass from annual leguminous species are given in the specialized literature. According to Burlacu G. *et al* (2002) *Vicia sativa* harvested in the flowering period contained: 170 g/kg DM, 10.5% ash, 18.4% CP, 28.2% CF, 58.2% DOM, 18.24 MJ/kg GE, 11.13 MJ/kg DE, 8.94 MJ/kg ME. Ates E. *et al* (2010) stated that field pea *Pisum arvense* contained 151.3g/kg CP, 31.42% ADF, 42.69 % NDF. Burlyaeva M.O. *et al* (2012) mentioned that the crude protein content in the *Lathyrus* species green forage was: 28.4% *Lathyrus cicera*, 24.4-27.2% *Lathyrus sativus*, 23.0% *Lathyrus ochrus* and 17.6% *Lathyrus tingitanus*. Ates S. *et al* (2013) revealed that *Vicia sativa* contained 21.5% CP, 31% NDF, 22% ADF, 60% IVDMD and *Vicia villosa* ssp. *dasycarpa* respectively 16.5% CP, 40% NDF, 31% ADF, 46% IVDMD. Vishnyakova M.A. *et al* (2014) found the following protein content in *Vicia* species green mass: 20.4-21.9% *Vicia sativa* and 20.60-23.38 % *Vicia villosa*. Heuze V. *et al* (2015, 2016, 2019) reports that the fresh mass nutritive value of *Vicia sativa* was: 193 g/kg DM, 23.0% CP, 2.5% EE, 25.4% CF, 36.7% NDF, 28.9% ADF, 6.1 % lignin, 9.8 % ash, 69.8% DOM, 18.6 MJ/kg GE, 12.4MJ/kg DE and 9.8 MJ/kg ME; *Lathyrus sativus* forage – 217 g/kg DM, 21.4% CP, 2.9% EE, 25.8% CF, 38.2% NDF, 27.1% ADF, 10.5% ash, 69.4% DOM, 18.5 MJ/kg GE, 12.3 MJ/kg DE and 9.7 MJ/kg ME; *Trigonella foenum-graecum* forage – 308 g/kg DM, 16.5% CP, 2.1% EE, 25.8% CF, 38.8% NDF, 30.3% ADF, 14.0% ash, 68.0% DOM, 17.4 MJ/kg GE, 11.4 MJ/kg DE and 9.1 MJ/kg ME. Uslu O.S. *et al* (2018), evaluating of the forage quality of some legume plants harvested in flowering stage, reported that *Lathyrus sativus* contains 19.37% DM with 21.51% CP, 2.00% EE, 24.83% ADF, 37.64% NDF, 8.31% ash, 72.60% OMD and 9.76 MJ/kg ME; *Vicia sativa* – 23.61% DM with 19.91% CP, 2.60% EE, 27.69% ADF, 31.54%

NDF, 9.28% ash, 72.79% OMD and 10.30 MJ/kg ME; *Trigonella foenum-graecum* – 25.27% DM with 20.17% CP, 1.49% EE, 20.24% ADF, 22.86% NDF, 9.85% ash, 72.87% OMD and 9.98 MJ/kg ME; *Medicago sativa* – 18.65% DM with 20.55% CP, 2.26% EE, 26.65% ADF, 35.50% NDF, 9.98% ash, 70.54% OMD and 9.79 MJ/kg ME, respectively. Akbay F. *et al* (2020) found that fenugreek grass, which was harvested at different harvest times, contained 19.34-22.44% DM with 15.57-19.43% CP, 1.38-1.90% EE, 25.39-35.31% ADF, 39.89-55.25% NDF, 8.03-10.71% ash, 64.26-73.40% OMD and 8.75-9.86 MJ/kg ME. Atis I. &Acikalin S. (2020) reported that the dry matter from the harvested forage of *Lathyrus sativus* contained 20.2-22.6% CP, 37.5-40.9% NDF, 24.1-26.6 % ADF RFV=155.5-174.3. Niu H. *et al* (2021) found that the chemical composition of the forage dry matter from *Medicago sativa* was: 17.27-21.16% CP, 33.14-43.89% NDF, 27.99-37.11 % ADF, 10.17-10.20% ash, but from *Trigonella foenum-graecum* – 17.75-23.55% CP, 26.42-37.79% NDF, 22.93-34.44% ADF, 10.92-11.0% ash, respectively.

Table 1

The biochemical composition and the fodder value of the green mass of the studied annual leguminous species

Indices	*Lathyrus sativus*	*Pisum sativum arvense*	*Trigonella foenum-graecum*	*Vicia hirsuta*	*Vicia sativa*
Crude protein, g/kg DM	251	142	216	208	194
Crude fibre, g/kg DM	215	371	244	316	297
Minerals, g/kg DM	124	89	119	113	102
Acid detergent fibre, g/kg DM	254	392	261	346	326
Neutral detergent fibre, g/kg DM	427	598	416	536	523
Acid detergent lignin, g/kg DM	34	65	37	50	48
Total soluble sugars, g/kg DM	150	91	160	64	109
Cellulose, g/kg DM	220	327	224	296	278
Hemicellulose, g/kg DM	173	206	155	190	187
Dry matter digestibility, %	85.1	62.2	81.7	70.7	71.1
Organic matter digestibility, %	80.1	59.3	75.2	64.5	66.8
Relative feed value	151	91	153	108	113
Digestible energy, MJ/ kg	13.51	11.58	13.41	12.22	12.50
Metabolizable energy, MJ/ kg	11.09	9.51	11.01	10.04	10.26
Net energy for lactation, MJ/ kg	7.10	5.53	7.02	6.05	6.28

Table 2

The biochemical composition and the fodder value of the hay of the studied annual leguminous species

Indices	*Lathyrus sativus*	*Pisum sativum arvense*	*Trigonella foenum-graecum*	*Vicia hirsuta*	*Vicia sativa*
Crude protein, g/kg DM	264	131	209	189	198
Crude fibre, g/kg DM	244	418	310	356	322
Minerals, g/kg DM	135	90	124	110	106
Acid detergent fibre, g/kg DM	294	436	326	393	345
Neutral detergent fibre, g/kg DM	475	645	501	606	510
Acid detergent lignin, g/kg DM	36	76	47	65	50
Total soluble sugars, g/kg DM	58	60	55	6	70
Cellulose, g/kg DM	258	360	279	328	295
Hemicellulose, g/kg DM	191	209	175	213	165
Digestible dry matter, %	74.5	53.1	71.7	59.8	70.5
Organic matter digestibility, %	68.4	51.6	63.4	54.8	65.5
Relative feed value	129	85	118	90	113
Digestible energy, MJ/ kg	12.95	10.96	12.50	11.57	12.24
Metabolizable energy, MJ/ kg	10.63	9.00	10.26	9.50	10.05
Net energy for lactation, MJ/ kg	6.65	5.02	6.28	5.51	6.05

Table 3

Biomethane production potential of green mass substrates from the studied annual leguminous species

Indices	*Lathyrus sativus*	*Pisum sativum arvense*	*Trigonella foenum-graecum*	*Vicia hirsuta*	*Vicia sativa*
Crude protein, g/kg	257.0	142.0	209.0	208.0	194.0
Minerals, g/kg	125.0	89.0	124.0	113.0	102.0
Nitrogen, g/kg	41.1	22.7	33.4	33.3	31.0
Carbon, g/kg	486.1	506.1	486.7	492.8	498.9
Ratio carbon/nitrogen	11.8	22.3	14.6	14.8	16.1
Hemicellulose, g/kg	183.0	206.0	175.0	190.0	187.0
Acid detergent lignin, g/kg	34.0	65.0	47.0	50.0	48.0
Biomethane potential, L/kg	378	305	347	343	343

The quality and productivity of hay mainly depends on the plants species, the growing period at which these plants have been harvested, on the botanical structure of the herbage, on the techniques and technology of hay preparation, on the conditions of its storage and on many other factors. Each factor and even combinations of them can have an impact on the nutritional value of hay. Analysing the results regarding the biochemical composition of the hay prepared from annual leguminous species, *Table 2*, we would like to mention that hay dry matter contained: 131-164 g/kg CP, 244-418 g/kg CF, 50-135 g/kg ash, 294-436 g/kg ADF, 475-645 g/kg NDF, 36-76 g/kg ADL, 258-360 g/kg Cel, 165-209 g/kg HC, 6-70 g/kg TSS. The nutritive value and the energy concentration of the prepared hay was 53.1-74.5% DMD, 51.6-68.4% DOM, RFV=85-129, 9.00-10.63 MJ/kg ME and 5.00-6.65 MJ/kg NEl. We found that during the process of preparing hay, there was an increase in the content of crude fibre, cellulose and a decrease in the total soluble sugar content, dry matter digestibility and relative feed value and energy concentration as compared to the initial green mass. The *Lathyrus sativus* hay was characterized by very high content of crude protein, ash, hemicellulose, but low content of acid detergent lignin and cellulose, which had a positive effect on digestibility, relative feed value and energy concentration. The hay prepared from *Trigonella foenum-graecum* was characterized by high level of crude protein and minerals, low level of cell wall fractions (NDF, ADF, ADL) and total soluble sugars, but optimal digestibility, nutritional and energy value of the feed. The amounts of crude protein and total soluble sugars became lower, but the concentration of cell wall increased significantly in the *Pisum sativum arvense* and *Vicia hirsuta* hays. The high concentration of total soluble sugars led to higher digestibility and energy value of the fodder. The *Vicia sativa* hay had high content of total soluble sugars and optimal content of crude protein and minerals, low level of acid detergent lignin.

Some authors mentioned various findings about the quality of hay prepared from annual leguminous species. Hadjipanayiotou M. *et al* (1983) mentioned that, in Cyprus, the chemical composition of *Vicia sativa* hay was 18.6% CP, 2.2% EE, 25.8% CF, 47% NDF, 33 % ADF, 6.3% lignin, 11.5% ash, 60% DOM, 11.0-12.0 MJ/kg DE. Poland C. *et al* (2003) reported that the nutritional quality of *Lathyrus sativus* hay was 18.2% CP, 48.6% NDF, 36.3% ADF, 60.6% TDN and – of *Medicago sativa* hay – 18.1% CP, 44.6% NDF, 35.0% ADF, 61.7% TDN. Burlacu G. *et al* (2002) mentioned that *Vicia sativa* hay contained: 850 g/kg DM, 10.0% ash, 16.5% CP, 2.6% EE 32.0% CF, 54.0% DOM, 18.22 MJ/kg GE, 10.39 MJ/kg DE and 8.35 MJ/kg ME. Ates S. *et al* (2013) reported that the quality of *Lathyrus sativus* hay was 21% CP, 32% NDF, 18% ADF, 75% DOMD and straw 14% CP, 45% NDF, 25% ADF, 60% DOMD, respectively. Vahdani N. *et al* (2014) reported that grass pea hay with seedpods contained: 953.7 g/kg DM, 908.3 g/kg OM, 232.4 g/kg CP, 43.0 g/kg EE, 318.5 g/kg CF, 397 g/kg NDF, 383 g/kg NDF_{OM}, 300 g/kg ADF; 96.5 g/kg HC, 13.4 g/kg TP,17.6 g/kg TT, 0.2 g/kg CT, 8.75% g/kg, 11.8 g/kg $_B$ –ODAP, 772.0 g/kg OMD, 10.7 MJ/kg ME. Das M. *et al* (2015) mentioned that the nutritional value of *Lathyrus sativus* hay was: 93.47% OM, 14.99% CP, 2.18% EE, 31.85% CF, 44.75% NFE, 58.08% NDF, 41.51% ADF; 9.3 % ADL, 16.57% HC, 32.21% Cel, 6.22% ash. Heuze V. *et al* (2015, 2016) mentioned that the hay made from *Lathyrus sativus* and *Vicia sativa* contained 19.1-19.7% CP, 1.8-2.9% EE, 24.5-28.5% CF, 38.6-43.4% NDF, 28.7-32.7% ADF, 6.5% lignin, 9.9-10.7 % ash, 13.3-14.5 g/kg Ca, 2.1-2.9 g/kg P, 64.4-68.3% DOM, 18.1-18.6 MJ/kg GE, 11.0-12.0 MJ/kg DE and 8.7-9.5 MJ/kg ME. According to Mihailović V. *et al* (2016), the hay yield and forage crude protein yield of some legume species grown in Serbia were: *Lathyrus sativus* 11.8 t/ha and 1882 kg/ha CP, *Vicia sativa* subsp. *sativa* 10.5 t/ha and 1674 kg/ha CP, *Vicia hirsuta* 4.5 t/ha and 723 kg/ha CP. Kaya E. (2021) evaluating of the forage quality of vetch species grown in a native pasture in Turkey, reported that the fodder value of *Vicia sativa* hay was 17.5% CP, 0.7% EE, 26.0% ADF, 48.6% NDF, 7.6% ash, 71.0% DOM and 9.5 MJ/kg ME. Turan N. (2021) mentioned that the concentrations of nutrient in *Vicia sativa* hay was 141-188 g/kg CP, 262–287 g/kg ADF, 364–435 g/kg NDF and 725–782 g/kg IVOMD.

The increasing energy demand that has been noticed worldwide, the risk of depletion of fossil energy sources and their injurious impact on environment led to our coal-based society recognizing the potential of renewable energy sources. The use of phytomass substrates for biogas production has recently become of major interest in Europe. Biogas plants produce not only methane for heat and electricity, but also digestate and fugate, which are believed to be good fertilizers, since they are rich in plant available nutrients such as nitrogen, phosphate and potash, and could serve as a replacement for fossil based mineral fertilizers in organic agriculture. Plant substrates may be used in biogas generators as fresh mass and as ensiled mass. The quality of green mass substrates from the studied annual leguminous species and their biochemical methane potential are presented in *Table 3*. The carbon to nitrogen ratio constitutes a basic factor governing the correct course of anaerobe digestion and methanogenic processes. Methanogenic

bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30:1 were found to be unsuitable for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, due to low pH, poor buffering capacity and high concentrations of ammonia in the substrate. The nitrogen content in the studied legume green mass substrates ranged from 21.7 to 41.1 g/kg, the estimated content of carbon – from 486.1 to 506.1 g/kg, the C/N ratio varied from 11.8 to 22.3. The biochemical methane potential of legume substrates varied from 305 l/kg VS to 378 l/kg VS. The best methane potential was achieved by *Lathyrus sativus* substrate. The *Pisum sativum arvense* substrates contained high level of acid detergent lignin 65 g/kg, which influence negatively the activity of bacteria and decomposition processes, thus, the biochemical methane potential achieved only 305 l/kg VS. It has been found that biomethane production potential of green mass substrates from *Trigonella foenum-graecum, Vicia hirsuta* and *Vicia sativa* achieved 343-345 l/kg VS.

According to Oleskowicz-Popiel P. (2010), the methane yield of *Vicia villosa* substrate was 279 L/kg VS. Wang G.& Schmidt J.E. (2010) reported that methane potential of vetch substrate achieved 320 L/kg VS, in clover substrate 290-390 L/kg VS, but in alfalfa 240 L/kg VS, respectively. Molinuevo-Salces B. *et al* (2012) found that the specific methane yield after 57 days of anaerobic digestion was 195L/kg VS in *Trifolium pratense* substrate and 186 l/kg VS in *Vicia sativa* substrate. Ahlberg I. & Nilsson T. (2006) reported that, in *Vicia villosa* biomass, there was very high protein content (25.6%) and it produced high methane yield (305-343 L/kg). Herrmann C. *et al* (2016) mentioned that forage pea substrates contained 253 g/kg DM, 9.7% CP, 1.8% EE, 45.3% NFE, 55.1% NDF, 42.0% ADF, 12.0% ADL, C/N=27 and the biochemical methane potential was 245.1 l/kg ODM. Pabón-Pereira C.P. *et al* (2020) mentioned that biochemical methane potential was 290 l/kg ODM in *Vicia sativa* substrates, 370 l/kg ODM in *Pisum sativum* substrates and 350 l/kg ODM in *Vicia faba* 350 l/kg ODM. Hunady I. *et al* (2021) calculated the theoretical methane yield and revealed that the values of biomass from *Galega orientalis, Lathyrus pratensis, Trigonella foenum-graecum* and *Melilotus alba* ranged from 0.161 to 0.172 m^3/kg VS, the methane yield of biomass from *Onobrychis viciifolia, Astragalus cicer, Dorycnium germanicum, Vicia sylvatica* ranged from 0.141 to 0.160 m^3/kg VS and the absolutely lowest value – 0.12-0.14 m^3/kg VS – was calculated for *Medicago sativa.* Petcu V. *et al* (2022) found that in winter peas C/N= 9.24-12.63 and in mixture of winter peas + triticale C/N=9.69-11.95.

CONCLUSIONS

The local ecotypes of the annual leguminous species *Lathyrus sativus*, *Pisum sativum arvense, Trigonella foenum-graecum, Vicia hirsuta* and *Vicia sativa* are an important biomass source, can be used as green mass and hay for farm animals or as co-substrate in biogas generators for the production of renewable energy.

The local ecotypes of these species can serve as initial material to be used for breeding and implementing new cultivars of annual leguminous crops for agricultural production.

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