THE QUALITY INDICES OF THE BIOMASS OF SOME *TRIFOLIUM* SPECIES UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

We studied the quality indices of the biomass of the local ecotypes of *Trifolium alpestre, Trifolium hibridum, Trifolium pannonicum, Trifolium pretense, Trifolium repens*. It was determined that the nutrient content and energy value of the dry matter of whole plants of the studied Trifolium species were characterized by the following indices: 144.9-206.9 g/kg CP, 25.8-31.7 g/kg EE, 224.4 -312.9 g/kg CF, 352.7-492.9 g/kg NFE, 85.6-125.0 g/kg ash, 11.4-22.9 g/kg Ca, 1.6-2.6 g/kg P, 17.86-18.43 MJ/kg GE, 8.31-9.96 MJ/kg ME and 4.63-5.76 MJ/kg NEl. The quality indices of the prepared hays were: 168.4-196.9 g/kg CP, 15.8-2.51 g/kg EE, 269.0-339.6 g/kg CF, 350.8-424.0 g/kg NFE, 94.2-124.6 g/kg ash, 12.6-22.6 g/kg Ca, 1.6-2.8g/kg P and 7.80-8.58 MJ/kg ME. The green mass substrates from the studied Trifolium species have C/N=14.89-21.10 and the biochemical methane potential varied from 260 to 271 l/kg ODM. The local ecotype of the studied Trifolium species can be used for the restoration of permanent grasslands and degraded lands, as a component of the mix of grasses and legumes for the creation of temporary grasslands. The harvested clover biomass can be used as forages for farm animals or as substrates in biogas generators for biomethane production.

Key words: biochemical composition, biomethane potential, green mass, hay, nutritive value, Trifolium species

The interest in agricultural systems based on crops of the *Fabaceae* family has increased regularly over the recent years due to their ability to form symbiotic relationship with nitrogen fixing bacteria, thus, they improve the physical properties of soil, form a large amount of organic raw material for circular economy and, besides, they are an important source of proteins, beneficial to human and animal nutrition (European Parliament Resolution, 2018).

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 Basarabia, the family *Fabaceae* Lindl. is represented by 146 species of 35 genera, including 20 species of the genus *Trifolium* L. (Izverscaia T., 2020).

Members of the genus *Trifolium* include fodder crops, medicinal plant, honey plants, cover crops, energy biomass crops, ornamentals, soil nitrifiers, dune stabilizers, important agricultural weeds. In this context, the plants of the genus *Trifolium* have gained a lot of attention, being studied in many research centres (Ates E., 2011; Kiraz A.B., 2011; Lang J.&Vejražka K., 2012; Zvereva G.K., 2016; Tambara A.A.C. *et al*, 2017; Stinner W. *et al*, 2017; Egan L.M. *et al*, 2021; Hunady I. *et al*, 2021; Ţîţei V., 2023; Belashova O.V. *et al*, 2024).

The aim of this study was to evaluate the quality indices of the biomass from the local ecotype of *Trifolium alpestre* L., *Trifolium hibridum, Trifolium pannonicum* Jacq., *Trifolium pretense* L., *Trifolium repens* L., as forages for farm animals or as substrates in biogas generators for biomethane production.

MATERIALS AND METHODS

The local ecotypes of *Trifolium* species: *Trifolium alpestre*, *Trifolium hibridum*, *Trifolium pannonicum*, *Trifolium pratense* and *Trifolium repens* maintained in monoculture in collections of the National Botanical Garden (Institute) of Moldova, Chişinău, served as research subjects. The green mass samples were collected in in the third growing season and the first cut was done in the early flowering stage. The harvested plants were chopped into 1.5-2.0 cm small pieces, with a laboratory forage chopper; the dry matter content was detected by drying the samples to a constant weight, at 105°C. The prepared hay was dried directly in the field. For chemical analyses, the plant samples were dried in a forced-air oven at 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) – by Kjeldahl method; crude fat (EE) – by Soxhlet method, crude cellulose (CF)– by Van Soest method; ash – in muffle furnace at 550°C and the nitrogen-free extract (NFE) was mathematically appreciated. The calcium (Ca) concentration of the samples was determined by using the atomic absorption spectrometry method, phosphorus (P) concentration – by spectrophotometric method.

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The gross energy (GE), metabolizable energy (ME), net energy for lactation (NEl) were calculated according to standard procedures:

GE= 23.9 xCP+39.8 xEE+ 20.1 xCF+17.5x NFE;
ME=14.07+0.0206 xEE-0.0147xCF - 0.0114 xCP;
NEl= 9.10+0.0098 xEE-0.0109 xCF-0.073xCP.

The carbon content of the substrates was determined using an empirical equation according to Badger C.M. *et al,* (1979). The biogas (BP) and the biomethane potential (MP), litre per kg of volatile solid matter (VS), were calculated using the gas forming potential of nutrients according to Baserga U. (1998), corrected by the nutrient digestibility.

RESULTS AND DISCUSSIONS

Providing high quality feed to meet the nutritional needs of farm animals is important for their growth and development, maintaining their health and wellbeing, as well as obtaining high quality animal products, which would increase their market value and would generate higher income for farmers, being an essential component of sustainable agriculture, food safety and security.

The biochemical composition and the fodder value of the green mass from the studied *Trifolium* species are shown in Table 1. We would like to mention that the nutrient content of the dry matter of whole plants of the studied *Trifolium* species was: 144.9-206.9 g/kg CP, 25.8-31.7 g/kg EE, 224.4 -312.9 g/kg CF, 352.7-492.9 g/kg NFE, 85.6-125.0 g/kg ash, 11.4-22.9 g/kg Ca, 1.6-2.6 g/kg P. A higher content of crude protein was found in the fodder from *Trifolium pretense* and *Trifolium pannonicum*. The green fodder of *Trifolium hybridum* and *Trifolium pretense* is characterized by higher content of fats, nitrogen-free extract and low content of crude cellulose as compared with *Trifolium pannonicum* and *Trifolium repens*. The green fodder of *Trifolium alpestre* has optimal concentration of crude cellulose and nitrogen free extract and lower – of crude protein and ash. The green fodder from *Trifolium hybridum, Trifolium pretense, Trifolium alpestre* have higher content of phosphorus, metabolizable energy and net energy for lactation as compared with *Trifolium pannonicum* and *Trifolium repens* green fodder.

Some authors mentioned various findings about the dry matter nutrient concentration of the green mass from *Trifolium* species. According to Acar Z. *et al*, (2001) the *Trifolium hybridum* plants contained 18.92% CP, 12.08 % ash, 1.34% Ca; *Trifolium pratense* – 17.74% CP, 13.70 % ash, 1.91% Ca and *Trifolium repens* 18.93% CP, 13.64 % ash, 1.39% Ca. Burlacu G. *et al*, (2002) revealed that the *Trifolium pratense* plants contained 183 g/kg DM, 90.8% OM, 18.6 % CP, 3.8% EE, 24.0% CF, 44.4% NFE, 9.0% sugars, 4% starch and 18.1 MJ/kg GE. Dewhurst R.J. *et al*. (2009) mentioned that *Trifolium pratense* plants contained 900 g/kg OM, 16.6% CP, 47.6 % NDF, 31.2% ADF with 69% OMD, 0.74 UFL/ kg, 86g/kg PDIE and 106 g/kg PDIN, but *Trifolium repens*, respectively, 887 g/kg OM, 22.9% CP, 32.0% NDF, 19.3% ADF with 80% OMD, 1.03 UFL/ kg, 102 g/kg PDIE and 147 g/kg PDIN. Tavlas A. *et al.*, (2009) reported that the *Trifolium pratense* genotypes contained 13.24 % CP, 32.15 % ADF, 42.97 % NDF with 63.86 % DDM, 59.39% TDN, RFV =139.6 and 0.61 Mcal/lb NEl. Ates E. (2011) mentioned that chemical content of dry matter from *Trifolium arvense* plants was 18.40-19.27% CP, 27.37-28.23% CF, 2.87-3.13% Ca, 0.27-0.28% P; from *Trifolium hybridum* plants – 18.97-19.57% CP, 23.83-24.67% CF, 1.92-2.13% Ca, 0.38-0.39% P; from *Trifolium medium* plants – 18.90-20.00% CP, 26.03-27.33% CF, 1.63-1.67% Ca, 0.43-0.44% P; from *Trifolium dubium* plants – 15.00-15.77% CP, 23.50-24.33% CF, 1.60-1.72% Ca, 0.35% P; from *Trifolium campestre* plants – 17.07-1.90% CP, 24.20-28.07% CF, 2.10-2.63% Ca, 0.36-0.37% P, respectively.

Homolka P. *et al*, (2012) found that the dry matter content, the concentrations of nutrients and energy of the red clover plants were 172.9 g/kg DM, 89.20% OM, 17.74% CP, 2.13% EE, 23.71 % CF, 41.80% NDF, 29.39% ADF, 4.95% ADL, 27.60% NSC with 75.4% *in vitro* digestibility of organic matter, 70.7% *in vivo* digestibility of organic matter, 17.74 MJ/kg GE, 11.84 MJ/kg DE, 9.57 MJ/kg ME and 5.67 MJ/kg NEl, but in lucerne forage, there was 237.1 g/kg DM, 89.03% OM, 14.55% CP, 2.06 % EE, 31.34% CF, 44.41% NDF, 34.48% ADF, 8.37% ADL, 28.01% NSC with 70.3% *in vitro* digestibility of organic matter, 65.6% *in vivo* digestibility of organic matter, 17.89 MJ/kg GE, 11.86 MJ/kg DE, 9.75 MJ/kg ME and 5.82 MJ/kg NEl. Lang J.& Vejražka K. (2012) mentioned that *Trifolium hybridum* yield was 9.19 t/ha DM with 21.8 % CP, 19.95% CF and 6.65 MJ/kg NEl; *Trifolium pratense* cultivars 2.75-16.42 t/ha DM with 17.35-21.26 % CP, 21.25-29.53% CF and 5.51- 6.14 MJ/kg NEl; *Trifolium repens* cultivars 1.89-8.35 t/ha DM with 22.72-24.06 % CP, 19.10-26.48% CF and 5.91- 6.6.56 MJ/kg NEl; *Trifolium ambiguum* yield was 2.73-3.17 t/ha DM with 17.28-18.38 % CP, 21.78-27.88% CF and 5.85-6.38 MJ/kg NEl. Küchenmeister K. (2013) reported that the nutrient content of *Trifolium repens* plants was: 24.0-254.4% CP, 31.5-36.6% NDF, 26.3-27.4% ADF, 6.1-6.7% WSC. Dandikas V. *et al*, (2015)

mentioned that the nutrient composition of the tested red clover mass was 80.5-85.1% OM, 13.3-23.3% CP, 1.5-2.7 % EE, 18.4-27.7% CF, 4.5-6.2% starch, 6.3-10.6% reducing sugars, 33.6-53.6% NDF, 27.9-39.8% ADF, 5.2-7.2% ADL.

Heuze V. *et al*, (2015, 2019), reported the average feed value of *Trifolium pratense* aerial part was: 190 g/kg dry matter, 19.7% CP, 3.5% EE, 22.4% CF, 36.4% NDF, 26.6% ADF, 4.1% lignin, 8.3% WSC, 10.4% ash, 14.4 g/kg Ca and 3.4 g/kg P, 74.1% DOM, 18.4 MJ/kg GE, 13.1 MJ/kg DE and 10.4 MJ/kg ME, but *Trifolium repens* 168 g/kg dry matter, 24.8% CP, 2.7% EE, 19.6% CF, 27.5% NDF, 22.1% ADF, 3.9% lignin, 9.2% WSC, 11.3% ash, 10.1 g/kg Ca and 3.3 g/kg P, 80.9% DOM, 18.3 MJ/kg GE, 14.2 MJ/kg DE and 11.1 MJ/kg ME. Golubeva O.A *et al*, (2016) mentioned that *Trifolium pratense* plants contained 12.0-15.0% CP, 22.2-27.8 % CF, 0.5-0.8 nutritive units/kg with 9.5-9.8 MJ/kg ME and 89-120 g digestible protein/ nutritive units, *Trifolium hybridum* – 16.0% CP, 25.8% CF, 0.7 nutritive units/kg with 9.1 MJ/kg ME and 114 g digestible protein/ nutritive units, but *Trifolium repens*, respectively, 20.8% CP, 32.1% CF, 0.8 nutritive units/kg with 9.9 MJ/kg ME and 118 g digestible protein/ nutritive units.

Teleuță A. & Ţîţei V. (2016) established that the biochemical composition and energy concentration of *Trifolium repens* plants was: 11.38% CP, 2.10% EE, 42.00% CF, 38.44% NFE, 6.08% ash and 8.05 MJ/kg ME.

Ergon A. *et al*, (2017) mentioned that the nutritive value of dry matter from *Trifolium pratense* was 17.8-20.6% CP, 34.2-39.5% NDF, 29.1-30.5% ADF, 14.9-17.0% WSC, 32.5-35.2% NFC, 5.89-6.31 MJ/kg NEl and from *Trifolium repens* 16.3-22.6% CP, 36.9-43.8% NDF, 27.6-29.3% ADF, 14.1-18.7% WSC, 30.7-32.3% NFC, 5.93-6.24 MJ/kg NEl. Tambara A.A.C. *et al*. (2017) reported that the nutrient content of dry matter from *Trifolium pratense* was 24.08% CP, 33.72% NDF and 19.97% ADF, but from *Trifolium repens* 23.94% CP, 30.75% NDF and 18.91% ADF.

Sheaffer C.C. *et al*, (2018) mentioned that *Trifolium hibridum* contained 17.1-24.4% CP, 28.8-42.0% NDF, 62.9-70.1% *in vitro* DM. Shamanin A.A. *et al.* (2019) revealed that the quality indices of the dry matter from *Trifolium pannonicum* was 111.49 g/kg protein, 221.92 g/kg fibres, 168.10 g/kg sugars and the energy value 9.01 MJ/kg ME.

Wróbel B. & Zielewicz W. (2019) reported that the nutritional value of red clover was: 16.37-17.61% CP, 36.67-38.02% NDF, 27.84-28.77% ADF, 4.47-4.64% ADL, 12.84-13.08% WSC, 23.20-24.30% Cel, 8.53-9.25% HC, 66.49-67.21% DDM. Nechaeva T. *et al*, (2020) mentioned that *Trifolium pannonicum* plants contained 190-230 g/kg dry matter with 14.8-18.5 % CP, 2.1-2.7% EE, 26.0-28.9% CF, 9.7-12.5% ash, 24.9 g/kg Ca, 2.0 g/kg P, 0.88-0.90 fodder units/kg and 10.5-10.7 MJ/kg ME. Hunady I. *et al*, (2021) found that *Trifolium alpestre* dry matter contained 92.57 % OM, 15.08% CP, 4.04 % EE, 24.74% CF, 41.69% NDF, 32.09% ADF; *Trifolium pannonicum* – 94.86 % OM, 13.57% CP, 3.81 % EE, 26.87% CF, 48.18% NDF, 37.98% ADF; *Trifolium rubens* – 96.06 % OM, 15.21% CP, 2.78 % EE, 26.62% CF, 42.98% NDF, 33.82% ADF. Belashova O.V. *et al*, (2024) mentioned that the nutrient concentration in dry matter from *Trifolium hibridum* plants was 216.4 g/kg CP, 6.99 g/kg P; from *Trifolium pratense* plants – 238.8 g/kg CP, 6.48 g/kg P; from *Trifolium repens* plants – 277.3 g/kg CP and 6.52 g/kg P, respectively.

Fodder conservation is necessary in most parts of Earth to maintain feed supply, particularly in autumn and winter seasons. Hay is a valuable fodder for different species of animals, and the nutritional value of hay depends on the plant species, the period and method of harvesting the plants, the drying and storage system of the hay. As a result of the research carried out, Table 2, it was established that the hay from the investigated species is characterized by an optimal concentration of nutrients. *Trifolium hibridum* hay has higher content of crude protein, nitrogen free extract, phosphorus and lower amount of fats and crude cellulose, with higher energy value than other species. *Trifolium pratense* hay has higher content of fats, and *Trifolium repens* contains higher amounts fats and crude cellulose, ash and calcium and lower energy value. *Trifolium pannonicum* hay has higher concentration of crude protein and nitrogen free extract as compared with *Trifolium pratense* and *Trifolium repens* hay.

Several literature sources describe the biochemical composition and nutritional performance of hay from *Trifolium* species. According to Medvedev P.F.& Smetannikova A.I. (1981), *Trifolium hybridum* hay contained 19.0% CP, 1.26 % EE, 26.7% CF, 35.5 % NFE, 7.2- % ash and *Trifolium repens* hay 17.9-23.7% CP. Kiraz A.B. (2011) reported that *Trifolium repens* hay contained 15.08% CP, 9.77% ash, 3.29% EE, 41.06 % NDF, 33.15% ADF, 63.07% DMD, 2.435 Mcal/kg ME and RFV=143, but *Trifolium incarnarium* hay – 16.74% CP, 10.69% ash, 1.95% EE, 38.48 % NDF, 36.40% ADF, 60.54% DMD, 2.346 Mcal/kg ME and RFV=146, respectively. Heuze V. *et al*, (2015, 2019) mentioned that red clover hay contained 18.3% CP, 2.5% EE, 27.4% CF, 37.7% NDF, 28.3% ADF, 6.0% lignin, 6.5% ash,

13.5 g/kg Ca, 9.0 g/kg P, 67.2% DOM, 19.0 MJ/kg GE, 11.9 MJ/kg DE and 9.5 MJ/kg ME, but white clover hay – 22.7% CP, 23.4% CF, 29.4% NDF, 28.8% ADF, 3.5% lignin, 12.3% ash, 65.1% DOM, 17.4 MJ/kg GE, 10.7 MJ/kg DE and 8.4 MJ/kg ME. Zvereva G.K. (2016) in a comparative study on the loss of aerial organs and their parts during haymaking and the wilting intensity of cut shoots in perennial legume plants, found that in *Galega orientalis*, *Trifolium pratense* and *Trifolium pannonicum* leaves are larger, the intensity of shedding of vegetative organs in hay is lower (9-13%), the amount of shed leaves in the leaf mass reached 2-6%, after mowing, their leaves lose moisture more quickly than petioles, but more intense loss of vegetative and generative organs was observed in *Medicago* and *Melilotus* plant species (22-30% of the total weight); the process of drying of shoots and leaves of legume grasses was uneven, *Medicago varia*, *Melilotus albus* and *Melilotus officinalis* plants had smaller leaves; the loss of their vegetative organs at drying was greater; their petioles dried out faster than the leaf blades. Nechaeva T. *et al*, (2020) mentioned that Hungarian clover hay had 11.2-15.9 % CP, 1.5-3.2% EE, 25.2-35.6% CF, 8.8-11.1% ash.

Table 1.

The biochemical composition and the fodder value of the green mass from the studied *Trifolium* species

Indices	*Trifolium hibridum*	*Trifolium pratense*	*Trifolium repens*	*Trifolium pannonicum*	*Trifolium alpestre*
Crude protein, % DM	*16.54*	*20.68*	*18.23*	*19.15*	*14.99*
Crude fats, % DM	*3.17*	*2.98*	*2.71*	*2.56*	*2.72*
Crude cellulose, % DM	*22.44*	*23.79*	*31.29*	*29.74*	*26.78*
Nitrogen free extract, % DM	*49.29*	*41.23*	*35.27*	*37.77*	*46.58*
Ash, % DM	*8.56*	*11.32*	*12.50*	*10.78*	*8.93*
Calcium, % DM	*1.14*	*1.48*	*2.28*	*1.72*	*1.16*
Phosphorus, %	*0.26*	*0.23*	*0.18*	*0.16*	*0.24*
Gross energy, MJ/ kg	*18.43*	*18.09*	*17.86*	*18.15*	*17.96*
Metabolizable energy, MJ/ kg	*9.96*	*9.15*	*8.31*	*8.41*	*9.38*
Net energy for lactation, MJ/ kg	*5.76*	*5.29*	*4.63*	*4.71*	*5.36*

Table 2.

The biochemical composition and the fodder value of the hay from the studied *Trifolium* species

Indices	*Trifolium hibridum*	*Trifolium pratense*	*Trifolium repens*	*Trifolium pannonicum*
Crude protein, % DM	*19.69*	*16.84*	*16.87*	*17.18*
Crude fats, % DM	*1.59*	*2.51*	*1.63*	*1.58*
Crude cellulose, % DM	*26.90*	*32.15*	*33.96*	*31.24*
Nitrogen free extract, % DM	*42.40*	*38.51*	*35.08*	*39.75*
Ash, % DM	*9.42*	*9.99*	*12.46*	*10.25*
Calcium, % DM	*1.26*	*1.82*	*2.26*	*1.75*
Phosphorus, %	*0.28*	*0.17*	*0.17*	*0.16*
Gross energy, MJ/ kg	*18.17*	*18.18*	*17.61*	*18.19*
Metabolizable energy, MJ/ kg	*8.58*	*8.30*	*7.84*	*8.20*
Net energy for lactation, MJ/ kg	*4.89*	*4.62*	*4.33*	*4.59*

Table 3.

The biochemical biomethane production potential of green mass substrates from the studied *Trifolium* species

Indices	*Trifolium hibridum*	*Trifolium pratense*	*Trifolium repens*	*Trifolium pannonicum*	*Trifolium alpestre*
Carbon, g/kg DM	*508.00*	*492.66*	*486.11*	*49567*	*505.94*
Nitrogen, g/kg DM	*26.46*	*33.09*	*29.17*	*30.64*	*23.98*
Ratio carbon/nitrogen	*19.20*	*14.89*	*16.66*	*16.18*	*21.10*
Biodegradable protein, g/kg DM	*125.70*	*157.17*	*138.55*	*145.54*	*113.92*
Biodegradable fats, g/kg DM	*19.02*	*17.88*	*16.26*	*15.36*	*16.32*
Biodegradable carbohydrates, g/kg DM	*493.00*	*445.62*	*453.40*	*443.43*	*486.74*
Biogas potential, L/kg VS	*501*	*498*	*476*	*471*	*485*
Biomethane potential, L/kg VS	*271*	*266*	*261*	*260*	*262*
Methane content, % DM	*54.20*	*53.80*	*54.80*	*55.10*	*54.00*

Biogas is obtained by the anaerobic digestion of organic matter in special installations called digesters, which are designed and built in different shapes and sizes, depending on the type and quantity of raw material used as substrate. These digestion tanks contain complex bacterial cultures that break down (digest) organic matter to produce biogas, which consists of a relatively high percentage of methane (CH_4), the main component of natural gas, as well as carbon dioxide (CO_2), hydrogen sulphide (H_2S), water vapour, traces of other gases and digestate – the by-product of the anaerobic digestion process, which has many useful applications, as a nutrient-rich fertilizer. The biomethane produced from plant mass has a great importance and can successfully replace natural gas to obtain electric power and heat, can be compressed and used as vehicle fuel. The stability and the productivity of biogas digesters are mostly influenced by nutrient content and its biodegradability, and the carbon to nitrogen ratio (C/N) of the substrate. It is known the optimal C/N ratio in substrate should range from 10 to 30, which does not affect the development of bacteria involved in anaerobic digestion. The quality indices of the green mass substrates from the investigated *Trifolium* species and their biochemical methane potential are shown in Table 3. The carbon to nitrogen ratio in the investigated *Trifolium* substrates varied from 14.89 to 21.10, therefore, it met the established standards. The calculated biochemical biomethane potential of investigated substrates varied from 260 l/kg to 271 l/kg VS. The best biomethane potential was achieved in *Trifolium hybridum* substrate due to the higher content of fats and carbohydrates.

Several publications have documented the biomethane potential of *Trifolium* substrates. According to Lehtomäki A. (2006) the methane potentials of the *Trifolium pratense* substrates were 280-300 l/kg VS. Adamovics A. (2014) mentioned that the methane yield of red clover was 222.8-245.4 l/kg. Wahid R. *et al*, (2015) reported that the cumulative methane yield in red clover substrates varied from 263 to 328 l/kg VS, but grass-clover mixture – from 320 to 352 l/kg, respectively. Dandikas V. *et al*. (2015) remarked that the studied fresh mass substrates from *Trifolium pratense* contained 13.3-23.3% CP, 5.2-7.2% ADL and methane yield reached 273-346 l/kg VS, but the substrates from *Trifolium repens* plants 17.3-29.0% CP, 6.5-8.8% ADL and the methane yield was 265-320 l/kg VS, respectively. Teleuță A. &Ţîţei V. (2016) reported that the calculated gas forming potential of the fermentable organic matter of the *Trifolium repens* ecotype was 470 l/kg VS and the methane yield – 247 l/kg VS. Santamaría-Fernández M. *et al*, (2018) revealed that the methane yield of red clover residual press cake after protein extraction was 219-375 l/kg. Stinner W. *et al*, (2021) mentioned that the green mass substrate from *Trifolium alexandrinum* had 69% degradability and 308 l/kg VS methane yields, from *Trifolium pratense* 66-72% degradability and 278-316 l/kg VS methane yields. Hunady I. *et al*, (2021) calculated the theoretical methane yield and revealed that the values of biomass from *Trifolium pannonicum*, *Trifolium rubens* and *Trifolium alpestres*, *Trigonella foenum-graecum* and *Melilotus albus* ranged from 0.130 to 0.140 m^3/kg VS, the methane yield of the biomass from *Onobrychis viciifolia*, *Astragalus cicer*, *Dorycnium germanicum* and *Vicia sylvatica* ranged from 0.141 to 0.160 m^3/kg VS. Lallement A. *et al*, (2022) revealed that the methane potential of millet substrate was 267.9 l/kg VS, but the mix of millet and clover reached was 309.7 l/kg VS. In our previous research (Ţîţei V., 2023), we found that the biochemical methane yield of *Trifolium alexandrinum* substrates was 327-340 l/kg VS and *Trifolium pratense* substrates 364-365 l/kg VS. Li W. *et al*, (2023) remarked that the red clover substrate contained 5.96 g/kg N, 71 g/kg HC, 353 g/kg Cel, 42 g/kg ADL with practical biomethane potential 276.87 l/kg VS.

CONCLUSIONS

The studied local ecotypes of *Trifolium alpestre*, *Trifolium hibridum*, *Trifolium pannonicum*, *Trifolium pratense* and *Trifolium repens* can serve as starting material in breeding and implementing new varieties of leguminous species in the production of protein rich forages, as well as feedstock for biomethane production as a source of renewable energy.

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