THE AGROECONOMICAL VALUE OF *Trifolium alexandrinum* AND *Trifolium pratense*, IN THE REPUBLIC OF MOLDOVA

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Abstract. The aim of this study was to evaluate the quality of harvested green mass, ensiled mass and hay from Romanian cultivars of berseem clover *Trifolium alexandrinum* 'Viorel' and red clover *Trifolium pratense* 'Select 2', grown in an experimental field of the National Botanical Garden (Institute), Chișinău, Republic of Moldova. It was determined that the dry matter nutrient content of the harvested mass varied among the clover species: 145-169 g/kg CP, 232-279 g/kg CF, 89-100 g/kg ash, 252-302 g/kg ADF, 422-479 g/kg NDF, 31-51 g/kg ADL, 140-222 g/kg TSS, 221-251 g/kg Cel, 170-177 g/kg HC with nutritive and energy values 65.4-69.2 % DDM, RFV=127-153, 12.84-13.52 MJ/kg DE, 10.54-11.10 MJ/kg ME and 6.56-7.12 MJ/kg NEl. The hay prepared from *Trifolium* species contained 168-183g/kg CP, 244-289 g/kg CF, 95-106 g/kg ash, 265-303 g/kg ADF, 415-437 g/kg NDF, 34-47 g/kg ADL, 113-161 g/kg TSS with 65.3-68.3 % DDM, RFV=139-153, 12.82-13.36 MJ/kg DE, 10.53-10.97 MJ/kg ME and 6.54-6.98 MJ/kg NEl. The nutrient content of ensiled clovers mass was: 147-206 g/kg CP, 226-266 g/kg CF, 112-159 g/kg ash, 239-283 g/kg ADF, 422-452 g/kg NDF, 29-43 g/kg ADL, 106-153 g/kg TSS, 210-250 g/kg Cel, 159-183 g/kg HC, 66.9-70.3 % DDM, RFV=138-155, 13.11-13.72 MJ/kg DE, 10.76-11.26 MJ/kg ME and 6.74-7.28 MJ/kg NEl The fresh and ensiled clover mass substrates for anaerobic digestion had optimal C/N=14-22 and biochemical methane potential varied from 327 to 375 l/kg ODM.

Keywords: biochemical composition, biomethane potential, fodder value, *Trifolium alexandrinum*, *T. pratense*.

Rezumat. Valoarea agroeconomică a speciilor de *Trifolium alexandrinum* și *Trifolium pratense* în Republica Moldova. Scopul prezentului studiu a constatat în evaluarea calității masei proaspete recoltate, a masei însilozate și fânului preparat din soiuri românești: trifoi de Alexandria *Trifolium alexandrinum* 'Viorel' și trifoi roșu *Trifolium pratense* 'Select 2' cultivate în cultură pură pe câmpul experimental din Grădina Botanică Națională (Institut), Chișinău, Republica Moldova. S-a stabilit că conținutul de nutrienți în substanță uscată a masei proaspete recoltate variază în dependență de specia de trifoi: 145-169 g/kg proteină brută (CP), 232-279 g/kg celuloză brută (CF), 89-100 g/kg cenușă, 252-302 g/kg fibre solubile în detergent acid (ADF), 422-479 g/kg fibre solubile în detergent neutru (NDF), 31-51 g/kg lignină sulfurică (ADL), 140-222 g/kg zaharuri solubile (TSS), 221-251 g/kg celuloză (Cel), 170-177 g/kg hemiceluloză (HC), 65.4-69.2 % substanță uscată digestibilă (DDM), valoare relativă furajeră RFV=127-153, 12.84-13.52 MJ/kg energie digestibilă (DE), 10.54-11.10 MJ/kg energie metabolizantă (ME) și 6.56-7.12 MJ/kg energie netă pentru lactație (NEl). Fânul preparat din speciile de *Trifolium* conține 168-183g/kg CP, 244-289 g/kg CF, 95-106 g/kg cenușă, 265-303 g/kg ADF, 415-437 g/kg NDF, 34-47 g/kg ADL, 113-161 g/kg TSS, 65.3-68.3 % DDM, RFV=139-153, 12.82-13.36 MJ/kg DE, 10.53-10.97 MJ/kg ME și 6.54-6.98 MJ/kg NEl. Conținutul de nutrienți în furajul murat fiind de 147-206 g/kg CP, 226-266 g/kg CF, 112-159 g/kg cenușă, 239-283 g/kg ADF, 422-452 g/kg NDF, 29-43 g/kg ADL, 106-153 g/kg TSS, 210-250 g/kg Cel, 159-183 g/kg HC, 66.9-70.3 % DDM, RFV=138-155, 13.11-13.72 MJ/kg DE, 10.76-11.26 MJ/kg ME și 6.74-7.28 MJ/kg NEl. Substraturile de masă proaspătă și masă murată pentru digestia anaerobă se caracterizează printr-un raport optimal de carbon și azot de C/N=14-22, iar potențialul biochimic de obținere a metanului variază de la 327 la 375 l/kg materie organică.

Cuvinte cheie: compoziția biochimică, potențial biometan, valoarea nutritivă a furajului, *Trifolium alexandrinum*, *T. pratense*.

INTRODUCTION

World plant biodiversity, namely, its conservation and prospects for practical use are becoming increasingly pressing problems in the 21st century. *Fabaceae* plants have gained a lot of attention recently because they contribute to the accumulation of biological nitrogen in soil, the improvement of physical and chemical properties of the soil, the formation and restoration of its structure and play an important role in increasing the quality of food and fodder, containing a significant amount of protein, vitamins and minerals. A lot of them are used as honey, medicinal and ornamental plants, also as cover crops and feedstock for biorefineries, as a source of renewable energy. In the European Union, the interest in leguminous plants has increased for several economic and environmental reasons (The resolution of the European Parliament, 2018; ECPGR, 2021).

The *Trifolium* genus comprises 244 accepted species names out of which 25 species are of agricultural importance. In the spontaneous flora of the Basarabia the family *Fabaceae* is represented by 146 species of 35 genera, including 20 species of the genus *Trifolilum* (IZVERSCAIA, 2020). In natural grasslands of România, there are 21 genera of legumes with 198 species and 581 taxa, including the *Trifolilum* genus with 38 species, 2 subspecies, 32 varieties and 71 forms, to which a huge number of ecotypes must be added, providing an image of the genetic resources which await investigation (MARUȘCA, 1999).

The most known and common species is red clover *Trifolium pratense* L., perennial plant, native to Europe. The root collar is multi-stemmed with numerous basal leaves (rosette), from whose axils the shoots start, usually branched, glabrescent or hairy, fistulous or full, with 3-9 internodes and a height of 35-85 cm. The habit is bushy, it may be tighter or looser, with 25-35 green or reddish shoots, of different shades and intensities, depending on their anthocyanin content; they are erect, semi-sprawling or sprawling. The leaves are alternate, compound, with three leaflets. The basal leaves have longer petioles, and those from the some – shorter petioles. The leaflets are sub-sessile,

ovate or elliptical, rarely – obovate, hairy on the lower side and on margins, 15-50 mm long and 14-18 mm wide; on the upper side on each leaflet, there is an arrow-shaped white or light-green spot, which covers up to 2/3 of the surface, named macula. The stipules at the base of the petiole are oval, acute at the tip and ciliated, fused with the petiole, pale, with visible green or reddish nerves. The flowers are hermaphrodite, 13-16 mm, grouped by 40-160 in spheroid heads, surrounded at the base by an involucre formed by two-three trifoliate leaves. The flowers are sessile, purple-red, solitary or double, bractate. They bloom in May - June; allogamous pollination. Fruit – a triangular ovate pod, monospermous, 1.8-2.5 mm long. The seeds are ovate or oblong-ovate, weakly edged, asymmetrical, with a yellow-brown tegument, with a violet base, with an obvious gloss, which fades with ageing and the colour turns brown. Immature seeds are yellow-green. The seeds are 1.6-2.3 mm long, 1.2-1.8 mm wide and 0.7-1.2 mm thick. The weight of 1000 seeds in diploid forms is 1.1-2.2 g, and in tetraploid forms it can exceed 3 g. The plant has a taproot, with numerous adventitious roots, on which there are nodules with the bacteria *Rhizobium leguminosarum bv.trifolii* that fix atmospheric nitrogen and consume the carbohydrates of the host plant; one plant has over 100 nodules, the capacity to fix atmospheric nitrogen - between 185-375 kg/ha per year. Red clover is a mesophyte, the amount of water required for germination reaches 120% of the weight of the seed, it possesses moderate tolerance to drought, for optimal development it needs an amount of rainfall of over 400 mm/year, which is higher as compared with alfalfa, but it easily tolerates long-term flooding (SAVATTI & ROTAR, 2014; HEUZÉ et al., 2015; IZVERSCAIA, 2020; REVENCO & ŢÎŢEI, 2021). The selected forms and the created Romanian cultivars of red clover have a productivity of 13-19 t/ha dry matter with 17.3-19.6% CP, 69% DMD, 1.07 UFL/kg, 1.530 kcal/kg net energy (MARUŞCA et al., 2011; SAVATTI & ROTAR, 2014). This species is almost always present in the mixtures of grassland species used in Europa, as well as in the scientific recommendations developed and implemented by the Research and Development Institute for Grasslands Braşov, Romania (MARUŞCA et al., 2014).

Trifolilum alexandrinum L. is known as berseem clover, Egyptian clover or Alexandria clover and is an annual legume species native to western Asia. It has erect or ascending sparsely hairy stems, 60–80 cm tall, hollow, branching at the base, with alternate leaves bearing 4-5 cm long and 2-3 cm broad leaflets. Flowers are yellowish-white and form dense, elliptical clustered heads about 2 cm in diameter. The flowers must be cross-pollinated by honey bees to produce seeds. The fruit is a pod containing one single yellow to purplish-red seed. It has a shallow taproot. It can withstand some drought and short periods of waterlogging. It does better than alfalfa in high moisture soils and is very productive under irrigation. It is moderately tolerant of salinity and can grow on a wide range of soils, though it prefers fertile, loamy to clay soils with mildly acidic to slightly alkaline pH (6.5-8). Berseem clover has some frost tolerance, down to -6°C and as low as -15°C for some cultivars. It has similar nutritive value to alfalfa, without causing bloat in ruminant species. It is a good protein source (15-20% DM) grazed fresh or cut for hay and/or ensiled. It can also be used as a cover and green manure crop due to its vigorous growth and nitrogen-fixation ability. It is “bee-friendly” since blossoms of berseem clover, unlike alfalfa, have no tripping mechanism and provide a diet meeting all their nutritional needs. Due to fast germination and tendency for winter-killing, berseem clover can be used as a “nurse crop” for alfalfa plantings. It is also suitable to sow in a mixture with winter cereals, such as oats, to make high quality silage or hay. Today, berseem clover is extensively cultivated in the Mediterranean Basin, the Indian sub-continent and southern USA, under irrigated and rainfed farming conditions due to its excellent feeding value, high growth rate and good regrowth potential after cutting or grazing. This species has an advantage over other annual legumes species, including annual medics and peas, of providing multiple harvests during the growing season (MEDVEDEV & SMETANNIKOVA, 1981; HEUZÉ et al., 2016). Introduced in Romania *Trifolilum alexandrinum,* as forage crop, has been researched in several university and scientific centres (ZAMFIR et al., 2001; MOGA et al., 2007; COJOCARIU et al., 2008; MARIAN et al., 2008).

No clovers cultivars are registered in the Catalogue of Plant Varieties of the Republic of Moldova.

The aim of this study was to evaluate the quality of harvested green mass, ensiled mass and prepared hay of the *Trifolium alexandrinum* and *Trifolium pratense* and the prospects of using them as feed for ruminant animals and as feedstock for the production of renewable energy.

MATERIALS AND METHODS

The Romanian cultivars of berseem clover *Trifolium alexandrinum* 'Viorel', created at the National Agricultural Research and Development Institute Fundulea, Romania and red clover *Trifolium pratense* 'Select 2', created at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania cultivated in the experimental plot of the “Alexandru Ciubotaru” National Botanical Garden (Institute), Chişinău, latitude 46°58′25.7″N and longitude N28°52′57.8″E, served as subjects of the research, and the traditional fodder crop – common sainfoin *Onobrychis viciifolia* 'Anamaria' created at the Research-Development Station for Pastures Vaslui, Romania was used as control. The experimental design was a randomized complete block design with four replications, and the experimental plots measured 50 m^2. The seeds were sown in late March, at a depth of 2.0 cm, at a distance between rows of 15 cm. The berseem clover green mass samples were collected in the flowering stage, red clover green mass samples were collected at the 1st cut, in the flowering stage, in the second growing season, while common sainfoin in the second growing season in the budding-flowering stage. The hay was dried directly in the field. The clover silages were prepared directly from harvested green mass, but common sainfoin haylage was produced from wilted green mass,

cut into small pieces and compressed in glass containers. The containers were stored for 45 days, and after that, they were opened and the organoleptic assessment and the determination of the organic acid composition of the persevered forage were done in accordance with the Moldavian standard (SM 108). The fresh mass and fermented fodder 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 analysing such indices as: crude protein (CP), crude fibre (CF), crude ash (CA), total soluble sugars (TSS), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL) 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) and cellulose (Cel), digestible dry matter (DDM), the 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 et al., (1979). The biochemical methane potential was calculated according to DANDIKAS et al., (2015).

RESULTS AND DISCUSSIONS

Analysing the results of the study on the harvested mass quality of the above-mentioned *Trifolium* species, Table 1, we found that the nutrient content and fodder energy value were: 145-169 g/kg CP, 232-279 g/kg CF, 89-100 g/kg ash, 252-302 g/kg ADF, 422-479 g/kg NDF, 31-51 g/kg ADL, 140-222 g/kg TSS, 221-251 g/kg Cel, 170-177 g/kg HC, 65.4-69.2 % DDM, RFV=127-153, 12.84-13.52 MJ/kg DE, 10.54-11.10 MJ/kg ME and 6.56-7.12 MJ/kg NEl, while for *Onobrychis viciifolia* fodder – 177 g/kg CP, 293 g/kg CF, 96g/kg ash, 309 g/kg ADF, 447 g/kg NDF, 49 g/kg ADL, 114 g/kg TSS, 260g/kg Cel, 135g/kg HC, 64.8 % DMD, RFV=135, 12.73MJ/kg DE, 10.45 MJ/kg ME, 6.48 MJ/kg NEl, respectively. The red clover plants contained an optimal concentration of crude protein and a low amount of structural carbohydrates, lignin, due to which it had higher digestibility, relative feed value and energy concentration as compared with the berseem clover. The biochemical composition and the fodder quality of *Trifolium alexandrinum* did not differ significantly in comparison with *Onobrychis viciifolia* fodder. Different results regarding the biochemical composition and the nutritive value of the harvested mass from *Trifolium* species are given in the specialized literature. According to MEDVEDEV & SMETANNIKOVA (1981) the *Trifolium alexandrinum* dry matter contained 16-23.50 % CP, 20.62 % CF, 15.87 % ash. HADJIPANAYIOTOU et al., (1983) mentioned that, in Cyprus, the dry matter content and chemical composition of *Trifolium alexandrinum* forage was 250 g/kg DM, 19.4 % CP, 2.4 % fat, 17.4 % CF, 35.0 % NDF, 27.0 %, ADF, 4.1 % lignin, 13.3 % ash, 1.6% Ca, 0.33%P, 61.0% OMD and 11.1 MJ/kg DE. BURLACU et al., (2002) remarked 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. COJOCARIU et al., (2008) reported that the chemical composition of the berseem clover forage was 23.4-29.8 % CP, 2.82-3.42 % fat, 30.45-32.25 % CF, 33.50-36.65 % ADF, 52.70-54.60 % NDF, 9.72-18.45 % ash, 1.28-1.40% Ca, 0.28-0.56%P. DEWHURST et al., (2009) compared the feed quality of clover species harvested in early flowering period and 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 et al., (2009) reported that red clover 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. MOHSEN et al., (2011) mentioned that chemical composition of berseem clover first cut mass was 16.93% CP, 3.11 % EE, 27.32 % CF, 38.41% NFE, 14.23% ash, while for second cut forage 18.40% CP, 3.28 % EE, 26.40 % CF, 37.20% NFE and14.70% ash. HOMOLKA et al., (2012) found that the dry matter content, the concentrations of nutrients and energy of the *Trifolium pratense* 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 *Medicago sativa* 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. DANDIKAS et al., (2015) mentioned that the nutrient composition of the tested red clover plants 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 et al. (2015, 2016), reported the average feed value of *Trifolium pratense* aerial part was: 19.0% 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 alexandrinum* 12.5% dry matter, 19.9% CP, 3.2% EE, 23.3% CF, 44.8% NDF, 27.6% ADF, 5.1% lignin, 5.1% WSC, 15.4% ash, 19.3 g/kg Ca and 2.7 g/kg P, 73.0% DOM, 17.4 MJ/kg GE, 12.2 MJ/kg DE and 9.6 MJ/kg ME. KUMAR et al., (2015) remarked that the chemical composition and nutritive value of berseem clover 89.91% OM, 17.74% CP, 2.83 % EE, 43.28% NDF, 24.08% ADF, 7.09 % ADL, 19.20% HC, 15.04% Cel, 10.09% ash, 8.51 MJ/kg ME, 614.4 g/kg TDDM, 637.6 g/kg TDOM. SALAMA (2015) revealed that the nutrient composition of berseem clover plants was 17.60% CP, 39.91% NDF, 27.20% ADF, 4.09% ADL. BOZHANSKA et al., (2016) mentioned that the chemical composition and energy nutritional value of *Trifolium alexandrinum* was 11.18% CP, 3.02 % EE, 33.44% CF, 42.86% NDF, 30.95% ADF,

6.13 % ADL, 11.91% HC, 24.82% Cel, 12.24% ash, 15.93 MJ/kg net energy, 6.71 MJ/kg exchangeable energy, 0.61 feed unit of milk and 0.55 feed unit of growth, but *Trifolium incarnatum,* respectively, 15.24% CP, 3.61 % EE, 21.68% CF, 31.16% NDF, 20.81% ADF; 3.08 % ADL, 10.35% HC, 17.73% Cel, 11.85% ash, 15.81 MJ/kg net energy, 7.34 MJ/kg exchangeable energy, 0.69 feed unit of milk and 0.53 feed unit of growth. In our previous research, TELEUŢĂ & ŢÎŢEI (2016), it was 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, but *Onobrychis viciifolia* plants 17.44% CP, 3.39 % EE, 33.50% CF, 39.43% NFE, 6.24% ash and 10.44 MJ/kg ME. TAMBARA et al., (2017) reported that the biochemical composition of dry matter from *Trifolium pratense* plants 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. BACCHI et al., (2021) mentioned that *Trifolium alexandrinum* herbages mowing in the incoming flowering stage contained 178.3 g/kg DM with 19.04 % CP, but in pod filling stage 290.2g/kg DM with 18.43 % CP. HUNADY 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, while *Onobrychis viciifolia* plants – 91.18 % OM, 12.47% CP, 2.72 % EE, 26.08% CF, 47.97% NDF, 34.48% ADF. ARIF et al., (2022) revealed that nutritive qualities under varying seed rates and phosphorus fertilization of berseem clover were characterized by 17.05-19.20% CP, 59.66-62.46 %TDN, RFV= 120.90-137.46 and 1.45-151 Mcal/ kg net energy for lactation. PATIDAR et al., (2022) found that berseem forage contained 10.16% ash, 17.69% CP, 1.84 % EE, 43.82 % NDF, 32.40% ADF, 11.46% starch with 57.14 % TDN, 17.34 MJ/kg GE, 10.79 MJ/kg DE and 9.00 MJ/kg ME. RADY et al., (2022) reported that nutritive value of *Trifolium alexandrinum* plants was 826.5 g/kg OM, 15.53% CP, 1.71 % EE, 13.69% NSC, 55.32 % NDF, 49.01% ADF, 6.31%HC, 33.50%Cel, 15.51% lignin, 759.4 g/kg TDDM, 733.9 g/kg TDOM. AHMED et al., (2023) mentioned that the dry matter content and chemical composition of *Trifolium alexandrinum* forage was 259.9 g/kg DM, 88.47 % OM, 16.45 % CP, 51.96 % NDF, 24.41 %, ADF, 5.84 % ADL, 23.57 % Cel, 25.55 % HC. ZAIŢ et al., (2023) found that that forage quality of red clover in monoculture was 15.72-17.01% CP, 40.37-49.57 % NDF, 32.97-38.66% ADF and RVQ=116.7-158.6, but in mixture of red clover and tall fescue respectively 13.57-15.43% CP, 51.05-60.89 % NDF, 30.07-36.80% ADF and RFQ=100.0-137.2 (Table 1).

Table 1. The biochemical composition and the feed value of green mass of the studied *Fabaceae* species.

Indices	*Trifolium alexandrinum*	*Trifolium pratense*	*Onobrychis viciifolia*
Crude protein, g/kg DM	145	169	177
Crude fibre, g/kg DM	279	231	293
Ash, g/kg DM	100	89	96
Acid detergent fibre, g/kg DM	302	252	309
Neutral detergent fibre, g/kg DM	479	422	447
Acid detergent lignin, g/kg DM	51	31	49
Total soluble sugars, g/kg DM	140	222	114
Cellulose, g/kg DM	251	221	260
Hemicellulose, g/kg DM	177	170	138
Digestible dry matter, g/kg DM	654	692	648
Relative feed value	127	153	135
Digestible energy, MJ/kg DM	12.84	13.52	12.73
Metabolizable energy, MJ/kg DM	10.54	11.10	10.45
Net energy for lactation, MJ/kg DM	6.60	7.12	6.48

Fodder conservation is necessary in most parts of Earth to maintain feed supply, particularly in autumn and winter seasons. Ensiling forages has several advantages relative to harvesting as hay. These include greater opportunity for mechanization, reduced labour cost and less chance of weather losses. During the sensorial assessment, it was found that the clovers silage had olive-brown colour with peculiar smell, similar to pickled vegetables, while the haylage prepared from common sainfoin consisted of yellowish-green leaves and yellow-green stems and it had a pleasant smell like pickled vegetables. The texture of the plant mass stored as silage and haylage was preserved well, without mould and mucus. The results regarding the quality of the ensiled forage are shown in Table 2. It has been determined the clovers silages had pH=4.16-4.22, lower than common sainfoin haylage. We would like to mention that red clover silage was characterised by higher content of crude protein, ash and reduced concentration of cell wall fractions (NDF, ADF, ADL) which had a positive effect on the digestibility, nutritional value and energy supply of the feed. It was found that, during the process of ensiling berseem clover, the level of crude protein, cellulose and hemicellulose did not modify essentially, the concentration of ash and total soluble sugars increased, but the level of fibres and lignin decreased in comparison with the initial green mass. In sainfoin haylage, the concentrations of digestible dry matter and energy were reduced as compared with clover silage. Several studies have evaluated the potential of *Trifolium* species for silage production. BURLACU et al., (2002) remarked that the silage from *Trifolium pratense* harvested in full flowering period contained 215g/kg DM, 87.6% OM, 14.5 % CP, 2.9% EE, 33.1% CF, 37.1% NFE, 45.1% ADF, 8.7 % lignin, 5.6 % HC and 17.8 MJ/kg GE, but prepared haylage respectively 350g/kg DM, 91% OM, 13.1 % CP, 2.8% EE, 33.2% CF, 42.9% NFE, 0.7% sugars, 5% starch and 18.2 MJ/kg GE. MUSTAFA & SEGUIN (2003) compared the feed quality of silages and remarked than berseem clover silage had pH=4.55 and contained 290 g/kg DM, 21.7% CP, 32.7 %

ADF, 50.7 % NDF, 14.5% ash, but alfalfa silage pH=4.87, 329 g/kg DM, 21.7% CP, 34.6 % ADF, 45.0 % NDF and 11.0% ash respectively. DEWHURST et al., (2009) found that the nutritive value of the silage prepared from *Trifolium pratense* plants was 900 g/kg OM, 18.9% CP, 41.3 % NDF, 32.1% ADF, while for the silage prepared from *Trifolium repens* it was 901 g/kg OM, 23.7% CP, 28.3 % NDF, 26.4% ADF respectively. GAAFAR et al., (2011) established that the nutritive value of *Trifolium alexandrinum* silage was: 15.3% CP, 2.5% EE, 25.2% CF, 44.3% NFE, 12.8% ash, 62.6% TDN, 69.1% DDM and 2760 kcal/kg DE. MOHSEN et al., (2011) reported that the chemical composition and nutritive value of the ensiled berseem clover first cut mass was 349 g/kg dry matter, pH=4.22, 15.0% CP, 3.51 % EE, 30.3 % CF, 39.5% NFE, 11.80% ash, 70.7% DDM, 72.1% DOM, 632 g/kg TDN, while the silage from second cut mass 357 g/kg dry matter, pH=4.15, 14.6% CP, 3.45 % EE, 31.0 % CF, 39.7% NFE, 11.20% ash, 69.7% DDM, 69.2% DOM, 617 g/kg TDN. HEUZE et al., (2015), mentioned that red clover silage contained 277 g/kg dry matter, 18.9% CP, 4.1% EE, 27.1% CF, 41.3% NDF, 32.4% ADF, 4.6% lignin, 10.4% ash, 7.9 g/kg Ca, 2.4 g/kg P, 68.6% DOM, 18.9 MJ/kg GE, 12.2 MJ/kg DE and 9.7 MJ/kg ME. MOLONEY et al., (2021), evaluating the quality of red clover silage, mentioned that, when prepared from first cut harvested mass, it contained 149 g/kgDM, 17.6 % CP and 614 g/kg DMD; from second cut mass, 183 g/kgDM, 21.3 % CP and 670 g/kg DMD, while from third cut mass 155 g/kgDM, 18.6 % CP and 660 g/kg DMD. AHMED et al., (2023) mentioned that the dry matter content, fermentation characteristics and chemical composition of *Trifolium alexandrinum* forage after 30-120 days of ensiling was 152.176.8 g/kg DM, pH= 4.5-4.9, 19.1-57.5g/kg lactic acid, 4.7-28.9g/kg butyric acid, 19.4-26.6 g/kg acetic acid, 88.03-88.25 % OM, 14.55-17.61 % CP, 49.39-53.93 % NDF, 33.08-38.48 %, ADF, 8.16-9.36 % ADL, 24.92-29.33 % Cel, 15.43-16.89% HC, 62.23%TDOM (Table 3).

Table 2. The biochemical composition and the nutritive value of the ensiled forage from the studied *Fabaceae* species.

Indices	*Trifolium alexandrinum*	*Trifolium pratense*	*Onobrychis viciifolia*
pH index	4.22	4.16	4.68
Crude protein, g/kg DM	147	206	142
Crude fibre, g/kg DM	266	226	312
Ash, g/kg DM	112	159	118
Acid detergent fibre, g/kg DM	283	239	317
Neutral detergent fibre, g/kg DM	452	422	470
Acid detergent lignin, g/kg DM	43	29	40
Total soluble sugars, g/kg DM	153	106	135
Cellulose, g/kg DM	250	210	277
Hemicellulose, g/kg DM	169	183	153
Digestible dry matter, g/kg DM	669	703	642
Relative feed value	138	155	127
Digestible energy, MJ/kg DM	13.11	13.72	12.63
Metabolizable energy, MJ/kg DM	10.76	11.26	10.37
Net energy for lactation, MJ/kg DM	6.74	7.28	6.38

Table 3. The biochemical composition and the feed value of hay from the studied *Trifolium* species.

Indices	*Trifolium alexandrinum*	*Trifolium pratense*
Crude protein, g/kg DM	168	183
Crude fibre, g/kg DM	289	244
Ash, g/kg DM	106	95
Acid detergent fibre, g/kg DM	303	265
Neutral detergent fibre, g/kg DM	437	415
Acid detergent lignin, g/kg DM	47	34
Total soluble sugars, g/kg DM	113	161
Cellulose, g/kg DM	256	231
Hemicellulose, g/kg DM	134	150
Dry matter digestibility, g/kg DM	653	683
Relative feed value	139	153
Metabolizable energy, MJ/kg DM	12.82	13.36
Digestible energy, MJ/kg DM	10.53	10.97
Net energy for lactation, MJ/kg DM	6.54	6.98

Hay is the best source of energy, protein, vitamins and minerals for farm animals. The nutritional value of hay depends on the plant species, the mowing period, on the techniques and equipment used for hay preparation, tstorage conditions, and many other factors. The nutritive and energy value of the prepared hays from the studied *Trifolium* species are shown in Table 3. We would like to mention that prepared clover hays contained 168-183g/kg CP, 244-289 g/kg CF, 95-106 g/kg ash, 265-303 g/kg ADF, 415-437 g/kg NDF, 34-47 g/kg ADL, 113-161 g/kg TSS with 65.3-68.3 % DDM, RFV=139-153, 12.82-13.36 MJ/kg DE, 10.53-10.97 MJ/kg ME and 6.54-6.98 MJ/kg NEl. *Trifolium pratense* hay was characterized by low content of fibres and lignin and a high level of crude protein, soluble sugar, relative feed value, metabolizable energy and net energy for lactation. Literature sources indicate variation in the chemical composition and nutritional value of hay from *Trifolium* species. GAAFAR et al., (2011) remarked that the nutritive value of the hay prepared from *Trifolium alexandrinum* was: 14.4% CP, 2.45% EE, 27.6% CF, 45.2% NFE, 10.3% ash,

61.1% TDN, 65.2% DDM and 2694 kcal/kg DE. DAS et al., (2015), reported that the nutritional value of *Trifolium alexandrinum* hay was: 88.10% OM, 15.69% CP, 2.26% EE, 28.59% CF, 41.56% NFE, 53.98% NDF, 37.90% ADF; 7.01% ADL, 16.08% HC, 30.89% Cel, 11.89% ash. HEUZE et al., (2015, 2016), 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, while berseem clover hay 15.7% CP, 2.4% EE, 26.9% CF, 49.3% NDF, 30.2% ADF, 13.7% ash, 21.9 g/kg Ca and 2.4 g/kg P, 63.1% DOM, 17.5 MJ/kg GE, 10.4 MJ/kg DE and 8.3 MJ/kg ME. ZVEREVA (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 grasses in the forest-steppe of West Siberia, found that in the leguminous plants *Galega orientalis*, *Trifolium pratense* and *Trifolium pannonicum*, leaves are larger, the intensity of shedding of vegetative organs in hay is less (9-13%), shed leaves in the leaf mass amounted to 2-6%, after mowing, their leaves lose moisture more quickly than petioles, and 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 (Table 4).

Table 4. The biochemical composition and the biomethane production potential of the studied *Fabaceae* species.

Indices	***Trifolium alexandrinum***		***Trifolium pratense***		***Onobrychis viciifolia***	
	green mass	**silage**	**green mass**	**silage**	**green mass**	**haylage**
Crude protein, g/kg DM	145.00	147.00	169.00	206.00	177.00	142.00
Nitrogen, g/kg DM	23.20	23.52	27.04	32.96	28.30	22.70
Carbon, g/kg DM	500.00	493.33	506.11	467.22	502.20	490.00
Ratio carbon/nitrogen	21.60	21.00	18.72	14.18	17.70	21.60
Acid detergent lignin, g/kg DM	51.00	43.00	31.00	29.00	49.00	40.00
Hemicellulose, g/kg DM	177.00	169.00	170.00	183.00	138.00	153.00
Biomethane potential, L/kg VS	327	340	364	375	291	311

Biomass is an attractive and convenient energy resource that can be used in the processes of decarbonisation and energy transition. The production of biogas by anaerobic digestion (biomethanation) of phytomasses is of growing importance in the context of renewable energy production, is socio-economically cost-efficient and environmentally efficient by reducing greenhouse gas emissions. It offers the possibility to use multiple feedstocks, and to meet different types of energy needs (heat, electricity, and fuel) and to provide fertilizers in organic farming systems. The carbon to nitrogen ratio is one of the basic factors governing the correct course of methane production by anaerobic digestion. 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 results regarding the quality of the studied legume substrates and their biochemical methane potential are presented in Table 4. In the clover green mass substrates, according to the C/N ratio, which constituted 19-22, the concentration of acid detergent lignin (31-51 g/kg) and hemicellulose (170-177g/kg) met the established standards; the biochemical methane potential of the studied clover green mass substrates varied from 327 to 364 l/kg ODM, as compared with 291 l/kg ODM in the sainfoin green mass substrate. As we have mentioned above, the process of ensiling decreased the lignin content, which had a positive effect on the activity of methanogenic bacteria. The biochemical biomethane potential of clover ensiled mass substrates reached 340-375 L/kg, the best biomethane potential was achieved in *Trifolium pratense* substrate. Several publications have documented the biomethane production potential of substrates from *Trifolium* species. According to LEHTOMAKI (2006). substrate from red clover harvested in the flowering period contained 92% OM, 3.2% N, 47.5 % C, C/N=15, 21.6% lignin with methane potential 300 l/kg VS, but timothy-clover grass harvested in the silage stage 93% OM, 3.4% N, 46.8 % C, C/N=14, 19.3% lignin with methane potential 380 l/kg VS. WAHID et al., (2015) found that pure red clover harvested in different cutting periods contained 91-94 % OM, 13.5-25.0% CP, 44.7-46.0 % C with 263-328 l/kg VS cumulative methane yield at 90 days, but grass-clover mixture contained 90-95% OM, 6.7-22.0% CP, 43.5-45.3 % C, 320-352 l/kg VS, respectively. DANDIKAS et al., (2015) remarked that *Trifolium pratense* fresh mass substrates contained 80.5-85.1% OM, 13.3-23.3% CP, 5.2-7.2% ADL and methane yield 273-346 l/kg VS, but *Trifolium repens* substrates – 80.5-85.1% OM, 17.3-29.0% CP, 6.5-8.8% ADL, 265-320 l/kg VS, respectively. TELEUȚĂ & ŢÎŢEI (2016) mentioned that the calculated gas forming potential of the fermentable organic matter of the studied *Fabaceae* species varied from 470 to 544 l/kg volatile solid matter (VS) and methane potential varied from 247 to 288 l/kg VS. STINNER et al., (2018) reported 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, from *Onobrychis viciifolia* 63-68% degradability and 267-290 l/kg VS methane yields, from *Medicago sativa* 61-75% degradability and 266-323l/kg VS methane yields. HUNADY 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 m3/kg VS. ZHANG et al., (2021) reported that the methane potentials of clover crop materials varied

from 140 to 556 l/kg VS, while the lucerne crop from 240 to 357 l/kg VS. LI 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, while the alfalfa substrate – 7.60 g/kg N, 117 g/kg HC, 343 g/kg Cel, 85 g/kg ADL, 269.50 l/kg VS, respectively.

CONCLUSIONS

The dry matter of *Trifolium alexandrinum* 'Viorel' and red clover *Trifolium pratense* 'Select 2' natural forage is characterized by 145-169 g/kg CP, 232-279 g/kg CF, 89-100 g/kg ash, 252-302 g/kg ADF, 422-479 g/kg NDF, 31-51 g/kg ADL, 140-222 g/kg TSS, 221-251 g/kg Cel, 170-177 g/kg HC with nutritive and energy values of 65.4-69.2 % DDM, RFV=127-153, 12.84-13.52 MJ/kg DE, 10.54-11.10 MJ/kg ME and 6.56-7.12 MJ/kg Nel.

The hay prepared from *Trifolium* species contained 168-183g/kg CP, 244-289 g/kg CF, 95-106 g/kg ash, 265-303 g/kg ADF, 415-437 g/kg NDF, 34-47 g/kg ADL, 113-161 g/kg TSS with 65.3-68.3 % DDM, RFV=139-153, 12.82-13.36 MJ/kg DE, 10.53-10.97 MJ/kg ME and 6.54-6.98 MJ/kg Nel.

The nutrient content and fodder value of ensiled clover mass was: 147-206 g/kg CP, 226-266 g/kg CF, 112-159 g/kg ash, 239-283 g/kg ADF, 422-452 g/kg NDF, 29-43 g/kg ADL, 106-153 g/kg TSS, 210-250 g/kg Cel, 159-183 g/kg HC, 66.9-70.3 % DDM, RFV=138-155, 13.11-13.72 MJ/kg DE, 10.76-11.26 MJ/kg ME and 6.74-7.28 MJ/kg Nel.

The fresh and ensiled clover mass substrates for anaerobic digestion had optimal C/N=14-22 and the biochemical methane potential varied from 327 to 375 l/kg ODM.

The Romanian cultivars of berseem clover *Trifolium alexandrinum* 'Viorel' and red clover *Trifolium pratense* 'Select 2' are of high agro-economical value and 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 mass can be used as forage for farm animals or as substrate in biogas generators for the production of renewable energy.

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