SOME BIOLOGICAL FEATURES AND BIOMASS QUALITY OF *LUPINUS ALBUS* AND *LUPINUS LUTEUS* IN MOLDOVA

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Abstract

Fabaceae species play a crucial role in natural ecosystems and agriculture, because they have the potential to symbiotic fixation of atmospheric nitrogen and soil carbon sequestration, improve biological activity and soil structure, increase the quality and quantity of food and feed, bring improvements resource efficiency in various biorefinery systems. We have studied biological features, biochemical composition and nutritive value, and have estimated the biomethane potential of aerial biomass of the *Fabaceae* species *Lupinus albus* and *Lupinus luteus*, which have been cultivated in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, R. Moldova, *Medicago sativa* and *Onobrychis viciifolia* were used as control variants. The results of our research revealed that the dry matter of harvested whole plants of *Lupinus* species contained 166-206 g/kg CP, 86-110 g/kg ash, 221-258 g/kg ADF, 337-339 g/kg NDF, 31-40 g/kg ADL, 190 - 218g/kg Cel and 116-141 g/kg HC. The nutritional value of *Lupinus* green mass:75.5- 80.9 % DDM, 72.9-76.6 % DOM, RFV 168-208, 13.45-13.96 MJ/kg DE, 11.04- 11.46 MJ/kg ME and 7.06-7.48 MJ/kg NEl. It has been found that the biomethane potential of the *Lupinus* substrates varied from 309 to 324 l/kg ODM.The annual species *Lupinus albus* and *Lupinus luteus* are a promising source of fodder and feedstock for biomethane production.

Keywords: biochemical composition, *Lupinus albus*, *Lupinus luteus*, nutritive value

People are currently confronting many global challenges. The global population is increasing at an exponential rate, leading to unprecedented crises, among which food and energy security, risk of climate change, air pollution and emissions greenhouse gases, rising prices of production means, decreasing farmland area and reducing the reserves of fossil energy and uncertainties about future reliability of supply are of prominent concerns. World protein needs are and will continue rising in the future due to the world population increase, the living condition improvement and the evolution toward an animal and plant protein based diet. To face the global crisis, particular attention has been paid to the reassessment of the value of neglected and underutilized crops, mobilization and domestication of new species would promote agricultural diversity, encourage scientists to create new varieties with increased genetic potential for productivity, quality and increased resistance to harmful biotic and abiotic factors, farmers search and apply agrotechnologies which would guarantee satisfactory yield, high quality and positive influence on the natural environment.

Fabaceae (*Leguminosae*) is the third largest family of flowering plants, after the *Orchidaceae* and *Asteraceae.* Economically, legumes, *Fabaceae*, represent the second most important family of crop plants after the grass family, *Poaceae*, grain legumes account for 27 % of world crop production and provide 33 % of the dietary protein consumed by humans, while pasture and forage legumes provide vital part of animal feed. They provide important sources of oil, fiber, and protein-rich food and feed while supplying nitrogen (N) to agro-ecosystems via their unique ability to fix atmospheric N_2 in symbiosis with the soil to create symbiotic relations with nitrogen fixing bacteria, as *Rhizobium* species, increasing soil carbon content, and stimulating the productivity of the crops that follow. Increasing the role of legumes plants are attractive and necessary in the context of sustainable development of agriculture, reconnection of crop and livestock production, their potential to contribute to the mitigation of climate change by reducing fossil fuel use or by providing feedstock for the emerging biobased economies where fossil sources of energy and industrial raw materials are replaced in part by sustainable and renewable biomass resources. Many legumes can be produced on marginal/surplus lands and on degraded or drastically disturbed soils (Duke J.A., 1981; El

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Bassam H, 2010; Jensen E.S. *et al*, 2012; Murphy-Bokern D. *et al*, 2017).

The genus *Lupinus* L. tribe *Genisteae*, *Fabaceae* family currently includes about 300 species. Most species grow on the American continent and 12-13 annuals species grow in the African and Mediterranean highland. Many *Lupinus* spp. are ornamental garden plants, and five species are cultivated on a larger scale as agricultural crops: *Lupinus albus*, *Lupinus anugustifolius*, *Lupinus cosentenii*, *Lupinus luteus* and *Lupinus mutabalis* in climates ranging from northern Europe and Russia, to the arid Australian plains and the Andean highlands. Bähr M. *et al*, (2014) believe that lupines are an alternative to soybeans, as they contain comparable amounts of proteins of a similar amino acid profile, but more fiber content, which is favorable from the dietary point of view, compared to beans.

The oldest record of *Lupinus albus* dates back to around 3500 years BC, in the Late Neolithic, was first cultivated as green manure, as forage and probably also for human consumption. In Germany in the 1930s, von Sengbusch identified natural sweet-seeded mutants, which heralded the beginning of modern *Lupinus albus* breeding (Gladstone J.S., 1970). White lupin, *Lupinus albus L.*, is annual, erect, branched, bushy, short-hairy herb up to 120 cm tall, with a strong taproot can grow 70 cm deep. Leaves alternate, digitately compound with 5–9 leaflets; stipules linear to narrowly triangular, up to 1 cm adnate to the base of the petiole; petiole 3.5–7(–12) cm long; leaflets obovate, 2–6 cm × 0.5–2 cm, cuneate at base, rounded and mucronate at apex, nearly glabrous above, hairy beneath. Inflorescence a terminal false raceme 3–30 cm long, many-flowered, lower flowers alternate, upper ones in whorls; peduncle short or absent. Flowers bisexual, papilionaceous; pedicel 1–2 mm long; calyx 8–14 mm long, densely hairy outside, tube 4 mm long, 2-lipped, upper lip entire, lower lip entire or slightly 3-toothed; corolla white to violet-blue, standard obovate, 15–18 mm × 8–12 mm, margins partly reflexed, wings obovate, 13–17 mm × 6–10 mm, keel ladle-shaped, 12–15 mm × 4 mm, beaked; stamens 10, all joined into a tube; ovary superior, 1-celled, style 7.5 mm long with a ring of small hairs below the stigma. The pods are 3-6 seeded, narrowly oblong, laterally compressed, (6-) 9-15 cm long × 1-2 cm wide, yellow in colour. The weight of 1000 seeds is 200-350 g. 2n = 50 (Kurlovich B.S., 2002; Jansen P.C.M., 2006; El Bassam H, 2010; Clark S., 2014). The average seed yield of white lupine ranges from 0.5 to 4 t/ha (Jansen P.C.M., 2006). In Romania, the seed yield was 3.033 kg/ha and the protein yield 1.077 kg/ha **(**David G. *et al*, 2014).

Yellow lupine, *Lupinus luteus* L., is an annual plant herb, rosetted in the beginning and subsequently becoming erect, with vigorous basal branching. It is strongly taprooted and its stalk up to 80 cm tall is densely hairy. The leaf consists of 7–9 (11) ovate-oblong or lanceolate leaflets, prolate at the basis, densely villous on both sides, sized 30–60 x 8–15 mm. Stipules of the rosetted leaves are crescent and chuffy on stalks, linear-obovate in shape. The inflorescence is a terminal spicate raceme up to 25 cm long, set on a peduncle of 5–12 cm, containing 6-10 whorls of 5 fragrant yellow papillonaceous flowers each. Floral bracts are small-sized, obovate, silky-pubescent, easily falling. The upper lip of the calyx is bipartite, the lower one has 3 small denticles. The corolla is 14-16 mm long, bright gold-yellow in colour. Petals are yellow, orange, or whitish; 9 stamens; 5 upper ones are longer. The fruits are densely hairy flat pods, 4-5 cm long x 1.1-1.3 cm wide. The pods contain 4-6 seeds. The seeds are reniform, smooth, white or white with brown to black speckles. The weight of 1000 seeds is 120-140 g (Kurlovich B.S. 2002; Terekhina N. V., 2008; El Bassam H., 2010). *Lupinus luteus* appeared in Russia about 1811 y. as ornamental plant. Being cultivated since the end of the 19th century, it occupies about 2 million hectares within the territory of the former USSR, but in the 21st century, it has been used to produce plant-based feed and its role in animal husbandry, in Russia, is limited (Lukashevich M.I. *et al*, 2018). Modern *Lupinus luteus* cultivars are resistant to pod shattering, though improvements are still necessary in Mediterranean-type environments, which experience hot, dry conditions at harvest time (Wolko B. *et al*, 2010).

Lupine has modeate requirements towards temperature; however, white lupine is more demanding than yellow lupine. Lupine is generally drought tolerant, the most resistant is the yellow lupine, the less resistant – the blue lupine, and the white lupine "requires" a humid and warm spring, then it withstands drought well. In terms of photoperiodism, annual lupine species are long-day plants. White lupine reacts less to the length of the day than the yellow lupine. The requirements of lupine to the soil are relatively low, due to the development of the root system (even over 2 m), the high capacity to solubilise phosphorus and other elements from combinations that, for other plants, are difficult to solve. Pollination is autogamous in white lupine and allogamous in yellow lupine.

Lupines are N-fixing legumes, and white lupine has been reported to fix 400 kg N/ha

(Jansen P.C.M., 2006), but yellow lupine can fix in one year between 150 to 169 kg N/ha (Lança A.C., 1993). Utilizing lupine as green manure helps to protect environments from pollution, go without expensive fertilizers and obtain ecologically clean products (Kurlovich B.S., 2002). In mixed cultures of bioenergy crops, lupines (*Lupinus albus* and *Lupinus angustifolius*) have the ability to mobilize trace elements and make these elements available for co-cultured species. Lupines mobilize trace elements by carboxylates and enzyme exudation and by lowering the pH value in the rhizosphere. In a comparison between white and blue lupine for trace element mobilization, white lupine was more effective than blue lupine and thus recommended for phytoremediation (Hentschel W. and Wiche O., 2016). Lupine is studied and cultivated in many regions of the world, used in animal feed as form of seeds, green forage and silage, in industry as raw material for the production of bioenergy and various chemicals, in human nutrition as an alternative protein and bioactive component source, as green manure and ameliorative plant growth and soil fertility of the sandy acid soils, in medicine and as an ornamental plant (Aniszewski T., 1993; Zraly Z. *et al*, 2006; Doležal P. *et al*, 2008; Bhardwaj H. *et al*, 2010; Wolko B. *et al*, 2010; Lucas M.M. *et al*, 2017; Abraham E.M. *et al*, 2017; Pietrzykowski M. *et al*, 2017; Prusinski J., 2017; Criste F.L., 2020; Struti D.I. *et al*, 2020). Lupine production and cultivated area worldwide for 2017 is estimated at about 1.610.969 tonnes and 930.717 ha respectively. The percentage of global production attributed to Europe increased remarkably from 17.6% in 2013 to 29% in 2017 (Abraham E.M. *et al*, 2017). In 2016, lupine in Russia was cultivated on an area of 135 thousand hectares (Lukashevich M.I. *et al*, 2018). This research was aimed at evaluating the biological features, biochemical composition of non-traditional annual species *Lupinus albus* and *Lupinus luteus* grown under the conditions of the Republic of Moldova, and the possibility to use them as fodder for ruminant animals or as biogas substrate.

MATERIALS AND METHODS

The annual *Fabaceae* species: white lupine, *Lupinus albus* L., and yellow lupine, *Lupinus luteus* L., which were cultivated in the experimental plot of the National Botanical Garden (Institute) of Moldova, Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″, served as objects of study; the traditional crops alfalfa, *Medicago sativa*, and common sainfoin, *Onobrychis viciifolia*, were used as control variants. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. Sowing was done in April at a depth of 4.0 cm on rows distance 45 cm, the sowing density of *Lupinus albus* was 80 seeds/ m^2 and *Lupinus luteus* – 110 seeds/m^2. 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 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. The leaves/stems ratio was determined by separating the leaves and flower from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). For chemical analysis plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with diameter of openings of 1 mm and some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), digestible dry matter (DDM), digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

The carbon content of the substrates was obtained using an empirical equation reported by (Badger *et al*, 1979). The biochemical biogas potential (Yb) and methane potential (Ym) were calculated according to the equations of Dandikas *et al,* 2014, based on the concentration of acid detergent lignin (ADL) and hemicellulose (HC):

$$Yb=727+0.25\ HC-3.93\ ADL$$

$$Ym=371+0.13HC-2.00ADL$$

RESULTS AND DISCUSSIONS

After the phenological observations, it was found that, the growth and development rates of the studied *Lupinus* species differed from those of the traditional leguminous forage crops. Thus, the seedlings emerged uniformly on the soil surface 15-17 days after sowing, or, 5-7 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. Emergence is epigeal, cotyledons emerge above ground before development of true leaves, and early seedling growth is considerably slower than later vegetative stages. Over a period of 18-23 days, the plants developed fine roots, which grew 20-23 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. *Lupinus albus* needed a shorter period to develop the rosette (17 days), but *Lupinus luteus* – a longer one (25 days). Then, the growth rate accelerated and allowed the development of an erect stem; bud initiation started. It was determined that the period of time from the emergence of plantlets till the formation of flower buds was shorter for *Lupinus albus* and constituted 52 days, but for the *Lupinus luteus* species – 61 days, the established phenological differences were maintained until the end of the growing season. In the next period, the main stem elongated and flowers, axillary buds near the inflorescence started their development into branches. During the bud formation till the full flower stage, a faster growth rate of aerial part was observed in *Lupinus albus.* In flowering stage, *Lupinus albus* plants reached 76.20 cm and *Lupinus luteus* – 64.4 cm, while the traditional leguminous forage crops reached 84.5-93.1 cm (*table 1*). The bio-morphological characteristics of the whole plant have significant impact on the forage productivity and quality. At the time of the harvest, *Lupinus albus* had the greatest mass of a single plant. The biomass productivity of *Lupinus luteus* was 2.64kg/m^2 green mass or 0.48 kg/m^2 dry matter with 75 % leaves and flower in fodder, but *Lupinus albus* - 3.92 kg/m^2 green mass or 0.66 kg/m^2 dry matter with 63 % leaves and flower. The *Lupinus* forages were richer in leaves, but poorer in dry matter, in comparison with the control variants.

Literature sources indicate considerable variation in yield both between individual lupine species and their cultivars. The forage yield reported by Bhardwaj H. *et al*. (2010) of white lupines in the United States ranged between 0.8-2 t/ha dry matter. In Serbia, the forage productivity of twelve studied white lupine cultivars on chernozem alkaline soils was 21.3-53.3 t/ha green mass or 3.6 – 8.7 t/ha dry matter (Mihailovic V. *et al,* 2008). In Poland, the white lupine yield harvested in flat pod stage was 20.4 t/ha green mass or 3.4 t/ha dry matter, but yellow lupine – 43.1t/ha green mass or 6.4 t/ha dry matter (Faligowska A. and Szukała J., 2009). Fikadu T.R., 2017, found that sweet white lupine high plant ranged from 58.96 to 85.63 cm, the harvested herbage – from 17.33 to 39.58 t/ha and the dry matter yield – from 2.53 to 4.85 t/ha, this variation in forage dry matter yield could be due to differences in the growth environment, planting spacing, harvest period and the lupine varieties evaluated. According to Lukashevich M.I. *et al,* (2018), the developed yellow lupine cultivars in the All-Russian Lupine Research Institute reached the productivity 55.41-64.76 t/ha green mass, 8.94 – 12.37 t/ha dry matter and 1.42 - 2.12 t/ha protein.

Table 1

Some agrobiological peculiarities of *Lupinus albus* and *Lupines luteus*

Species	Plant height, cm,	Leaf and flower, g		Stem, g		Yield, kg/ m^2	
		green mass	dry matter	green mass	dry matter	green mass	dry matter
Lupinus albus	76.2	24.04	4.34	17.72	2.55	3.92	0.66
Lupinus luteus	64.4	18.40	3.23	5.36	1.08	2.64	0.48
Medicago sativa, first cut	84.5	5.38	1.38	4.92	1.41	20.8	0.56
Onobrychis viciifolia, first cut	93.1	12.50	2.86	10.10	2.49	25.8	0.61

The optimum use of forage resources in animal diets depends on the availability of detailed information on their chemical composition, biological properties and nutritional value, which may vary between plant species and varieties. Analyzing the results of the green mass quality of the studied *Lupinus* species (*table 2*), we found that the dry matter content and its chemical composition varied in comparison with alfalfa and common sainfoin

It has been proved that proteins have high biological value for growth and serve as structural elements in all plant tissues. In the animal body, they are utilized for growth, replacement of old, damaged or worn-out cells/tissues and formation of milk. They are of particularly great value to young growing animals and lactating ruminants (McDonald P. *et al*, 2010). It has been found that *Lupinus luteus* is characterized by high content of protein in dry matter (20.6%). *Lupinus albus* has about the same amount of protein in the fodder (16.6%) as *Onobrychis viciifolia*, but a lower one in comparison with *Medicago sativa.* 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 and prevent the emergence and development of diseases of animals (McDonald P. *et al,* 2010). We could mention that *Lupinus albus* has low content of minerals (8.6%), but *Lupinus luteus* high content of minerals (11.0%) in comparison with traditional leguminous forage crops. Plant cell walls provide the basic mechanical support that allows plants to stand upright, play important roles in plant responses to various abiotic stresses, such as drought, flooding, heat, cold, and salt and is essential in stress sensing and signal transduction. Cell wall components such as NDF, ADF, cellulose, hemicellulose and lignin are very important limiting factors to the feeding processes and to the ability of the animal to utilize the consumed forage. Carbohydrates are the most important source of energy and are the main precursors of fat and sugar (lactose) in milk. The level of structural carbohydrates were substantially reduced in the *Lupinus* fodder: 221-258 g/kg ADF, 337-339 g/kg NDF, 31-40 g/kg ADL, which had a positive effect on dry and organic matter digestibility, relative feed value and energy content.

Table 2

The biochemical composition and fodder value of the *green mass of Lupinus albus* and *Lupinus luteus*

Indices	*Lupinus albus*	*Lupinus luteus*	*Medicago sativa*	*Onobrychis viciifolia*
Crude protein, g/kg DM	166	206	172	166
Minerals, g/kg DM	86	110	91	96
Acid detergent fiber, g/kg DM	258	221	347	309
Neutral detergent fiber, g/kg DM	399	337	510	447
Acid detergent lignin, g/kg DM	40	31	58	49
Digestible dry matter, g/kg DM	755	809	623	669
Digestible organic matter, g/kg DM	729	766	579	615
Relative feed value	168	208	118	142
Digestible energy, MJ/ kg DM	13.45	13.96	12.20	12.73
Metabolizable energy, MJ/ kg DM	11.04	11.46	10.03	10.46
Net energy for lactation, MJ/ kg DM	7.06	7.48	6.04	6.48

The nutritive value and energy value of *Lupinus luteus* was RFV= 208, 13.45 MJ/kg DE, 11.04 MJ/kg ME and 7.06 MJ/kg; *Lupinus albus*-RFV=168, 13.45MJ/kg DE, 11.04MJ/kg ME and 7.06MJ/kg NEl; *Onobrychis viciifolia* - RFV=142, 12.73MJ/kg DE, 10.46MJ/kg ME and 6.48MJ/kg NEl; *Medicago sativa*- RFV=118, 12.20MJ/kg DE, 10.03MJ/kg ME and 6.04MJ/kg NEl, respectively. Some authors mentioned various findings about the green mass quality of the *Lupinus* ssp. The harvested whole lupine plant in wax ripeness stage of the seed were characterized by 187.2 g/kg DM, 208.2 g/kg CP, 221.7 g/kg of CF, 290.4 g/kg ADF, 410.9 g/kg NDF, 140.5 g/kg of starch, 31.5 g/kg WSC, 60.18 % rumen degradability of crude protein (Doležal P. *et al*, 2008). According to Faligowska A. and Szukała J., 2009, the harvested white lupine green forage in flat pod stage contained 16.9% DM, 16.3 % CP, 2.44% fat, 24.1% CF, 49.1% nitrogen-free extract, 7.94% ash, but yellow lupine green forage – 16.9% DM, 17.6 % CP, 1.39% fat, 29.3% CF, 42.3% nitrogen-free extract, 9.38% ash. Bhardwaj H.L *et al*, (2010) remarked that the chemical composition of white lupine forage varied in dependence of growing location: crude protein – from 167.1 to 217.8 g/kg and acid detergent fiber – from 189.2 to 303.9 g/kg. In Chile, the chemical composition of lupine forages harvested in early bloom period was: 17.1 % CP, 18.7% CF, 29.9% NDF with 81.2% IVOMD and 12.0 MJ/kg ME, but in mid-bloom period – 14.9 % CP, 19.3% CF, 27.7% NDF, 80.8% IVOMD and 12.2 MJ/kg ME (Valderrama X., Anrique R., 2011). Faligowska A. *et al*, (2014), reported that white lupine green mass was characterized by 158-253 g/kg DM, 141-154 g/kg CP, 261.8-266.1 g/kg NDF and 399.9-422.1 g/kg WSC, but prepared lupine silage – 218.6-220.9 g/kg DM, 151.4-152.5 g/kg CP, 319.4-351.4 g/kg NDF, 7.3-13.7 g/kg WSC with pH=4.5-4.6.

Heuzé (2019), revealed that the aerial part of *Lupinus albus* contained 20.2% dry matter, 21.53% CP, 23.5% CF, 31.1% NDF, 25.6% ADF, 3.1% ether extract, 4.1% lignin, 8.0 % ash, 71.7% organic matter digestibility, 13.0 MJ/kg DE, 10.3 MJ/kg ME. Baizán G. S. *et al* (2015) reported that, under the climatic conditions of Spain, the nutrient contents of *Lupinus albus* grown in monoculture was: 10.63% CP, 50.07% NDF, 26.94.22% ADF with 74.51% IVOMD and 10.51 MJ/kg ME, but in mixtures with Italian lupine – 17.85% CP, 45.02% NDF, 29.22% ADF with 67.65% IVOMD and 9.60 MJ/kg ME. Fikadu T.R. (2017) remarked that nutritional value of sweet lupine cultivars was 21-24% CP, 47-49% NDF, 34-39% ADF, 5.2-5.6% ADF, 66.9-69.1% IVOMD, with metabolizable energy content 9.04-9.44 MJ/kg.

Lukashevich M.I. *et al*, (2018) mentioned that green forage from yellow lupine cultivars contained 15.9-17.9 % CP, 2.16- 2.54% fat, 0.030-0.041% alkaloids, 23.68-28.85 mg/g carotene. During our previous research, it has been found that the chemical composition and the nutritive values of *Lupinus perennis* dry mass were: 144.2 g/kg CP, 39.1 % g/kg EE, 254.4 g/kg CF, 105.6 g/kg minerals, 456.7 g/kg NFE, 0.96.2 nutritive units, 9.87 MJ/kg metabolizable energy and 105.3 g/kg digestible protein (Ţîţei V. *et al*, 2015).

In a biobased economy, fossil resources are replaced by biomass for the production of industrial chemicals, transportation fuels, electricity, heat, and other products. Second-generation biofuels, power and heat generated by combustion and production of industrial raw materials could be based on legume biomass and residues. Legumes have high contents of constituents (*table 3*) other than carbohydrates, which may be relevant in biorefinery concepts in which the different components could be used for a variety of biobased products. Anaerobic digestion is an optimal conversion technology containing legume biomass since the valuable content of N, P, and other nutrients is exploited efficiently via the digestate biofertilizer. High C/N biomass feedstock low in crude protein and fat, such as maize and grasses, could benefit by being enriched with crude protein by mixing legume foliage to improve efficiencies. But sole crop legume biomass with a high N content would not be optimal either because the concentration of NH_4 in the reactor may become too high and stop the digestion process. Currently, biogas is produced from monoculture maize feedstock in many European countries, but legume-based energy crops for biogas should be integrated in sustainable crop rotations. The ratio of the content of carbon and nitrogen (C/N) of the raw material is essential in the production of biogas.

Table 3

The biochemical biogas and biomethane production potential of *Lupinus albus and Lupinus luteus* substrates

Indices	*Lupinus albus*	*Lupinus luteus*	*Medicago sativa*	*Onobrychis viciifolia*
Crude protein, g/kg DM	166	206	172	166
Minerals, g/kg DM	86	110	91	96
Nitrogen, g/kg DM	26.6	33.0	27.5	26.6
Carbon, g/kg DM	507.7	494.4	505.5	502.2
Ratio carbon/nitrogen	19.1	15.0	18.4	18.9
Cellulose, g/kg DM	218	190	289	260
Hemicellulose, g/kg DM	141	116	163	138
Acid detergent lignin, g/kg DM	40	31	58	49
Bio gas potential, L/kg VS	605	634	540	569
Biomethane potential, L/kg VS	309	324	276	291

The production of biomass from legumes for energy purposes is considered an important element of sustainable agriculture. The ratio of the content of carbon and nitrogen (C/N) of the raw material is essential in the production of biogas. We could mention that the nitrogen content in the studied legume substrates ranges from 2.66% to 3.3 %, the estimated content of carbon – from 49.44% to 50.77 %, the C/N ratio varied from 15 to 19. The essential differences were observed between the content of cellulose, hemicellulose and lignin. *Lupinus luteus* substrate is characterized by low cell wall compounds. The *Lupinus albus* substrate contained acceptable amounts of hemicellulose and lignin, in comparison *Medicago sativa* substrate. Dobre P. *et al*, (2014), mentioned that 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.

The gas forming potential of the studied substrates varied from 540 to 664 litre/kg VS. The best biogas yield was achieved in *Lupinus luteus* substrates with methane potential yield of 324 l/kg VS, the lowest – in the biomass of *Medicago sativa*. The methane yield per ha of studied *Lupinus* species reached 1864 m^3/ha on *Lupinus albus* and 1384 m^3/ha *Lupinus luteus* substrate.

According to Lehtomäki A., 2006, the methane yield for lupine was 360 l/kg. Carvalho L. *et al*, (2013) remarked that the yellow lupine silage substrate made it possible produce 665 m^3/t biogas and 409 m^3/t methane, the annual methane yield was 6871 m^3/ha, but oilseed radish silage produced 447 m^3/t, 294 m^3/t and 1600 m^3/ha. Dubrovskis V. *et al*, (2011) mentioned that biogas potential of large leaf lupine (*Lupinus polyphyllus* L.) was 520 l/kg, the methane content was 61.9 % or 322 l/kg and an annual energy productivity 82 GJ/ha. Hensgen F., Wachendorf M., (2016) remarked that in anaerobic digestion tests the ensiled *Lupinus polyphyllus* in pure and mixture with semi-natural grassland mean methane yields between 251 and 270 l/kg VS.

Pakarinen A. *et al*, (2012), indicated the fresh white lupine produced the highest methane yield (343 ± 33 dm^3 /kg TS), mainly due to its highest amount of proteins. Kintl A. *et al*, 2019 reported that lupine silage has a methane yield of 244 l/kg, maize silage – 327 l/kg, but highest methane yield, 330 l/kg, was detected in mixed silage of 10% lupine and 90% maize.

CONCLUSIONS

Under the climatic conditions of the Republic of Moldova, *Lupinus albus* plants grow and develop more intensively in comparison with *Lupinus luteus.*

In the flowering stage, the productivity of *Lupinus luteus* achieved 2.64 kg/m^2 green mass or 0.48 kg/m^2 dry matter and *Lupinus albus* – 3.92 kg/m^2 green mass or 0.66 kg/m^2 dry matter, respectively.

The dry matter of harvested *Lupinus* species whole plants contained 166-206 g/kg CP, 86-110 g/kg ash, 221-258 g/kg ADF, 337-339 g/kg NDF, 31-40 g/kg ADL, 190 -218g/kg Cel and 116-141 g/kg HC.

The nutritional value of *Lupinus* green mass: 75.5- 80.9 % DDM, 72.9-76.6 % ODM, RFV 168-208, 13.45-13.96 MJ/kg DE, 11.04- 11.46 MJ/kg ME and 7.06-7.48 MJ/kg NEl.

The *Lupinus* green mass substrates for anaerobic digestion characterized by optimal C/N ratio and hemicelluloses and low amount of lignin, the biomethane potential varied from 309 to 324 l/kg ODM.

Lupinus luteus green mass was characterized by high content of protein, ash and low cell wall compounds, which had a positive effect on matter digestibility, nutritive value and biomethane potential.

These *Lupinus* species can serve as starting material in crop improvement and implementation of new leguminous species for animal fodder diversification and feedstock for anaerobic digestion and biomethane production.

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