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SOME AGROBIOLOGICAL PECULIARITIES AND POTENTIAL USES OF *GLYCYRRHIZA GLABRA L.* AND *ONOBRYCHIS ARENARIA* (KIT.) DC. IN THE REPUBLIC OF MOLDOVA

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Abstract

The agrobiological features, the quality of the green mass and haylage from the local ecotype of Glycyrrhiza glabra and Onobrychis arenaria have been the subject of a recent study. It has been found that the dry matter of harvested green mass of the studied crops contained 162-167 g/kg CP, 302-307 g/kg CF, 88-100g/kg CA, 467-471 g/kg NDF, 317-321 g/kg ADF, 48-54 g/kg ADL, 267-269 g/kg Cel, 150 g/kg HC, 100-103 g/kg TSS, 64.7-67.7 % DDM, 54.3-59.9 % ODM, RFV= 125-127, 10.23-10.28 MJ/kg ME and 6.34-6.56 MJ/kg NEl. The biochemical composition of the dry matter of the haylage and its nutritive value: 155-184 g/kg CP, 267-301 g/kg CF, 114-123 g/kg CA, 429-550 g/kg NDF, 299.333 g/kg ADF, 47-50 g/kg ADL, 249-288 g/kg Cel, 130-187 g/kg HC, 79-91 g/kg TSS, 62.4-67.8 % DDM, 53.6-54.0 % ODM, RFV= 113-142, 10.20-10.57 MJ/kg ME and 6.20-6.59 MJ/kg Nel. We found that the studied substrates for anaerobic digestion have C/N=16.54-19.85, optimal amount of lignin and hemicelluloses, the biochemical methane potential varied from 283 to 301 l/kg ODM. The studied ecotype had optimal quality as fodder for livestock and as substrate for biomethane production.

Key words**:** *biochemical composition, biochemical methane potential, Glycyrrhiza glabra, haylage, nutritive value, Onobrychis arenaria*

INTRODUCTION

Expanding the range of cultivated plants is essential for a sustainable fodder production, which is a key factor for the welfare and achieving the full productive potential of different species and breeds of animals. Besides, such plant species can also be a source of biomass used as raw material for various industries. Perennial legume species provide high quality, energy-protein nutrients as forage for grazing, freshly mowed or as preserved fodder and, at the same time, due to their ability to fix atmospheric nitrogen and mobilize phosphorus in the soil, can contribute to maintaining soil fertility. Under the conditions of an arid climate and intensification of soil salinization processes, the plants of the family *Fabaceae* Lindl (syn. *Leguminosae* Juss.), the genera *Glycyrrhiza* L. and *Onobrychis* Adans. show high tolerance and stable productivity and can more rationally use soil and water resources.

Liquorice – *Glycyrrhiza glabra L.* (sin. *G. glandulifera* Waldst. & Kit., *G. hirsuta* Pall., *G. pallida* Boiss. & Noe., *G. violacea* Boiss. & Noe., *Liquiritia officinarum* Medik.) is a herbaceous, perennial plant, native to southern Europe and the Middle East, also found in the local wild flora of the Republic of Moldova. The stems are erect or ascending, covered with glandular spines, vigorous, branched in the upper part, with 5-25 branches, having a shrub-like appearance, 125-205 cm tall, rough to the touch. The leaves are pale green, whitish on the underside, 5-20 cm long, with short hairy pedicel, imparipinnate compound, with 5-9 pairs of ovate or wide-elliptic leaflets, with numerous glands that secrete a sticky liquid. The stipules are small, lanceolate, and fall during the flowering period. The leaves are similar to acacia leaves. The flowers grow from the axil of the leaves, are bisexual, 8-10 mm long, blue, purple or lilac, with short pedicel, grouped by 50-80 in elongated racemes. Liquorice blooms in June and the fruits ripen in July-August. It is a

honey plant with a potential of 50-100 kg/ha honey. The fruits are brown, erect pods covered with hairs, flat, 1.5–2.5 cm long and 4-6 mm wide, with 3-5 seeds – 2-3 mm long and 3-4 mm wide, with a very hard coat, slightly glossy, greyish-green or brown. The weight of 1,000 seeds is 2-3 g. Seed viability: 3-4 years. In the soil, the plant develops a strong tap root system, which reaches a depth of 1.5-2.0 m, often down to 7 m. It produces a thick, spindle-shaped main rhizome, from which grow cylindrical stolons, brown on the outside, yellow on the inside, 1-2 m long, from which many roots and new shoots grow [22].

Sand sainfoin, *Onobrychis arenaria* (Kit.) DC. (sin. *O. borysthenica* (Sirj.) Klokov, *O. brachypus* Vassilcz. Klokov *O. tanaitica* Spreng., *Hedysarum arenarium* Kit.) is a species occurring in the spontaneous flora of different areas of Eurasia, it is often found in the forest-steppe and steppe areas of our region. It was first cultivated in late 19th century in Ukraine from local wild populations. A considerable part of the globally cultivated varieties of sainfoin were created by crossing with *Onobrychis arenaria* species. It is a perennial plant, with erect, whitish stems, with 7-8 internodes, glabrous or slightly pubescenct, 40-90 cm tall and 3-5 mm in diameter at the base. It has pinnate leaves, with 6-15 pairs of elliptical or linear-lanceolate leaflets. The leaflets are 10-30 mm long and 2-5 mm wide, deep green or greenish-gray. The stipules are free, brown, with ciliated edges. The inflorescences are spiciform or fusiform racemes, 5-9 cm long, slightly narrower at the tip. The flowers have 2.5-3.2 mm long bracts, hairy, 2 mm long peduncle, the calyx is 4.3-5.1 mm long, and the bright pink or reddish pink corolla – 8-10 mm the banner is equal or sometimes shorter than the keel, the wings reach 1.9-2.6 mm. *O. arenaria* blooms in May-June. Allogamous pollination is characteristic of this species. Its honey potential is 90-140 kg/ha honey. The plants bear fruits in July. The fruit is a pod longer than calyx, rarely equal to it, semicircular, with a well-defined net-shaped nervation along the disc and along the suture, with short, brown or gray-green teeth, monosperm, indehiscent. The weight of 1,000 fruits is 15-20 g. The seeds are kidney-shaped, brown-green or yellow-gray, slightly shiny. The weight of 1,000 seeds is 9-11 g. The plant has a taproot, growing very deep into the soil, down to 10 m, it also develops oblique or vertical rhizomes, the adventitious roots grow mostly at a depth of 50-100 cm. The roots have a large number of nodules with the bacterium *Rhizobium simplex*, very resistant to high temperatures and long-term drought, the ability to fix atmospheric nitrogen is 200-300 kg/ha annually. Root secretions have a strong ability to dissolve carbonates and phosphates [22].

The goal of this research was to evaluate the agrobiological peculiarities, the quality of the fresh mass and the haylage of *Glycyrrhiza glabra* – licorice and *Onobrychis arenaria* – sand sainfoin.

MATERIALS AND METHODS

The local ecotypes of liquorice – *Glycyrrhiza glabra* and sand sainfoin – *Onobriychis arenaria* grown on an experimental plot in the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chişinău, served as research subjects. *Onobriychis arenaria* was harvested in the flowering stage and *Glycyrrhiza glabra* – in the budding stage. The fresh mass yield, the leaf content and the dry matter were assessed. The 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 biochemical composition of the haylage were done in accordance with the Moldavian standard SM 108 [19]. The fresh mass and haylage 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 ash (CA), total soluble sugars (TSS), acid detergent lignin (ADL), neutral detergent

fibre (NDF), acid detergent fibre (ADF), digestible dry matter (DDM) and organic digestible matter (ODM), which were determined by near infrared spectroscopy (NIRS) using the PERTEN DA 7200 NIR analysis system, according to standard methods. The analyses were done at the Research-Development Institute for Grasslands Brasov, Romania. Other indices, such as the concentration of cellulose (Cel) and hemicellulose (HC), the digestible energy (DE), the metabolizable energy (ME), the relative feed value (RFV) and the net energy for lactation (NEl) were calculated using standard equations. The carbon content in the organic matter was determined according to the method mentioned by Badger et al. [5], and the biomethane production potential – according to Dandikas et al. [7].

RESULTS AND DISCUSSIONS

At the time of harvesting, the local ecotype of *Onobrychis arenaria* reached 96 cm in height, it yielded 39.5 t/ha fresh mass (6.56 t/ha dry matter), which contained 64.8% leaves and inflorescences. *Glycyrrhiza glabra* plants developed slower, reaching on average 140 cm at the beginning of June. The yield was 33.7 t/ha fresh mass (9.67 t/ha dry matter), with a leaf content of 58.4%. According to Matolinets [15], the productivity of *Onobrychis arenaria*, in the second year of vegetation, was 4.83-5.92 t/ha dry matter. Astafiev et al. [4] mentioned that *Glycyrrhiza glabra* yielded 19.7-22.0 t/ha in lands without irrigation and 55 t/ha in lands with irrigation, and *Glycyrrhiza echinata* yielded 23.4-25.3 t/ha and 58 t/ha, respectively. Hetman & Veklenko [12] mentioned a sand sainfoin yield of 45.9-49.6 t/ha fresh mass, in Ukraine.

At the time when the containers with *Onobrychis arenaria* haylage were opened, it was found that the conserved mass had maintained consistency, the leaves were dark green and the fragments of stems – light olive, with a pleasant smell and aroma of vegetables and pickled fruits. The *Glycyrrhiza glabra* haylage consisted of fragments of yellow stems with green leaves, with pleasant aroma of caramelized fruits.

The results of the assessment of the biochemical composition and nutritional value of fresh and conserved fodder are presented in Table 1. It was found that the natural sand sainfoin feed contained a higher amount of crude protein and a low amount of lignin, which are indicators of a high nutritional value, as well as metabolizable energy and net energy for lactation. In the process of producing haylage from *Glycyrrhiza glabra* plants, we detected an increase in the content of crude protein and ash, and a decrease in the content of cellulose, hemicellulose, lignin and soluble sugars. On the other hand, in *Onobrychis arenaria* haylage, there was a reduction in the crude protein content, but the cellulose and hemicellulose contents increased significantly. This fact affected negatively the digestibility, energy supply and relative feed value.

Table 1. The biochemical composition and nutritional value of fresh mass and haylage from *Glycyrrhiza glabra* and *Onobrychis arenaria*

Indices	*Glycyrrhiza glabra*		*Onobrychis arenaria*	
	fresh mass	haylage	fresh mass	haylage
Crude protein (CP), g/kg	162	184	167	155
Crude fibre (CF), g/kg	307	267	302	301
Acid detergent fibre (ADF), g/kg	321	299	317	333
Neutral detergent fibre (NDF), g/kg	471	429	467	520
Acid detergent lignin (ADL), g/kg	54	50	48	47
Cellulose (Cel), g/kg	267	249	269	286
Hemicellulose (HC), g/kg	150	130	150	187
Total soluble sugars (TSS), g/kg	103	79	100	96
Crude ash (CA), g/kg	100	123	88	114
Digestible dry matter (DDM), %	64.7	67.4	67.7	62.4
Organic digestible matter (ODM), %	54.3	53.6	59.1	54.0
Digestible energy (DE), MJ/kg	12.46	12.87	12.52	12.42
Metabolizable energy (ME), MJ/kg	10.23	10.57	10.28	10.20
Net energy for lactation (NEl), MJ/kg	6.34	6.59	6.56	6.20
Relative feed value (RFV)	125	142	127	113

Source: Own calculation.

In the literature, there are data on the feed quality of the studied species, presented by different authors. Thus, Nohashkieva [18] reported that the mixture of *Glycyrrhiza glabra* with *Elymus repens* contained 16.43% protein, 2.09% fats, 20.18% crude fibre, 30.57% nitrogen free extract, 10.84% ash. Kamalak [13] mentioned that *Glycyrrhiza glabra* leaves contained 16.19-26.93% CP, 20.74-29.07% ADF, 1.57-10.83% tannins, 58.70-70.59% DDM, 10.14-12.12 MJ/kg ME. Alexeyeva [2] determined that the aerial biomass of the researched ecotypes of *Glycyrrhiza glabra* had a concentration of 12.80-21.40% crude cellulose, 15.67- 25.67% crude protein, 6.80-11.50% soluble sugars, 5.67- 17.40% minerals and 1.30-1.70%

flavonoids, including 9.61-16.30 g/kg Ca, 0.14-0.43 g/kg P, 5.27-9.05 g/kg Mg, 3.20-15.25 mg/kg Cu 18.90-50.62 mg/kg Zn, 22.80-104.10 mg/kg Mn, 112-486 mg/kg Fe and 1.30-1.70% flavonoids. Astafiev et al. [4] in a comparative study, established that the *Glycyrrhiza glabra* feed contained 8.09-8.31% crude protein, 3.01-6.62% fats, 24.87-25.86% crude cellulose, 7.13-9.49% ash, 53.07% nitrogen free extract, 33.94 mg/kg carotene; *Glycyrrhiza uralensis* – 10.04-12.92% crude protein, 0.32-3.47% fats, 21.80-22.65% crude cellulose, 5.51-6.12% ash, 58.57% nitrogen free extract, 55.33 mg/kg carotene; *Glycyrrhiza echinata* – 12.85% crude protein, 8.520% fats, 25.54% crude cellulose, 6.53% ash. Toderich et al. [23] mentioned that liquorice plants had a content of 20.7% crude protein, 4.2% fats, 33.4% crude cellulose, 33.3% nitrogen free extract, 7.51% ash and 18.4 MJ/kg crude energy, and that alfalfa plants contained 16.1%, 1.6%, 11.6%, 60.8%, 9.1% and 17.4 MJ/kg, respectively. During our previous research [21], it was established that *Glycyrrhiza glabra* has a nutrient concentration of 13.80% CP, 3.65% fat, 29.40% crude cellulose, 5.40% ash, 32.30% ADF, 49.50% NDF, 5.90% ADL, 26.4% Cel, 16.7% HC, 47.75% SEN, 65.4% DDM, 56.7% ODM, RFV= 122. Gryazeva [11] reported that the fresh mass of *Onobrychis arenaria* included 238.4-244.6 g/kg dry matter, containing 18.57-19.31 % crude protein, 29.18-29.41 % crude cellulose, 41.93-44.02 % nitrogen free extract, 2.29-2.36 % fats and 5.95-6.90 % ash; it was compared with alfalfa, which contained 248.9-269.6 g/kg dry matter with 18.62-20.66% crude protein, 31.18-32.60% crude cellulose, 37.20-39.17% nitrogen free extract, 2.49-2.52% fats and 7.02- 9.09% ash. In an earlier study [20], it was found that *Onobrychis arenaria* contained 16.6% crude protein, 1.0 % fats, 32.4 % crude cellulose and 34.6 % nitrogen free extract. Coşman et al. [6] conducted a research on the biochemical composition of *Onobrychis arenaria* hay and found that it contained 13.94% crude protein, 2.65% fats, 35.35% crude cellulose, 31.73% nitrogen free extract, 6.98% ash, 0.6 nutritive units/kg, 135 g digestible protein per nutritive unit. Matolinets [15] established that *Onobrychis arenaria* feed in the second year of vegetation contained 12.32-17.26% crude protein, 1.60-2.88% fats, 21.51-27.40% crude cellulose, 5.28-10.13% sugars, 5.80-7.28% ash, 10.07-11.13 MJ/kg metabolizable energy and 0.82-1.00 nutritive units/kg dry matter. Morozkov & Maisak [17] indicated that *Onobrychis arenaria* haylage contained 16.96% crude protein, 4.86% sugars, 2.59% fats, 26.15% crude cellulose, 10.63 g/kg Ca, 2.79 g/kg P, 22.90 mg/kg carotene and 9.50 MJ/kg metabolizable energy. Voloshin et al. [25] found that the concentration of nutrients and energy in the first-cut fresh mass of *Onobrychis arenaria* cultivars was as follows: 14.51-17.70% crude protein, 27.13-28.82% crude cellulose, 2.47-2.72% fats, 6.09-6.44% sugars, 6.13-6.79% minerals, 92.25-137.11 mg/% carotene, 0.78-0.83 nutritive units/kg, 9.81-10.12 MJ/kg metabolizable energy, for comparison, alfalfa contained 17.29% crude protein, 32.02% crude cellulose, 3.22% fats, 8.4% minerals, 3.12% sugars, 100.88 mg /% carotene, 0.69 nutritive units/kg and 9.24 MJ/kg metabolizable energy. Dronova et al. [9] mentioned that *Onobrychis arenaria* plants contained 17.7%% crude protein, 25.0% crude cellulose, 35.6% nitrogen free extract and 3.55% fats. Demydas et al. [8] in a comparative study evaluating different species of the genus *Onobrychis*, assessed the nutrient content of natural fodder, thus, it was found that *Onobrychis arenaria* feed contained 20.5-20.6% crude protein, 21.5-21.9% crude cellulose, 4.16-4.22% fats, 8.09-8.15% ash, 46.00% nitrogen free extract, 13.2-13.3 g/kg calcium and 6.2-6.5 g/kg phosphorus. In the same study, other sainfoin species were evaluated: *Onobrychis viciifolia*, which contained 19.3-19.4% crude protein, 21.2-21.6% crude cellulose, 48.00% nitrogen free extract, 3.48-3.62% fats, 7.80-7.98% ash, 13.4-13.5 g/kg calcium and 5.2-5.6 g/kg phosphorus, and *Onobrychis transcaucasica* – 20.1-20.3% crude protein, 21.5-21.6% crude cellulose, 4.07-4.20% fats, 46.00% nitrogen free extract, 8.06-8.16% ash, 12.6-13.3 g/kg calcium and 6.4-6.6 g/kg phosphorus. Hetman & Veklenko [12] mentioned a productivity of

1.58-1.73 t/ha of crude protein and 89.6-89.9 GJ/ha of metabolizable energy.

The biomethane produced by anaerobic digestion of organic matter is used for obtaining heat, electricity, fuel for internal combustion engines, and the digestate is used as an efficient fertilizer in organic agriculture. The raw material (organic substrate) is the determining factor in biogas production, and the nutrient content and the carbon-nitrogen ratio (C/N) play a key role in the microbial decomposition of organic matter and biomethane productivity [5, 7, 24]. Foam formation is considered one of the most common problems in biogas reactors, and the use of mixtures of legumes and plant species containing tannins would minimize the formation of foam in biogas reactors, with a beneficial effect on methane production efficiency, and environmental protection [1, 14, 16]. The studied substrates for the production of biomethane in anaerobic digesters, Table 2, are rich in protein and have a C/N ratio = 16.54-19.85, moderate content of hemicellulose and lignin, which provide a biomethane production potential of 283-301 l/kg organic matter. It was found that during the conservation process, the lignin content changes and the potential for obtaining biomethane increases in both species. Fardad et al. [10] mentioned that *Glycyrrhiza glabra* substrates have C/N = 19.36 and a biomethane potential of 89.8 l/kg. Amaleviciute-Volunge et al. [3] reported that the methane potential of sainfoin substrate was 277.7 L/kg. In our previous research [13, 14] we found out that the biomethane potential in the fermentable biomass of *Onobrychis arenaria* reached 288 l/kg and of *Glycyrrhiza glabra* – 298 l/kg.

Table 2. Biochemical methane potential of substrates from *Glycyrrhiza glabra* and *Onobrychis arenaria*

Indices	*Glycyrrhiza glabra*		*Onobrychis arenaria*	
	fresh mass	haylage	fresh mass	haylage
Crude protein (CP), g/kg	162	184	167	155
Acid detergent lignin (ADL), g/kg	54	50	48	47
Carbon, g/kg	500.00	487.22	506.67	492.22
Nitrogen, g/kg	25.92	29.44	26.72	24.80
Carbon/nitrogen ratio (C/N)	19.29	16.54	18.96	19.85
Biomethane potential, l/kg MO	283	288	295	301

Source: Own calculation.

CONCLUSIONS

The local ecotypes of *Glycyrrhiza glabra* (liquorice) and *Onobrychis arenaria* (sand sainfoin) are of economic and social interest, to be used in diversifying the range of multi-purpose cultivated plants, which can be grown on poorly productive lands, due to their ability to symbiotically fix nitrogen, in addition, they can be helpful in preventing soil erosion, and the harvested fresh mass can be used to produce various types of fodder for livestock and as substrates for biogas plants, in the production of renewable energy, and as soil fertilizers in organic farming.

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