BIOCHEMICAL CHARACTERISTICS OF THE ASTERACEAE SPECIES SILAGE AND POSSIBLE USE AS A FEEDSTOCK FOR LIVESTOCK AND BIOGAS PRODUCTION IN REPUBLIC OF MOLDOVA

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Abstract. *Silage is very palatable to livestock and can be fed at any time, they have also been used as substrate in biogas production. We studied the quality of silage prepared from non-traditional plant species of Asteraceae family: elecampane (Inula helenium), Jerusalem artichoke (Helianthus tuberosus), eastern purple coneflower (Echinacea purpurea) grown in experimental land of the Botanical Garden (Institute) of the Academy of Sciences of Moldova and compared it with the control – sunflower (Helianthus annuus). The silage obtained from these species, by organoleptic characteristics (smell, colour and consistency) and biochemical indices (pH, content and correlation of organic acids, chemical composition of the dry matter), largely meets the standards. The silage prepared from Asteraceae species contains 0.12- 0.23 nutritive units supplied with metabolizable energy for cattle 1.22- 2.32 MJ/kg, the digestible protein content is of 51-104 g/nutritive unit. The highest methane production was achieved with the sunflower, Helianthus annuus silage substrate (290.8 L/kgVS), followed by the Echinacea purpurea substrate (258.4 L/kgVS), the Inula helenium substrate produced 236.4 L/kgVS and the Helianthus tuberosus - 231.8 L/kgVS.*

Key words*: Asteraceae* species, biochemical composition, biomethane, nutritional value, silage

INTRODUCTION

Continuous population growth, depletion of fossil energy resources, food shortages caused by climate change, reduction and degradation of agricultural lands require identification of new sources to overcome these global problems.

Agriculture remains an important sector in the national economy of the Republic of Moldova, which plays an important role in providing the population with food products and also with raw materials for other sectors, providing about half of export earnings. The Republic of Moldova has virtually no fossil energy resources, 88-93% being imported.

One of the major problems concerning the revitalization and development of the animal breeding sector, both globally and locally, is the growth and diversification of fodder production, balanced in terms of quantity and quality throughout the year, according to the physiological needs of animals and productivity indices. An increase in production of protein substances can be achieved by broadening the diversity of crops, by extending the areas for cultivation, by creating new varieties with high genetic potential of productivity, quality and high tolerant to weeds, pests, diseases, drought and frost, have good winter hardiness and be able to grow with low nutrient and energy input. In the world's flora, there are over 50 000 species of plants that animals use as food and about 150 species are cultivated. This huge reserve allows the mobilization of new species that would expand the range of crops, increase

the productivity and elevate the quality of feed (KSHNIKATKINA ET AL.,2005; MEDVEDEV AND SMETANNIKOVA, 1981).

The increased use of biomass as a source of energy will contribute to reduce CO_2 emissions, increase energy security and support sustainable development and regeneration of rural areas. Biogas production from agricultural raw materials is a key technology for an environmentally friendly production of renewable energy. The conservation and storage of fytomass is also a necessary factor for the quality, using the substrate continuously as feedstock for biogas production. The digestate serves as an excellent fertiliser and soil improver of high quality replacing mineral fertilizer (DANDIKAS ET AL, 2014; HEIERMANN ET AL., 2009; WEILAND , 2003).

Ensiled (pickled) plants play an essential role in livestock feeding, especially in preparing homogeneous rations throughout the year , but in recent decades, they have also been used as substrate in biogas production. Corn silage is one of the most common, but frequent droughts, rising prices of seeds, agricultural equipment, fuel and fertilizers have a negative impact on the productivity and the cost of corn.

Identification, mobilization and research of new plant species of local and world flora, their cultivation, including the production of fodder and of renewable energy, have been a strategic direction of the research conducted within the Botanical Garden (Institute) of the Academy of Sciences of Moldova.

As a result of the research on mobilization, new plant species, with high and stable productivity, which can be successfully processed into silage, have been identified: sweet sorghum, amaranth, giant knotweed etc. (COŞMAN, 2014; TITEI ET AL.,, 2012). The species of the *Asteraceae* Bercht. & J.Pres family are of particular interest due to their biological peculiarities, productivity and tolerance to abiotic factors. That's why these promising species were chosen as subjects of a comparative study on the ensiling process, silage quality, the possibility of using it as fodder or as substrate in biogas production.

Native to Central North America, sunflower, *Helianthus annuus* L. is cultivated worldwide, from Russia to South America, is one of the important oilseed crops, can be sown in places where maize cultivation is difficult, sunflower forage is usually fed as silage. Sunflower is known as a melliferous plant (70-130 kg/ha of honey) and as an energy plant, in several countries, being created various cultivars especially for these purposes (DANDIKAS ET AL, 2014; HEUZÉ ET AL., 2015; ION ET AL., 2014). The stems are a poor feed and are usually either ploughed in or used as fuel (IVANOVA ET AL., 2015).

Elecampane, *Inula helenium L.*, is native to Europe and Asia, from Spain to Xinjiang Province in western China, and naturalized in some areas of North America. It develops strong, straight, hairy stems, branched at the top, 1.5-2.3 m tall. At the base of the plant, there are large, oval-oblong leaves, with toothed margin, long petiole, and the upper leaves have no petiole (sessile), are oval, alternate, green on the upper side and whitish on the underside. Flowers are grouped in large, yellow, 5-7 cm broad flower heads, attractive to many insect species. Elecampane has a long history of use as a medicinal herb. It is especially effective in treating coughs, consumption, bronchitis and many other chest infections as well as disorders of the digestive system. The root contains 40-50% levulose polymers, 95% of which is inulin and alantolactone, which is strongly anthelmintic. Studies regarding the use of elecampane as fodder for livestock and as biomass for renewable energy production have been initiated recently (IVANOVA ET AL., 2015; KSHNIKATKINA ET AL.,2005).

The Jerusalem artichoke, *Helianthus tuberosus* L., native to North America, has strong, vigorous stems, sometimes branched at the base, 2.5-3.0 m tall, but can reach even 5 m,

large leaves with a rough texture, hairy, which decrease in size from the base to the top. Flower heads with bright yellow petals resemble sunflower with a bunch of small florets (10-20) in the center. In the underground part, there are unevenly shaped tubers, round or elongated, with bumps, ranging in size between 2 and 10 cm, their color can vary from brown to white, red and even purple. It is an alternative plant, useful in many ways. It is interesting because of the high productivity and possibilities of cultivation on marginal land, the ability to absorb carbon from the air and to release oxygen, the suitability for the creation of effective green belts around industrial centers. Jerusalem artichoke is a good source of fructose, useful in food and pharmaceutical industry. This species is studied in Europe, Asia and America as forage and energy plant (HEIERMANN ET AL., 2009; KSHNIKATKINA ET AL., 2005; ŢÎŢEI ET AL., 2013).

Eastern purple coneflower, *Echinacea purpurea* (L.) Moench., native to North Ameriaca, is a plant with branched stems, which looks like a bush with coarse hairs and reddish brown spots. The leaves are linear-lanceolate, entire, 3-6 cm wide, with three arched ribs and coarse hairs. Its flowers are grouped in solitary flower heads, at the stem tips. Tubular flowers are arranged in a cone, surrounded by ligulate flowers, pink to deep red in colour. The species is highly appreciated in the pharmaceutical industry, because it contains active ingredients, considered natural antibiotics, it is effective against viruses, bacteria, in combating respiratory and genitourinary infections. Also, eastern purple coneflower strengthens the immune system during convalescence and stimulates hepatobiliary transport, helping to detoxify the body. Added to livestock rations, the green mass of this species has a stimulating effect on growth process, compensates for the deficiency of amino acids, vitamins and micronutrients that supports the physiological and productive processes in animals (KSHNIKATKINA ET AL.,2005; SAMORODOV AND POSPELOV , 1999). Eastern purple coneflower can be grown successfully in the Republic of Moldova, where the plants go through all ontogenetic stages and provide the necessary amount of seeds for propagation and production of the desired raw material.

MATERIALS AND METHODS

Plants of the *Asteraceae* family, grown on experimental land in the Botanical Garden (Institute) Academy of Sciences of Moldova, were used as research subjects: 3 year-old *Inula helenium*, *Helianthus tuberosus* and *Echinacea purpurea*. Sunflower, *Helianthus annuus* was used as the control variant. The green mass of sunflower, elecampane and eastern purple coneflower was harvested in the flowering stage, and the Jerusalem artichoke – during the formation of flower heads. The silage was prepared and evaluated in the Institute of Biotechnology in Animal Husbandry and Veterinary Medicine. The green mass was shredded and compressed in well sealed glass containers. After 30 days, the containers were opened, the organoleptic characteristics were analyzed and the biochemical composition of the silage was determined in accordance with the Moldavian standard SM 108. Dry matter or total solid (TS) content was detected by drying samples up to constant weight at 105 °C. Crude protein – by Kjeldahl method; crude fat – by Soxhlet method, crude cellulose – by Van Soest method; ash – in muffle furnace at 550 °C. Nitrogen-free extractive substance (NFE) was mathematically appreciated, as difference between organic matter values and analytically assessed organic compounds. Organic dry matter or volatile solids (VS), was calculated through differentiation, the crude ash being subtracted from dry matter. The biogas and biomethane, liter per kg of volatile solids (L /kg VS), were calculated using the gas forming

potential of nutrients according to BASERGA, 1998 and digestible index nutrients according to MEDVEDEV AND SMETANNIKOVA, 1981.

RESULTS AND DISCUSSIONS

Ensiling is one of the most effective methods of conservation of succulent fodder. It retains most of the nutrients and other valuable qualities of green fodder (succulence, dietetic qualities and high digestibility). The investigated green mass of the plants of the *Asteraceae* family, prepared for the production of silage, is distinguished by a different leaf and dry matter content compared with sunflower, and no juice leakage was observed during fermentation. When opening the glass vessels with silage made from sunflower, *Helianthus tuberosus* and *Echinacea purpurea*, there was no gas or juice leakage from the preserved mass but, from the vessels with silage made from *Inula helenium*, carbon dioxide – a by-product of fermentation – was intensively eliminated.

During the organoleptic assessment, it was found that the colour of the *Helianthus annuus* silage was homogeneous, yellow-green, but the silage made from *Inula helenium* – green-olive leaves and yellow-green stems. Characteristically, about 10 minutes after opening, under the presence of atmospheric air, the colour of the silage obviously darkened. In the *Helianthus tuberosus* silage, the chopped stems were yellow-green, and the leaves – dark green with shades of brown, but the elecampane silage was homogeneous, dark-brown to black. *Helianthus annuus* silage has a scent similar to the smell of fresh pine wood, but the silage made from *Inula helenium* and *Helianthus tuberosus* has a pleasant smell, specific to pickled fruits, and *Echinacea purpurea* silage – a pleasant fruit smell with an aroma of dried plums. The consistency of the silage *Asteraceae* species was retained, in comparison with the initial green mass, without mould and mucus.

It is known that the microflora of a living plant is totally different from that of the future silage. During the process of ensiling, epiphytic bacteria (microflora from the plant) develop in the fodder and produce organic acids, lactic acid bacteria – lactic acid, a natural preservative of the silage. But, on the plant, lactic acid bacteria, which pickle the fodder, are not always the most numerous, but can be other types of microorganisms that can taint the fodder. As a result of the performed analysis, it was determined that the pH index of the prepared silage was: sunflower – 3.93, *Inula helenium* – 4.93, the *Helianthus tuberosus* – 4.25 and *Echinacea purpurea* – 4.29. Except the *Inula helenium* silage, the pH index of the other studied species meets the standard SM 108 for the 1st class quality.

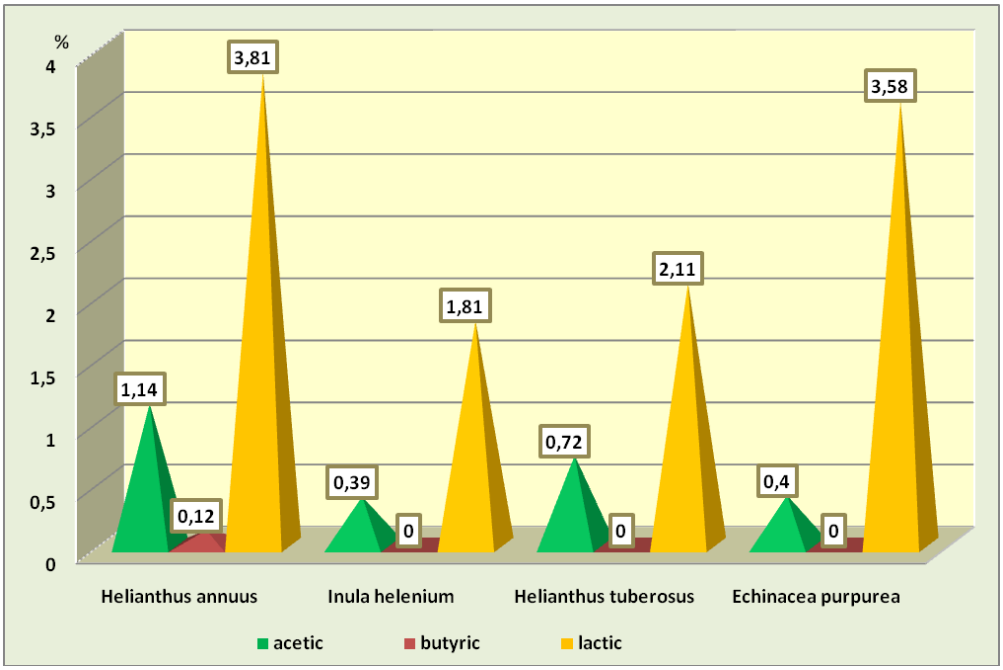

Figure 1. The total content of organic acids in the silage made from plants of the Asteraceae family

Analyzing the data regarding the overall content of organic acids (Figure 1), we conclude that the concentration of organic acids is higher in the sunflower and *Echinacea purpurea* silage and lower – in *Inula helenium* and *Helianthus tuberosus* silage. The lactic acid concentration in the sunflower silage reaches 3.81%, *Echinacea purpurea* silage – 3.58%, *Helianthus tuberosus* silage – 2.11% and *Inula helenium* silage – 1.81%. The *Echinacea purpurea* and *Inula helenium* silage is characterised by a lower acetic acid concentration. Butyric acid has not been found in the silage prepared from new plant species, but it is present in its free form in the sunflower silage – 0.12%. Lactic and acetic acids are present in all samples of silage, being predominantly in fixed state, which is desirable because organic acids in the fixed state contribute more to the preservation of nutrients in the silage.

The share of the lactic acid from the total amount of organic acids accumulated in the silage is obviously higher and varies from 74.6% in the *Helianthus tuberosus* silage to up to 89.9% in the *Echinacea purpurea* silage. As previously mentioned, butyric acid has a share of 2.37% in the sunflower silage. Thus, the data regarding the correlation of lactic, acetic and butyric acids in the silages made from new Asteraceae species are of high quality and can be consumed by animals.

The dry matter content in the obtained silage is low and varies from 14.19% in *Inula helenium* silage, 16.65% – *Helianthus tuberosus*, up to 24.5% – *Echinacea purpurea*, and the control, sunflower – 18.05%. Among all the nutrients in the fodder, the most important and also the most valuable are proteins, which cannot be substituted by any other nutrients from the fodder.

Analyzing the data on the biochemical composition of the dry matter from the silage, shown in Fig. 2, we find that a higher raw protein content, in comparison with the control, is in the silage made from *Inula helenium* (12.49%) and *Inula helenium* (10.58%). The fats from fodder provide energy to the body and contribute to the quality of animal products. Among the studied species, *Inula helenium* provides a very high fat content in the silage, exceeding the control *Helianthus annuus*.

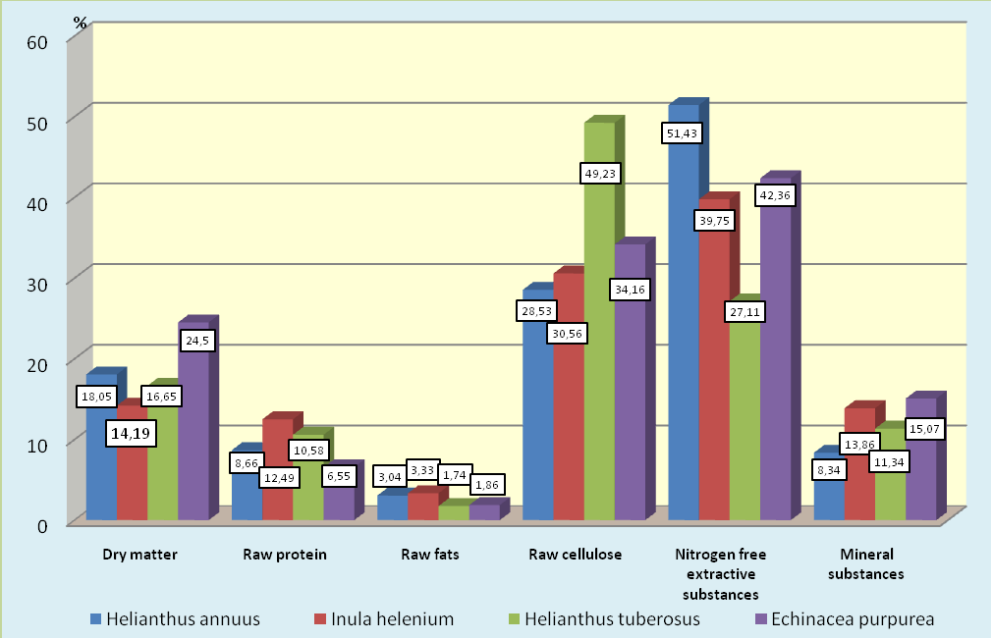

Figure 2. The dry matter content and its chemical composition in the silage made from plants of the Asteraceae family

The silage prepared from *Helianthus tuberosus* is characterized by a very high content of raw cellulose and a low content of nitrogen-free extractive substances, which negatively affects the nutritional quality. There is a moderate content of raw cellulose and nitrogen-free extractive substances in *Inula helenium* silage. All the studied species are rich in minerals in comparison with the control; a higher content is in *Echinacea purpurea* silage.

The organic matter content, the biochemical composition and the digestibility reflect the nutritional value of the silage. We could mention that the silage made from studied Asteraceae species (Table 1) contains 0.12- 0.23 nutritive units supplied with metabolizable energy for cattle 1.22- 2.32 MJ/kg, the digestible protein content is of 51-104 g/nutritive unit. Silage prepared from *Inula helenium* and *Helianthus tuberosus* contains 0.12-14 nutritive units, due to the low content of dry matter, in comparison with 0.19 nutritive unit *Helianthus annuus*, but the digestible protein content of this species met the zootechnical standards. *Echinacea purpurea* silage surpass the *Helianthus annuus* at the content nutritive unit and metabolizable energy for cattle.

Table 1.

Nutritional value of the *Asteraceae* species silage

Indicators	*Helianthus annuus*	*Helianthus tuberosus*	*Inula helenium*	*Echinacea purpurea*
1 kg silage contains: - nutritive units - metabolizable energy for cattle, MJ/kg **Digestible protein**, g/ nutritive unit	 0.19 1.92 60	 0.14 1.38 92	 0.12 1.22 104	 0.23 2.32 51

Table 2.

Stoichiometric gas production potential of biomass (silage) of the *Asteraceae* species

Indicators	*Helianthus annuus*	*Helianthus tuberosus*	*Inula helenium*	*Echinacea purpurea*
Biogas, L /kg VS	552.6	436.0	439.8	489.8
Biomethane, L /kg VS	290.8	231.8	236.4	258.4
Methane content, %	52.6	53.2	53.8	52.8

As the fermentation process in the rumen has many similarities with the biogas production process. The most suitable plant species for the production of biogas are those which are rich in degradable carbohydrates, lipids and proteins. In order to supply biogas crops over the entire year it is necessary to preserve the material (DANDIKAS ET AL, 2014; HEIERMANN ET AL., 2009; WEILAND , 2003).

In our experiments, (Table 2), the highest methane production was achieved with the sunflower, *Helianthus annuus* silage substrate (290.8 L/kgVS), followed by the *Echinacea purpurea* substrate (258.4 L/kgVS). The *Inula helenium* substrate produced 236.4 L/kgVS and the *Helianthus tuberosus* - 231.8 L/kgVS. Values obtained are in good accordance to DANDIKAS ET AL, 2014 who observed biomethane values for sunflower silage of 285-340 L/kgVS and HEIERMANN ET AL., 2009, for *Helianthus tuberosus* silage- 252 L/kgVS.

CONCLUSIONS

The conducted research makes it possible to conclude the following:

- the conservation of plants of the Asteraceae family (*Inula helenium*, *Helianthus tuberosus* and *Echinacea purpurea*) by ensiling is a way of obtaining high quality fodder – the silage, by organoleptic characteristics (smell, colour and consistency), meets the standards for a high quality fodder with the specific characteristics of each plant species;
- the biochemical indices (pH, content and correlation of organic acids, chemical composition) of the silage prepared from these species largely meet the standards established in the Republic of Moldova.
- the silage prepared from *Inula helenium* and *Helianthus tuberosus* contains 0.12-14 nutritive units, due to the low content of dry matter, in comparison with 0.19 nutritive unit *Helianthus annuus*, but the digestible protein content of this species met the zootechnical standards (93-104 g/ nutritive unit), *Echinacea purpurea* silage surpass the *Helianthus annuus* at the content nutritive unit and metabolizable energy for cattle.

- the highest methane production was achieved with the *Helianthus annuus* silage substrate (290.8 L/kgVS), followed by the *Echinacea purpurea* substrate (258.4 L/kgVS), the Inula helenium substrate produced 236.4 L/kgVS and the *Helianthus tuberosus* 231.8 L/kgVS.

The research conducted in the Republic of Moldova is among the first ones on the use of new plant species of the Asteraceae family for silage production and it is necessary to continue the thorough research in order to obtain accurate scientific data regarding biological peculiarities, agrotechnical practices, harvesting, conservation and efficient use of feedstock for livestock and biogas production.

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