BIOLOGICAL FEATURES OF *ISATIS TINCTORIA* AND THE QUALITY OF ENSILED BIOMASS UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

CARACTERISTICI BIOLOGICE ALE *ISATIS TINCTORIA* ȘI CALITATEA BIOMASEI ENSILATE ÎN CONDIȚIILE REPUBLICII MOLDOVA

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Summary. *We studied some biological features and the quality of the silage prepared from Isatis tinctoria plants cultivated in the experimental plot of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chisinau, R. Moldova. In the second growing season the local ecotype of Isatis tinctoria was characterized by fast growth and development rate, reaching 118-147 cm in height, with a content of leaves and inflorescences of 57%.*

It has been determined that the dry matter of ensiled mass from the local ecotype of Isatis tinctoria was characterized by 196 g/kg CP, 160 g/kg ash, 314g/kg CF, 335 g/kg ADF, 521 g/kg NDF, 37g/kg ADL, 298 g/kg Cel, 201g/kg HC with nutritive value 628 g/kg DDM, 10.16 MJ/kg ME, 6.18 MJ/kg NEl and RFV=112, this fact indicates an optimal quality of the silages prepared for ruminants. The biochemical methane production potential of Isatis tinctoria silage substrates reached 361 L/kg VS.

The ensiled biomass from the local ecotype of Isatis tinctoria can be used as alternative forage for ruminant animals, or as substrates in biogas generators for the production of renewable energy.

Keywords: *biochemical composition, biochemical methane potential, biological features, Isatis tinctoria, silage*

INTRODUCTION

Because the food consumed by humans and animals usually does not contain a sufficient amount of protein for a healthy diet, it is necessary to expand the areas where protein-rich crops are cultivated, by mobilizing new species that would expand the assortment of both: crops for the production of food for humans and fodder necessary for the development of the animal husbandry sector and poultry farming, as well as raw material for various industries, including the production of renewable energy. The family *Brassicaceae* Burnett (syn. *Cruciferae* Juss.) includes 372 genera and 4.060 accepted species. Most of them are herbaceous annuals, biennials and perennials, warm season shrubs and trees; some are used as agricultural crops and ornamental plants. Most of the species occur in the temperate zone of the Northern Hemisphere (particularly in the Mediterranean and Irano-Turanian regions) and much less in the Southern Hemisphere. In the native flora of Bessarabia, the Brassicaceae family is currently represented by 48 genera with 97 species.

The genus *Isatis* Tourn. ex Lis., *Brassicaceae* family, includes 92 accepted species, most of them native to the Mediterranean region east to central Asia. In the spontaneous flora of Republic of Moldova there are four species of this genus, namely *Isatis campestris* Stev ex DC,. *Isatis taurica* Bieb., *Isatis praecox Kit. ex Trat.* and *Isatis tinctoria* L. [1].

Isatis tinctoria L., commonly known as dyer's woad, is native to the steppe and desert areas of the Caucasus region, Central Asia, as well as eastern Siberia and western Asia and which has been cultivated in south-western Europe, since ancient times, as a source of blue dye for textiles, extracted from its leaves. It also occurs in the local wild flora in meadows, on roadsides or along railways. Dyer's woad develops erect stems, glabrous or hirsute, branched in the upper part, reaching 50-150 cm in height. It has grey-green leaves of different size and shape, depending on their position on the stem. Thus, the basal leaves are long petiolate and oblanceolate, 5-18 cm long and 0.8-4 cm wide, covered with simple hairs, and the upper ones are sessile and sagittate at the base, narrower than the basal leaves, 2-10 cm long and leaf size gradually decreases towards the top. The flowers are grouped in a raceme inflorescence, sometimes forming a large terminal panicle, with four yellow petals, 3-6 mm in diameter and 3.5 cm long, tetradynamous androecium, consisting of six stamens with two filaments shorter than the others, actinomorphic, hermaphrodite. It blooms at the end of April – the first days of May, it bears fruit at the end of June. The fruit – an indehiscent, winged, hanging, oblong-obovate, elliptic-obovate or flattened silique, glabrous or sparsely hairy, dark brown to black when ripe, 8-18 mm long and 2-7 mm wide with a single median seed. The weight of 1000 fruits is about 6-8 g. The seeds are small, red-orange, the weight of 1000 seeds – 2-3 g. The root system is a taproot consisting of a cylindrical, slightly sinusoidal, yellowish-green or yellowish-brown main root, wrinkled longitudinally and transversely lenticellate, which can grow to a depth of 1.5 m or more, with adventitious roots of 20-30 cm, which extend laterally to 30-45 cm. It is a biennial or perennial living up to 3-5 years, a long-day plant, undemanding to the type and structure of the soil, hardy, resistant to drought. It exhibits a higher tolerance to the climatic conditions of our country, as compared with autumn cereal crops and rapeseed. Despite being not very demanding to soils, it develops better on chernozems with medium cohesion. It reacts well to fertilization with organic and mineral fertilizers. It is propagated by seeds. Tolerates partial shade. Dyer's woad can be sown in pure culture, in the field, in mid-August, with seeders designed for rapeseed, with the rate of 4-8 kg/ha seeds at a depth of 1.5-2.0 cm, or with seeders designed for vegetables and beets with the rate of 24-30 kg/ha fruits at a depth of 3-4 cm and a distance between rows of 45 cm. It can also be sown in spring, preferably in March, performing soil leveling. The seedlings obtained from seeds sown in the soil in August emerge evenly in 2-3 days, those from whole fruits – after 6-9 days. Twenty days after the emergence of seedlings, the growth rate is intense and the plants enter the winter season with a strong rosette of 20-25 leaves. At the end of February – the first days of March, when the air temperature is above 3°C, the plants restart active growth. Generative shoots with flower buds are formed during April. It provides an extra-early source of pollen and nectar for bees and other insects, with a honey production potential of 70-100 kg/ha. It can also be sown in spring mixed with spring cereal crops or perennial grasses. Fresh mass productivity reaches 20-35 t/ha and fruit productivity – 50-800 kg/ha [2].

Since prehistoric times, *Isatis tinctoria* leaves have been used as a natural dye. It is also an important medicinal plant: its leaves are used in traditional medicine mainly for the treatment of infections, specifically: encephalitis, upper respiratory infection and gastroenteritis. The root extract has antibacterial, antiviral and antiparasitic properties and is also used to treat infections [3].

Isatis tinctoria has been studied in several research centers for various purposes: as fodder plant to obtain early spring forage with high content of crude protein, as honey plant, as energy plant etc. [4; 5; 6; 7; 8; 9; 10; 11; 12; 13].

The main goal of our research was to evaluate the growth and development rates of the local ecotype of *Isatis tinctoria* and the quality of the preserved mass as animal feed and as substrate for obtaining biomethane as a renewable energy source.

MATERIALS AND METHODS

The local ecotype of *Isatis tinctoria* (dyer's woad), grown in pure culture in the experimental sector of the "Alexandru Ciubotaru" National Botanical Garden (Institute), Chişinău, 46°58′25.7″ latitude and N28°52′57.8″E longitude, served as research subject and *Festuca pratensis* (meadow fescue) was used as control. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. The samples for conservation by ensiling were taken in the second year of vegetation, in mid-May. The harvested plants were chopped into small pieces (about 1.5-2.0 cm) with a laboratory forage chopper, compressed in well-sealed glass containers, stored at ambient temperature (18-20 °C) for 45 days, to allow complete fermentation to occur. The dry matter content was detected by drying samples up to constant weight at 105°C. For biochemical analyses, the silage samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with mesh diameter of 1 mm. Some assessments of the main biochemical parameters: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), have been made by near infrared spectroscopy (NIRS) using PERTEN DA 7200 in the

Research-Development Institute for Grassland Brasov, Romania. 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 calculated according to Badger et al. [14]. The biochemical methane potential was calculated according to the equations of Dandikas et al. [15].

RESULTS AND DISCUSSIONS

In the second year of vegetation, local ecotype of *Isatis tinctoria* (dyer's woad) came out of dormancy when the average recorded temperatures were above 5-7ºC. New leaves grew from the crown bud in the rosette, characterized by fast growth and development rates, forming by 5-9 generative shoots per plant, reaching 118-147 cm in height in the flowering stage. *Isatis tinctoria* started the flowering stage 12 days earlier than *Brassica napus oleifera*, so, it was valuable for bees as a pollen source. It also reached this stage 18 days earlier than *Festuca pratensis*. The *Isatis tinctoria* fodder harvested in the flowering period contained 57% leaves and inflorescences. In the harvested *Festuca pratensis* biomass, the content of leaves and inflorescences was only 43%.

The results regarding the biochemical composition of the dry matter of *Isatis tinctoria* silage are presented in Table 1. It was established that the *Isatis tinctoria* silage was characterized by very high content of crude protein and minerals, as compared with the *Festuca pratensis* silage. The content of cellulose and hemicellulose in the ensiled mass of *Isatis tinctoria* was much lower, but the content of acid detergent lignin was higher as compared with the *Festuca pratensis* silage. Thus, the low content of cell walls had a positive effect on the nutritional value and energy supply of *Isatis tinctoria* silage.

Table 1. The biochemical composition and nutritional value of the silage prepared from the studied species

Indices	*Isatis tinctoria*	*Festuca pratensis*
Crude protein, g/kg DM	196	80
Crude fibre, g/kg DM	314	394
Minerals, g/kg DM	160	96
Acid detergent fibre, g/kg DM	335	411
Neutral detergent fibre, g/kg DM	521	718
Acid detergent lignin, g/kg DM	37	22
Cellulose, g/kg DM	298	389
Hemicellulose, g/kg DM	201	307
Digestible dry matter, g/kg DM	628	569
Digestible energy, MJ/ kg	12.37	11.32
Metabolizable energy, MJ/ kg	10.16	9.29
Net energy for lactation, MJ/ kg	6.18	5.31
Relative feed value	112	74

In the specialized literature, different data are mentioned regarding the biochemical composition and nutritional value of *Isatis tinctoria* fodder. According to Тищенко in the woad silage prepared for plants harvested in the fruiting stage, 1 kg contained 0.11 nutritive units 21.3 g crude protein and 8.01 mg of carotene, but the woad haylage contained 0.16 g nutritive units and 41.4 g of crude protein per kilogram [7]. According to the results of the research conducted by Милашенко when ensiling the green mass of woad harvested in the early fruiting stage, 1 kg of feed contained 248 g/kg dry matter, 21.1 g/kg crude protein, 1.1 g/kg sugar, 8, 1 mg/kg carotene, 2.91 g/kg calcium, 0.49 g/kg phosphorus, 0.22 nutritive units, and 1 kg of haylage contained 437 g/kg dry matter, 40.9 g/kg crude protein, 2.3 g/kg sugar, 13.5 mg/kg carotene, 8.47 g/kg calcium, 1.12 g/kg phosphorus and 0.27 nutritive units [10]. During our previous studies, we found that *Isatis tinctoria* silage prepared from wilted green mass harvested in the seed development stage contained 137.8 g/kg CP, 31.5 g/kg EE, 354.8 g/kg CF, 368.2 g/kg NFE, 107.6 g/kg ash with fodder value 0.23 nutritive units/kg silage and 2.30 MJ/kg metabolizable energy silage and 110 g/ nutritive units digestible protein [16].

Among the fuels from plant biomass, biogas is of great importance and can successfully replace fossil fuels to obtain electric power and heat. The production of energy biomass by anaerobic digestion is carried out in biogas plants, using a wide range of bacteria, which results in methane gas as a fuel for the production of heat and electricity and carbon dioxide, and the residue called 'digestate' is rich in macro- and micro-nutrients and is widely used as a fertilizer in organic farming. The results on the quality of the investigated silage substrates and the biochemical biomethane potential are presented in Table 2. The carbon/nitrogen ratio is a basic factor that influences the correct course of fermentation and the biomethane yield. Methanogenic bacteria need an optimal ratio of carbon to nitrogen for their metabolic processes, thus, ratios higher than 30:1 were found to be inappropriate for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, because of low pH, poor buffering capacity and high concentrations of ammonia in the substrate. The nitrogen concentration in the silage substrate from *Isatis tinctoria* plants reached 31.36 g/kg and the estimated carbon content of 466.67 g/kg, the C/N ratio = 14.88, and in the silage mass substrate prepared from *Festuca pratensis* plants – 12.80 g/kg nitrogen, 466.67 g/kg carbon, C/N ratio = 39.20. As mentioned above, the *Isatis tinctoria* silage has higher lignin content and less hemicellulose than *Festuca pratensis* silage, a fact that can influence the activity of methanogenic bacteria. The biochemical methane potential per amount of organic mass of the investigated silage substrates did not vary essentially, being 361-367 l/kg, but per dry matter the methane biochemical potential of the silage substrate from *Isatis tinctoria* plants was lower – 304 l/kg. Carchesio et al. [17] remarked that the anaerobic digestion test showed that, in *Isatis tinctoria* herbaceous substrates, the net methane production was 153.1 l/kg VS, with 33% estimated degree of conversion. Ţîţei [16] reported that the gas forming potential of organic digestible matter varied from 438 l/kg in the *Isatis tinctoria* green mass harvested in the flowering period to 450 l/kg in the seed development period, but the calculated methane content in the biogas decreased from 56% to 54%; the calculated biogas capacity of *Isatis tinctoria* silage can reach value 464 l/kg organic substance with 54% methane content Ababii et al. [18] remarked that *Isatis tinctoria* green mass substrate had methane yields 356 l/kg VS, while *Sinapsis alba* green mass substrate had a yield of 349 l/kg and *Raphanus sativus var. oleifera* green mass substrate had a yield of 379 l/kg.

Table 2. The biochemical biomethane production potential of the investigated substrates

Indices	*Isatis tinctoria*	*Festuca pratensis*
Crude protein, g/kg DM	196.00	80.00
Nitrogen, g/kg DM	31.36	12.80
Ash, g/kg DM	160.00	96.00
Carbon, g/kg DM	466.67	502.20
Ratio carbon/nitrogen	14.88	39.20
Acid detergent lignin, g/kg DM	37.00	22.00
Hemicellulose, g/kg DM	201.00	307.00
Biomethane potential, L/kg VS	361.43	367.00
Biomethane potential, L/kg DM	303.60	331.77

CONCLUSIONS

The local ecotype of the species *Isatis tinctoria* is a high-potential plant for diversifying the assortment of agricultural crops grown to produce fodder and energy biomass. The biomass harvested together with other traditional crops could be ensiled and added to the diet of domestic ruminants, but also as a substrate for biomethane production stations.

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