AGROBIOLOGICAL PECULIARITIES AND PROSPECTS FOR VALORIFICATION OF WOAD, *ISATIS TINCTORIA* L., IN MOLDOVA

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Abstract

We studied agro biological peculiarities, chemical composition and nutritional value and evaluated the capacity to produce biogas from aerial biomass of the woad, *Isatis tinctoria* L., family *Brassicaceae* Burnett, which was cultivated on the experimental land of the Botanical Garden (Institute) of Academy of Sciences of Moldova. It was established that the species *Isatis tinctoria*, in the first growing season, was characterised by slow growth and development, produced a basal rosette of leaves and a strong taproot, but in the second growing season, it had an accelerated growth and development rate. This species started flowering 10-15 days earlier than oilseed rape, so, it was valuable for bees as a pollen source. In the flowering period (the second half of April), woad branched stalks reached up to 105-110 cm high, the natural forage yield reached 23.0 t/ha of natural forage with a high degree of foliage (50%), in the seed development period (the end of May) – 35.0 t/ha of natural forage. The chemical composition of solids of green mass of *Isatis tinctoria* in the flowering period is represented as follows: 20.18% raw protein, 4.46% raw fats, 32.40% raw cellulose, 12.25% minerals, 30.76% nitrogen free extracts, but in the seed development period – 12.00%, 3.08%, 38.83%, 8.56% and 37.53%, respectively. The nutritional value in the flowering period was 0.11 nutritive units/kg, 1.14 Mj/kg metabolizable energy and 165.6 g/ nutritive units digestible protein, but, in the seed development period – 0.14 nutritive units /kg, 1.43 Mj/kg and 100 g/ nutritive units gestible protein, respectively. The *Isatis tinctoria* silage prepared from wilted green mass harvested in seed development period, was distinguished by homogeneous dark-brown colour, pleasant smell of pickled cabbage, 0.23 nutritive units./kg and 2.30 Mj/kg metabolizable energy, 110 g/ nutritive units digestible protein, but corn silage – 0.30 nutritive units/kg, 2.64 Mj/kg and 40g/ nutritive units digestible, respectively.
The calculated biogas capacity of woad can reach values of 438-464 l/kg organic substance with 54-56% methane.
Taking into consideration the presented scientific results, the species *Isatis tinctoria* is promising as a crop with multiple utility for founding fodder-melliferous and melliferous-energy plantations, besides; it can be used for green manure.

Key words: agro biological peculiarities, biochemical composition, biogas yield, fodder value, *Isatis tinctoria*, woad

The production of great amounts of high quality plant material at low costs is an important factor for the revival of the agriculture and national economy of the Republic of Moldova. It is therefore, necessary to develop modern cultivation techniques for important traditional crops, but taking into account the expansion of degraded soil and the frequency of droughts, it is necessary to identify, mobilize and introduce new species with multiple utility that have gained importance as a new agricultural tool in recent years.

The family *Brassicaceae* Burnett is a monophyletic group of about 338 genera and some 3709 species distributed worldwide. It includes many economically important ornamental and crop species: vegetables or sources of industrial and cooking oils, condiments, forage and energy biomass (biodiesel and biogas). This crop produces large roots which promote soil aeration and water infiltration by diminishing. Certain species from the *Brassicaceae* family produce glucosinolates as secondary metabolites with toxic properties, affecting nematodes, diseases and weeds (Bohinc T. *et al*, 2012.)

The problem of forage with high protein content is still an actual one in livestock farming. One way of strengthening the fodder base is the use more green fodder. Therefore, to get green fodder is necessary promote the system of fodder production that ensures continuous feeding of animals from early spring to late autumn, but early spring green forage were winter rye and triticale. In recent years, good competitors of these crops are brassicas – forage plants known for their rapid growth, great biomass production and nutrient scavenging ability. They provide an excellent source of energy and protein, are a valuable tool

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for meeting the changing feed requirements of livestock, throughout the year. Feed supply and livestock performance can be manipulated through the use of different forage species. Forage rape (*Brassica napus* ssp. *biennis*) is a popular forage crop capable of producing a large bulk of forage in a short period, but not as winter hardy as triticale and kale (*Brassica oleracea* ssp. *acephala*). Therefore, to obtain early spring forage, woad, *Isatis tinctoria*, is of great interest (Kshnikatkina A.N. *et al*, 2005; Pimonov K. I. *et al*, 2010; Milashenko A.V., 2012) *Isatis tinctoria L.* (syn. *Isatis indigotica* Fortune*)*, commonly known as woad, dyer's woad or glastum is a European native, biennial or short-lived perennial herbaceous plant depending on local environmental conditions, with erect stem, hastate leaves and yellow flowers clustered in racemes (Al-Shehbaz I.A. *et al*, 2006). *Isatis tinctoria* combines valuable biological features, such as drought tolerance and winter hardiness, high content of crude protein and the formation of early, suitable for mowing, vegetative mass. In early spring, *Isatis tinctoria* was a good pasture plant, especially for sheep, when ewes with lambs that were grazing woad gained weight by 41% and lambs – 21%. In spring, after the regrowth of woad within 30-35 days, the harvest reached 35-45 t/ha of green mass, with nutritional value that was not lower than in the pea-oat mixture, it was used for feeding cattle and sheep, preparing grass fodder and silage. It is a beautiful honey plant, blooming much earlier than other honey plants. According to the content of protein, *Isatis tinctoria* is superior to corn, rye, triticale and rape (Vavilov P.P., Kondratyev A.A., 1975; Medvedev P.F., Smetannikova A.I., 1981; Pimonov K.I. *et al*, 2010). Among the positive properties of woad, is the possibility of sowing it several times during the growing season, the characteristics that make it an ideal pioneer crop to be grown on hills and marginal lands and the possibility to use it for various purposes (Milashenko A.V., 2012). Since prehistoric times, the leaves have been used as a natural dye. *Isatis tinctoria* is an important medicinal plant: its leaves are used in traditional medicine mainly for the treatment of infections, specifically: encephalitis, upper respiratory infection and gastroenteritis. *Isatis tinctoria* root extract has antibacterial, antiviral, and antiparasitic properties and is also used to treat infections (Galletti S. *et al*, 2013).

This research was aimed at evaluating some biological peculiarities, yield, biochemical composition of the natural fodder and silage of *Isatis tinctoria* and the possibility to use it as fodder in animal husbandry or as biogas substrate under the conditions of Moldova.

MATERIAL AND METHOD

The local ecotype *Isatis tinctoria* collected from spontaneous flora and cultivated on the experimental land of the Botanical Garden (Institute) served as object of study, the traditional fodder crops: alfalfa, *Medicago sativa* (green mass), and corn, *Zea mays* (silage) – control variants. 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 second half of April) and in the seed development period (the end of May). The green mass yield was measured by weighing. The *Isatis tinctoria* silage was prepared from wilted green mass in the seed development period (2 days after mowing) and evaluated in accordance with the Moldavian standard SM 108. Dry matter, or total solids (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. Organic dry matter, or volatile solids (VS), was calculated through differentiation, the crude ash being subtracted from dry matter. Nitrogen-free extract (NFE) was mathematically appreciated, as difference between organic matter values and analytically assessed organic compounds (Petukhov E.A. *et al*, 1989). The biogas and biomethane, litre per kg of volatile solids (l/kg VS), were calculated using the gas forming potential of nutrients (Baserga U., 1998) and digestible index of nutrients (Medvedev P.F, Smetannikova A.I., 1981).

RESULTS AND DISCUSSIONS

Seeds of woad *Isatis tinctoria* readily germinated at 3 to 25°C. *Isatis tinctoria* plantlets emerged at the soil surface 5-12 days later compared with other brassica forage crops. Over the next 50- 40 days, the growth and development of the aerial part of the plant was very slow, then it accelerated and, until the end of the growing season, produced a basal rosette with long-petioled leaves, 8-15 cm long and 2-4 cm wide. The blue-green leaves had small, soft, fine hairs. The root system was dominated by a strong taproot, which in some plants exceeded 1.0 m in depth, with lateral roots on the upper 18-27 cm that spread laterally about 40 cm.

In the first growing season woad wasn't harvested, but was suitable for grazing.

The species *Isatis tinctoria*, in the second year started vegetating when temperatures were above 3-5ºC, new leaves grew from the crown bud in the rosette, distinguished by an accelerated growth and development rate. 5-7 stems developed

from each rosette, the top of each stem was branched, with many yellow flowers. Wood grows early in the season, often before crops of grain, pasture, or alfalfa emerge. This early growth gives dyer's wood a competitive advantage; it uses early spring moisture and nutrients to grow quickly and produce a large root system. Later, during the growing season, its large taproot draws moisture from deep in the soil to give it an even greater advantage over shallow-rooted species, including many natives. This species started flowering 10-15 days earlier than oilseed rape, so, it was valuable for bees as a pollen source.

Figure 1 *Isatis tinctoria,* flowering period

In the second half of April, during the flowering period (*figure 1*), the branched stalks of *Isatis tinctoria* reached up to 105-110 cm high, the natural forage yield reached 23.0 t/ha of natural forage *(table.1).* The harvested fodder was richer in leaves (50%), but poorer in dry matter (13%). It was found that *Isatis tinctoria*, in the flowering period, was characterized by high content of protein (20.18%), fat (4.46%) and minerals (12.25%), and optimal content of raw cellulose (32.40%) in dry matter, probably due to the report leaves/stems of harvested fodder. It was determined that 100 kg of *Isatis tinctoria* natural fodder contained 11 nutritive units, 114 MJ/kg metabolizable energy, 1.5 kg digestible protein. The harvested green mass of *Isatis tinctoria* can be an excellent supplement, especially to low protein and fat forages such as straw and corn silage.

At the end of May, when the traditional forage crop alfalfa is at the beginning of flowering period and reaches the optimal harvest time, *Isatis tinctoria* is already in the seed development period. The yield of green mass, harvested during this period, reached 35 t/ha and the amount of dry matter and nutrients was considerably higher as compared with the previous period, and was about the same as in alfalfa. Analyzing the data regarding the chemical composition of dry matter in the fodder harvested during this period, a reduction in the content of protein, fat and minerals was found, which was probably related to the ratio leaves/stems. As compared with alfalfa, the dry matter of *Isatis tinctoria* is characterized by higher content of fat and cellulose and lower – of protein and ash. The amount of protein in a nutritive unit meets the zootechnical standards.

Some authors mention similar findings about the quality of *Isatis tinctoria* fodder. So, in 2005, as a result of a research conducted in Penza region, Russia, it was found that the dry matter content of green fodder was 22.3-24.9% raw protein, 3.0-3.6% raw fats, 10.8-12.5% raw cellulose, 13.9-15.4% minerals, 48.5% nitrogen free extracts and the nutritive value reached 0.31 nutritive units/kg green fodder (Kshnikatkina A.N. et .al., 2005); in Western Siberia, Russia - productivity reached 49.2 t/ha green mass, 5.71 t/ha dry matter, 4850 nutritive units and 1256 kg/ha protein (Milashenko A.V., 2012).

The specialized literature states that the fresh mass of *Brassicaceae* plants can be used to prepare silage (Kshnikatkina A.N., et. al, 2005; Pimonov K. I. et al, 2010; Milashenko A.V., 2012), but due to the high moisture content, it is recommended to mow the green mass and let it wilt before ensiling.

Figure 2. Isatis tinctoria silage

Table 1

Biological peculiarities, productivity, biochemical composition and nutritional value of *Isatis tinctoria*

Indices	*Isatis tinctoria* flowering period	*Isatis tinctoria* seed development period	*Medicago sativa* flowering period
Plant height, *cm*	110	105	83
Yield of natural fodder, *kg/m²*	2.30	3.50	2.48
Yield of dry matter, *kg/m²*	0.30	0.58	0.60
Content of leaves in the fodder,%	50	32	44
Biochemical composition dry matter:			
- raw protein,%	20.18	12.00	16.66
- raw fats,%	4.46	3.08	1.88
- raw cellulose,%	32.40	38.83	34.24
- nitrogen free extracts,%.	30.71	37.53	37.22
- mineral substances,%	12.25	8.56	10.00
1kg of natural fodder contains:			
- nutritive units	0.11	0.15	0.20
- metabolizable energy, Mj/kg	1.14	1.43	2.10
- dry matter, g	130.50	166.60	243.00
Digestible protein, g/nutritive unit	165.6	100.00	154.07
Nutritive units, t/ha	2.53	5.25	4.95
Digestible protein, kg/ha	415	529	764

The investigated *Isatis tinctoria* silage (*figure 2*), prepared from wilted green mass harvested in the seed development period, was distinguished by homogeneous dark-brown colour, pleasant smell specific of pickled cabbage, optimal leaf and low dry matter content compared with *Zea mays*, and no juice leakage was observed during fermentation.

As a result of the performed analysis (*table 2*), it was determined that the pH index of the *Isatis tinctoria* silage was 4.74, but the control variant, corn silage – 4.12. The concentration of total organic acids is higher in the *Isatis tinctoria* silage (4.57%), butyric acid has not been found and lactic acid predominates (3.49%). Lactic and acetic acids are present in silage, being predominantly in fixed state, which is desirable because organic acids in fixed state contribute more to the preservation of nutrients in the silage. The standard requirements for a silage made of traditional plants provide a level of lactic acid of the total organic acids at least 50-55% for the quality class I (Cosman S. *et al*, 1996).

Table 2

Biochemical composition and nutritional value of the *Isatis tinctoria* silage

Indices	*Isatis tinctoria*	*Zea mays*
Dry matter,%	25.72	29.16
Biochemical composition:		
- raw protein,%	13.78	6.52
- raw fat,%	3.15	3.23
- raw cellulose,%	35.48	20.30
- nitrogen free extracts,%	36.82	65.66
- minerals,%	10.76	4.26
1 kg of silage contains:		
- nutritive units	0.23	0.30
- metabolizable energy, MJ/kg	2.30	2.64
Digestible protein g/ nut. unit	110	40
pH of the silage	4.74	4.12
Total organic acids,%	4.57	3.38
Acetic acid,% dry matter	1.08	0.62
- free acetic acid,%	0.38	0.27
- fixed acetic acid,%	0.70	0.35
Lactic acid,% dry matter	3.49	2.76
- free lactic acid,%	0.81	1.00
- fixed lactic acid,%	2.68	1.76
Carotene, mg/kg	10.67	9.30

Analyzing the data on the chemical composition of the dry matter from the *Isatis tinctoria* silage, we found a low content of nitrogen free extracts (36.82%) and higher content of raw cellulose (35.48%), raw protein (13.78%) and

minerals (10.76%) in comparison with the control, which influenced the nutritive and energy value, so 1 kg of *Isatis tinctoria* silage contained 0.23 nutritive units and 2.30 MJ metabolizable energy, the digestible protein content was 110 g/nutritive unit, but the corn silage (milk-wax stage of ripeness) contained 0.30 nutritive units, 2.64 MJ and 40 g digestible protein/nutritive unit, respectively.

Forage has the potential to play a significant role in the supply of vitamins to ruminants. Carotene is vitamin A precursor, plays a vital role in bone growth, reproduction and immune system health. We could mention that the carotene content in the *Isatis tinctoria* silage is at high level, in comparison with corn silage.

Due to the increasing concern upon the effect of greenhouse gases and crude oil price, biogas has become of major interest as an alternative energy source. The capability of biomass methanization is tightly associated with organic matter content, chemical composition and nutrient digestibility (degrees of conversion) of the feedstock, harvesting time and plant species (Amon T. et al. 2007). Catch crops may form a potential biomass resource for biogas production (Molinuevo-Salces B. *et al*, 2013).

Table 3

Gas forming potential of the of nutrients *Isatis tinctoria*

Indicators	Green mass (April)	Green mass (May)	Silage
Organic digestible matter , g/kg	555	569	586
Biogas, liter /kg VS	438	450	465
Methane, liter /kg VS	245	242	251
Methane,%	56	54	54
Methane yield, m^3/ha	735	1400	1431

Organic digestible matter (ODM) is an important factor influencing biogas and methane yield. The gas forming potential of organic digestible matter varied from 438 l/kg in biomass harvested in the second half of April, the flowering period, to 450 l/kg VS – in the seed development period (*tab.3*), but the calculated methane content in the biogas decreased from 56 to 54%, reaching from 245 l/kg to 242 l/kg. The substrate from *Isatis tinctoria* silage characterized by high level gas forming potential (450 l/kg VS) and methane production were achieved 1431 m^3/ha.

In the Italian context, the anaerobic digestion test showed that in woad herbaceous substrates the net methane production was 153.1 l/kg VS, with 33% estimated degrees of conversion (Carchesio M. et al, 2014).

CONCLUSIONS

The species *Isatis tinctoria*, in the first growing season, was characterised by slow growth and development, produced a basal rosette of leaves and a strong taproot, but in the second growing season, it had an accelerated growth and development rate. In the flowering period (April) stalks reached up to 105-110 cm high, the natural forage yield reached 23.0 t/ha of natural forage with a high degree of foliage (50%), in the seed development period (the end of May) – 35.0 t/ha of natural forage.

The *Isatis tinctoria* dry matter, in the flowering period, contained 20.18% raw protein, 4.46% raw fats, 32.40% raw cellulose, 12.25% minerals and 30.76% nitrogen free extracts, but in the seed development period – 12.00%, 3.08%, 38.83%, 8.56% and 37.53% respectively.

The nutritional value, in the flowering period, was 0.11 nutritive units /kg, 1.14 Mj/kg metabolizable energy and 165.6 g/nutritive unit digestible protein, but in the seed development period – 0.14 nutritive units /kg, 1.43 Mj/kg and 100 g/ nutritive unit digestible protein, respectively.

The *Isatis tinctoria* silage prepared from wilted green mass harvested in the seed development period, was distinguished by homogeneous dark-brown colour and pleasant smell of pickled cabbage, 0.23 nutritive unit /kg and 2.30 MJ/kg metabolizable energy, 110 g/ nutritive unit digestible protein, but the corn silage – 0.30 nutritive unit /kg, 2.64 MJ/kg and 40 g/ nutritive unit digestible protein, respectively.

The gas forming potential of organic digestible matter varied from 438 l/kg in the 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% (245 l/kg to 242 l/kg VS). The best results of methane production were achieved for *Isatis tinctoria* silage (1431 m^3/ha).

Taking into consideration the presented scientific results, the local ecotype of the species *Isatis tinctoria*, is promising as a crop with multiple utility for founding fodder-melliferous and melliferous-energy plantations, as well, it can be used as green manure.

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