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THE QUALITY OF FRESH AND ENSILED BIOMASS OF *Brassica napus oleifera* AND PROSPECTS OF ITS USE

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Abstract

The genus Brassica, family Brassicaceae, contains 38 species and many of them are known as important agricultural and horticultural crops, which provide edible roots, leaves, stems, buds, flowers and seeds, some are used as human food in many different forms, and some species are used as fodder and cover crops. We investigated some biological peculiarities and the quality of fresh and ensiled biomass of Brassica napus subsp. oleifera, which was cultivated on the experimental land in the National Botanical Garden (Institute), Chisinau. The green mass of Brassica napus oleifera was mowed in the flowering stage and some of its main biochemical parameters were assessed. Thus, crude protein (CP), ash (CA), acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM) and digestible organic matter (DOM) have been determined by near infrared spectroscopy (NIRS) technique using 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, and the sensorial and chemical characteristics of the prepared silage were determined in accordance with the standard for forage quality analysis SM 108. It has been determined that the fresh mass of Brassica napus oleifera contained 14.4% DM, 227 g/kg CP, 97 g/kg CA, 442 g/kg NDF, 285 g/kg ADF, 41 g/kg ADL, 244 g/kg cellulose, 157 g/kg hemicellulose, RFV=140, 13.07 MJ/kg DE, 10.73 MJ/kg ME and 6.75 MJ/kg NEl. These characteristics indicate a good quality of the natural feed for ruminants. The prepared silage contained dark-green leaves with yellow-green stems, the concentration of nutrients was: 231 g/kg CP, 152 g/kg CA, 467 g/kg NDF, 307 g/kg ADF, 34 g/kg ADL, 273 g/kg cellulose, 160 g/kg hemicellulose, RFV=129, 12.77 MJ/kg DE, 10.58 MJ/kg ME and 6.50 MJ/kg NEl. The biochemical methane potential of Brassica napus oleifera substrates reached 309-324 L/kg organic matter.

Key words: *biochemical methane potential, Brassica napus oleifera, feed value, fresh mass, silage.*

INTRODUCTION

Adequate nutrition is essential for the reproduction and productivity of livestock. The forages should provide a stable and balanced diet for farm animals and meet the needs of ruminants to maintain their health and to improve the production of meat, milk and wool. Renewable energy sources coming from agricultural crops could play an important role in terms of energy supply and positive environmental effects. Biogas has become important as an alternative source of energy, because of its decentralized approach.

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. The genus *Brassica* L. includes 38 species, are native to Europe and temperate Asia and are especially common in the Mediterranean region, and the domestication of some of them dates back to antiquity. Many species are known as important agricultural and horticultural crops, which provide edible roots, leaves, stems, buds, flowers and seeds, some species are used as human food in many different forms, while others are also used as fodder, energy and cover crops. The most remarkable members include *Brassica napus* L., *Brassica oleracea* L., *Brassica juncea* (L.) Czern., *Brassica nigra* (L.) W.D.J. Koch., *Brassica rapa* L.

Rapeseed, *Brassica napus* L. subsp. *oleifera* DC., is an herbaceous annual, cultivated mainly for its oil-rich seeds, but also as forage and energy crop, with high potential as a honey plant, occurring commonly in areas with dry, temperate-continental climate. The stem is erect, branched, 70-130 cm tall, vigorous. The leaves are bright green, covered with hairs, with well-defined veins, alternating, the basal ones are petiolate, lyrate, pinnatisect, but the middle and the upper ones - sessile, lanceolate or oblong-lanceolate, covered with a waxy layer. The inflorescence is a raceme with bright yellow hermaphroditic flowers, the flowering stage lasts 15-21 days, and the pollination is predominantly allogamous, entomophilous. The potential honey 242.2-324.8 kg/ha (Ion et al., 2012). The fruit is linear cylindrical silique, 3-5 cm long, with 10-30 round black-bluish to brown-bluish seeds; the weight of 1000 seeds is 3.5-6.5 g. The rapeseed has a tap root with few branches, which penetrates into the soil up to 300 cm deep, the main mass of roots is located at 25-45 cm depth. Winter cultivars of rapeseed utilize environmental resources more effectively and better protect the soil against erosion and nutrient leaching in autumn and winter, are predominant in Europe, their seed productivity, which is 20% to even 60% higher in comparison with spring varieties. Rapeseed oil is variously used in cooking, as an ingredient in soap and margarine, can be made into biodiesel, the residue after oil extraction is used for fodder. *Brassica* species have gained great importance as cover and fodder crops in cropping systems, due to their many environmental and agronomic benefits (Haramoto & Gallandt, 2004; Bell et al., 2020). The incorporation of rapeseed biomass can provide the soil with 7.7 t/ha of organic matter, 280 kg/ha N, 37 kg/ha P, 35.4 kg/ha Mg, 354.0 kg/ha K, 147.1 kg/ha Ca, 0.02 kg/ha Cu, 0.23 kg/ha Zn and 0.02 kg/ha Mn (Ţîţei & Mazăre, 2019). The cultivation of winter fodder rapeseed should be reconsidered, both in terms of fodder value and agroeconomic importance, in cropping systems, yields of 35-50 t/ha of green mass can be obtained. Winter rapeseed can be fed to animals very early (middle of April) and plays an important role in maintaining a continuity of fresh food supply for livestock (Dragomir, 2004).

The aim of the current study was to evaluate some biological peculiarities, the quality of fresh and ensiled mass of winter rapeseed *Brassica napus* subsp. *oleifera* DC., as feed for ruminant animals, as well as substrate for the production of biomethane.

MATERIALS AND METHODS

Brassica napus subsp. *oleifera* cv. ‘Albatros’, which was 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 subject of study and the traditional crops alfalfa, *Medicago sativa*, and common sainfoin, *Onobrychis viciifolia*, were used as controls. The experimental design was a randomised complete block design with four replications, and the experimental plots measured 10 m^2. *Brassica napus* was sown in late August at a depth of 2.0 cm in rows at a distance of 15 cm; the sowing density was 60 germinable seeds per m^2. The plant growth, development and productivity were assessed according to methodical indications. The green mass was harvested in the flowering period. The green mass yield was measured by weighing. The dry matter content was detected by drying samples up to constant weight at 105°C. The leaf/stem ratio was determined by separating the leaves and flowers from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). For ensiling, the green mass was shredded and compressed in well-sealed containers. After 45 days, the containers were opened, and the sensorial and chemical characteristics of prepared silages were determined in accordance with standard laboratory procedures and the Moldavian standard SM 108 for forage quality analysis. 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 of the main biochemical parameters were assessed: crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF) and acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM), digestible organic matter (DOM) were determined by the 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 determined using an empirical equation according to Badger et al., 1979. The biochemical biogas potential (Yb) and the 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):

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

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

RESULTS AND DISCUSSIONS

While conducting the research on the biological peculiarities *of Brassica napus*, we observed that the emergence of the first seedlings occurred 4 days after sowing, and the abundant emergence – during the next 7-8 days. In late autumn, when the average temperatures dropped below 0°C, the rapeseed plants developed a rosette of 6-8 large leaves, and in the underground part - the main root, which was 8-10 mm thick and extended to a depth of over 25 cm. After a period of winter dormancy, the plants resumed growth in the first days of March. The first generative shoots emerged in late March - early April, and the flower buds - in the second half of April. The full flowering stage occurred in the first half of May.

Plant height, stem thickness and leaf/stem ratio have significant impact on the yield, but also affect the forage quality. Some results regarding the bio-morphological characteristics and the structure of the harvested aerial plant biomass are presented in Table 1.

At the harvest time, the height of *Brassica napus* plants was 125.1 cm, while the traditional leguminous forage crops reached 84.5-93.1 cm. *Brassica napus* had the largest weight of a single plant among the studied species. The forage yield of *Brassica napus* reached 58.2 t/ha green mass or 8.5 t/ha dry matter with 59.7% leaves and flowers, but the controls, *Medicago sativa* and *Onobrychis viciifolia* (first cut), respectively – 20.8-25.8 t/ha green mass or 5.6-6.1 t/ha dry matter with 46.5-50.5% leaves and flowers. The *Brassica napus* forage was richer in leaves, but poorer in dry matter, in comparison with the leguminous forage crops.

As a result of the research conducted in Hungary, Miko (2009) determined that the productivity of autumn rapeseed varied from 24.0 to 150.6 t/ha green mass, or 4.2-15.6 t/ha dry matter. Antanasovic (2012) mentioned that, in Serbia, autumn rapeseed could yield 77.1 t/ha green mass or 6.9 t/ha dry matter.

Table 1. Some agrobiological peculiarities and the structure of the green mass of the studied species

Plant species	Plant height, cm	Stem, g		Leaf, g		Yield, t/ha	
		fresh mass	dry matter	fresh mass	dry matter	fresh mass	dry matter
Brassica napus	125.1	123.74	11.81	120.97	17.52	58.2	8.5
Medicago sativa, first cut	84.5	5.38	1.38	4.92	1.41	20.8	5.6
Onobrychis viciifolia, first cut	93.1	12.50	2.86	10.10	2.49	25.8	6.1

Table 2. The biochemical composition and nutritive value of the harvested green mass of the studied species

Indices	*Brassica napus* fresh mass	*Brassica napus* silage	*Medicago sativa* fresh mass	*Onobrychis viciifolia* fresh mass
Crude protein, g/kg DM	227	231	172	166
Ash, g/kg DM	97	152	91	96
Acid detergent fibre, g/kg DM ,	285	307	347	309
Neutral detergent fibre, g/kg DM	442	467	510	447
Acid detergent lignin, g/kg DM	41	34	58	49
Total soluble sugars, g/kg DM	170	6	146	243
Cellulose, g/kg DM	244	273	289	260
Hemicellulose, g/kg DM	157	160	163	138
Digestible dry matter, g/kg DM	752	758	623	669
Digestible organic matter, g/kg DM	709	666	579	615
Relative feed value	140	129	118	142
Digestible energy, MJ/ kg	13.07	12.77	12.20	12.73
Metabolizable energy, MJ/ kg	10.73	10.58	10.03	10.46
Net energy for lactation, MJ/ kg	6.75	6.50	6.04	6.48

Analyzing the results of the fresh mass quality of the *Brassica napus* (Table 2), we found that dry matter contained 227 g/kg CP, 97 g/kg ash, 285 g/kg ADF, 442 g/kg NDF, 41 g/kg ADL, 170 g/kg TSS, 244 g/kg Cel, 157 g/kg HC, with 75.2 % DMD, 70.9 % OMD, RFV=140, 13.07 MJ/kg DE, 10.73 MJ/kg ME, 6.75 MJ/kg NEl, but the leguminous forage crops - 166-

172 g/kg CP, 91-96 g/kg ash, 309-347 g/kg ADF, 447-510 g/kg NDF, 49-58 g/kg ADL, 146-243 g/kg TSS, 260-289 g/kg Cel, 138-163 g/kg HC, with 62.3-66.9% DMD, 57.9-61.5% OMD, RFV=118-142, 12.20-12.73 MJ/kg DE, 10.03-10.46 MJ/kg ME, 6.04-6.48 MJ/kg NEl. The natural fodder of *Brassica napus* was characterised by very high concentration of crude protein, optimal amounts of total soluble sugars and hemicellulose and lower content of acid detergent lignin, which had a positive effect on its digestibility and was able to meet the energy needs of farm animals.

Some authors mentioned various findings about the green mass quality of *Brassica napus.* According to Westwood & Mulcock (2012), the harvested whole plants contained 67.2% leaves and 31.3% stems, 14.3% DM, 10.8% CP, 2.9% lipids, 23.2% NDF, 20.3% ADF, 27.3% WSC, 9.1% ash. Çaçan & Kokten (2017) found that the chemical composition and feed value of the green mass of rapeseed was 15.5-18.1% protein, 40.7-44.1% ADF, 46.4-50.5% NDF, 54.5-57.2% DMD, RFV=101.8-114.9. Gül et al. (2019) reported that canola fresh mass contained 23.66% dry matter with 14.65% CP, 55.33% NDF, 44.80% ADF, 10.16% ADL, 4.94% WSC, 11.39% ash, 54.0% DMD, 60.79% OMD, RFV=90.77 and 8.93 MJ/kg metabolizable energy. Heuze et al. (2019) remarked that the average feed value of rapeseed fresh aerial part was: 12.1% dry matter, 15.8% protein, 3.8 % fats, 19.3% raw cellulose, 28.9% NDF, 23.2% ADF, 4.5% lignin, 14.7% ash, 21.7 g/kg calcium and 5.8 g/kg phosphorus, 79.2% digestible organic matter, 17.6 MJ/kg gross energy, 13.6 MJ/kg digestible energy and 10.6 MJ/kg metabolizable energy.

Forage preservation is a key element for productive and efficient ruminant livestock farms, which provides a uniform level of high quality feed for ruminants throughout the year. Silage is the main preserved green succulent roughage fed to domestic animals. When opening the glass vessels with *Brassica napus* silage, carbon dioxide, a by-product of fermentation, was eliminated in moderate amounts from the preserved mass. During the organoleptic assessment, it was found that silage had agreeable colour, dark-green leaves and yellow-green stems, with specific aroma, somewhat like the smell of pickled cabbage; the consistency was retained in comparison with the initial fresh mass, without mould and mucus. It has been found that the concentration of nutrients and energy in silage was: 231 g/kg CP, 152 g/kg ash, 307 g/kg ADF, 467 g/kg NDF, 34 g/kg ADL, 6 g/kg TSS, 273 g/kg Cel, 160 g/kg HC, with 75.8% DMD, 66.6% OMD, RFV=129, 12.77 MJ/kg DE, 10.58 MJ/kg ME, 6.50 MJ/kg NEl. As compared with the initial fresh mass, the silage of *Brassica napus* had high concentration of ash, cellulose and very low content of total soluble sugars, which had a negative impact on digestibility, relative feed value and net energy for lactation.

Literature sources indicate considerable variation in the chemical composition and nutritional value of *Brassica* silages. According to Balakhial et al. (2008), the dry matter content and the biochemical composition of rapeseed silage was: 17.82% DM, pH = 4.78, 15.68% CP, 52.33% NDF, 32.33% ADF, 12.00% ash, 6.00% fats, 733.3 g/kg DMD, but rapeseed silages treated with different levels of urea and molasses - 18.79-20.21% DM, pH = 4.70-5.23, 15.64-16.67% CP, 47.66-50.66% NDF, 31.60-34.66% ADF, 11.67-12.67% ash, 3.33-4.33% fats, 586.6-760.0 g/kg DMD. Neely et al. (2009) reported that rapeseed silages contained 21.1% CP, 20.7% NDF, 19.9% ADF, 3.33-4.33% fats, 16.6% ash, RFV = 324. Sánchez et al. (2014) found that the dry matter content and the concentrations of nutrients and energy in *Brassica napus* wilted mass were: 308.6 g/kg DM, 277.7 g/kg CP, 302.2 g/kg NDF, 264.9 g/kg ADF, 225.7 g/kg ash, 202.0 g/kg NFC, 535.6 g/kg TDN, 1.33 Mcal/kg NEl, 2.16 Mcal/kg ME, but in the prepared silage: pH = 4.2, 346 g/kg DM, 126.8 g/kg lactic acid, 110.4 g/kg acetic acid, 7.4 g/kg propionic acid, 259 g/kg CP, 258 g/kg NDF, 235 g/kg ADF, 205 g/kg ash, 315 g/kg NFC, 598 g/kg TDN, 1.48 Mcal/kg NEl, 2.38 Mcal/kg ME. Herrmann et al. (2016) studied the biochemical composition of silages made of various crops in Germany and remarked that *Brassica napus* silage contained 213-364 g/kg dry matter with 89.7-92.0% organic matter, pH 3.8-4.5, 3.7-10.6% lactic acid, 1.1-2.8% acetic acid, 0-0.3% butyric acid, 8.9-11.3% CP, 3.4-17.2% fat, 41.2-53.4%

NDF, 34.6-51.2% ADF and 5.7-11.4% ADL. Gül et al. (2019) mentioned that rapeseed silage contained 21.79% dry matter with pH=4.63, 3.6 g/kg lactic acid, 14.31% CP, 54.32%NDF, 41.20% ADF, 19.37% ADL, 1.4% WSC, 13.85% ash, 56.80% DMD, 61.45% OMD, RFV = 97.24 and 7.94 MJ/kg metabolizable energy. Heuze et al. (2019) revealed that rapeseed silage contained 17.2% dry matter, 16.4% protein, 3.8% fats, 18.7% raw cellulose, 29.9% NDF, 23.8% ADF, 5.4% lignin, 19.0% ash, 9.8 g/kg calcium and 0.9 g/kg phosphorus and 17.6 MJ/kg gross energy.

Table 3. The biochemical biogas and biomethane production potential of the investigated substrates

Indices	*Brassica napus* fresh mass	*Brassica napus* green mass	*Medicago sativa* fresh mass	*Onobrychis viciifolia* fresh mass
Minerals, g/kg DM	97	152	91	96
Nitrogen, g/kg DM	36.3	37.0	27.5	26.6
Carbon, g/kg DM	501.7	471.1	505.5	502.2
Ratio carbon/nitrogen	13.8	12.7	18.4	18.9
Cellulose, g/kg DM	244	273	289	260
Hemicellulose, g/kg DM	157	160	163	138
Acid detergent lignin, g/kg DM	41	34	58	49
Bio gas potential, L/kg VS	605	633	540	569
Biomethane potential, L/kg VS	309	324	276	291

Plant biomass may be used for biogas production directly after harvest and as ensiled substrates. Anaerobic decomposition will produce methane, carbon dioxide, some hydrogen and a final product that can be used as a fertilizer. Methanogenic bacteria need a suitable ratio of carbon to nitrogen for their metabolic processes, ratios higher than 30:1 were found to be unsuitable for optimal digestion, and ratios lower than 10:1 were found to be inhibitory, due to low pH, poor buffering capacity and high concentrations of ammonia in the substrate. Dobre 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 results regarding the quality of the *Brassica napus* substrates and the potential for obtaining biomethane are shown in Table 3. The nitrogen content in the studied *Brassica napus* substrates ranged from 3.63% to 3.7%, the estimated content of carbon - from 47.11% to 50.17%, the C/N ratio varied from 12.7 to 13.8, but in the green mass substrates of leguminous crops, there was 2.66-2.75% nitrogen, 50.22- 50.55% carbon and C/N = 18.4-18.9. Essential differences were observed between the lignin contents. The *Brassica napus* substrates contained acceptable amounts of hemicellulose and low amounts of lignin; the biochemical methane potential reached 309-324 l/kg VS. The best methane potential was achieved in *Brassica napus* silage substrate - 324 l/kg VS, the lowest - in the fresh mass substrate of *Medicago sativa*.

According to Zubr (1986), the methane potential of fresh rapeseed substrate was 334 l/kg, but - of rapeseed silage substrate – 330 l/kg. Cleemput (2011) found that the methane values of hybridized winter rapeseed substrates ranged from 275.38 to 396.71 l/kg ODM. Murphy et al. (2011) reported that oilseed rape produced 2.5-7.8 t/ha dry matter and the measured methane yield was 240-340 m^3/t VS. Herrmann et al. (2016) mentioned that, in rapeseed silage substrates, the C/N ratio was 24-31, the methane content - 57.3-62.8 % of the produced biogas, and the biochemical methane potential was 244.2-276.3 l/kg ODM.

CONCLUSIONS

Under the climatic conditions of the Republic of Moldova, autumn rapeseed, *Brassica napus oleifera*, grows and develops optimally. In the flowering stage, early May, the forage yield can reach about 58.2 t/ha green mass or 8.5 t/ha dry matter.

The forage dry matter contains 227 g/kg CP, 97 g/kg ash, 285 g/kg ADF, 442 g/kg NDF, 41 g/kg ADL, 170 g/kg TSS, 244 g/kg Cel, 157 g/kg HC, with 75.2% DMD, 70.9% OMD, RFV=140, 13.07 MJ/kg DE, 10.73 MJ/kg ME, 6.75 MJ/kg NEl.

Brassica napus silage contains 231 g/kg CP, 152 g/kg ash, 307 g/kg ADF, 467 g/kg NDF, 34 g/kg ADL, 6 g/kg TSS, 273 g/kg Cel, 160 g/kg HC, with 75.8% DMD, 66.6% OMD, RFV=129, 12.77 MJ/kg DE, 10.58 MJ/kg ME, 6.50 MJ/kg NEl.

The biochemical methane potential of fresh mass and silage substrates of *Brassica napus oleifera* reaches 309-324 L/kg organic matter.

Brassica napus oleifera can be used as alternative feed for ruminants and as substrate for biomethane production.

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