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THE QUALITY OF FODDERS FROM BIRD'S-FOOT-TREFOIL, *LOTUS CORNICULATUS* L. UNDER THE CONDITIONS OF MOLDOVA

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Abstract

The aim of this study was to determine the green mass productivity, the biochemical composition and the fodder value of a perennial legume – bird's-foot-trefoil, Lotus corniculatus cv. Doru, grown in an experimental field of the National Botanical Garden (Institute), Chișinău. In the third growing season, the bird's-foot-trefoil was characterized by high growth rate and regenerative capacity after mowing, making it possible to mow it four times per season. The annual productivity was 44.50 t/ha green mass or 9.70 t/ha dry matter. It was determined that the quality of the green mass varied depending on the harvest time: crude protein 143.5-194.5 g/kg DM, crude fats 30.1-44.8 g/kg DM, crude cellulose 261.7-357.0 g/kg DM, nitrogen free extract 338.3-456.1 g/kg DM, sugars 37.5-61.0 g/kg, starch 18.1-37.8 g/kg, carotene 48.45-77.00 mg/kg, calcium 10.6-14.9 g/kg and phosphorus 2.2-3.3 g/kg. The biochemical and fodder value of the prepared haylages from first and second cuts were: pH 4.46-4.70, lactic acid 34.1-37.2 g/kg DM, acetic acid 5.7-9.4 g/kg DM, butyric acid 0.2 g/kg DM, organic matter 891.8-902.2 g/kg DM, crude protein 170.9-188.8 g/kg DM, crude fats 41.6-44.6 g/kg DM, crude cellulose 332.2-358.9 g/kg DM, nitrogen free extract 298.6-357.5 g/kg DM, sugars 0.87-1.86%, starch 0.91-1.76%, carotene 14.0-24.0 mg/kg, calcium 10.0-10.8 g/kg DM and phosphorus 2.7-3.1 g/kg DM. The prepared hay from first and second cuts contained 18.89-20.76% crude protein, 2.56-2.74% crude fats, 31.62-32.42 % crude cellulose, 33.86-36.33 % nitrogen free extracts, 10.49-11.01 % ash.

Key words: *biochemical composition, fodder value, green mass, hay, haylage, Lotus corniculatus, productivity*

INTRODUCTION

One of the principal sources of protein of the human diet is of animal origin. The increasing demand for food of animal origin which is caused by the steady growth of the world´s population can and must be met by improving animal performance. Animal husbandry implies a continuous process that requires a constant supply of high quality feed. Feed costs can account for 65% of livestock production expenses. Animal husbandry production is based on natural and cultivated pastures. In recent years, a lot of attention has been paid to improving the quality and reducing the production costs of forages (Marin et al., 2016). In many countries, various fodder species are studied, bred, cultivated and used as sources of protein, essential amino acids, biologically active substances, to provide a balanced diet for animals and thus to increase their productivity and, in addition, to improve soil fertility, to restore degraded grassland and marginal lands (Kshnikatkina et al., 2005). Leguminous plants typically contain more crude protein and less neutral detergent fiber than grasses, which increases the rate of forage digestion, resulting in greater intake. Perennial forage legumes represent one of the highest quality solutions to the constant demand for plant protein in animal husbandry. The inclusion of tanniferous plant species in forage production would be beneficial (Waghorn et al., 1998; MacAdam and Villalba, 2015).

Lotus is the largest genus of the tribe *Loteae*, family *Fabaceae*, comprises 140 species with worldwide distribution. Some of the *Lotus* species show a great potential for adaptation to a number of abiotic stresses, infertile and acidic soils, relevant components of grassland

ecosystems in environmentally constrained areas, can be regarded as pioneer forage legumes and are moderately tolerant to soil salinity (Díaz et al., 2005; Escaray et al., 2012).

Bird's-foot trefoil, *Lotus corniculatus,* is undoubtedly the species considered to have the greatest agricultural importance and the widest distribution. It is a perennial herbaceous plant, similar in appearance to some *Trifolium* species. The plant root system consists of a deep taproot with numerous secondary roots with good lateral spread. Roots can produce new shoots. Growth form ranges from prostrate to erect with numerous stems (10-60 cm) arising from a basal, well-developed crown and branches arising from leaf axils. Primary growth comes from the crown, but re-growth comes from buds formed at nodes on the stubble left after defoliation. Leaves are pentafoliate, alternately on short stalks with the two leaflets at petiole base resembling stipules. The asymmetrical, pointed leaflets are mainly glabrous and more slender and paler green than those of greater lotus. Inflorescences have up to eight yellow flowers and are umbel-like cymes at the end of long, auxiliary branches. The flowering period is indeterminate; consequently, seed develops over an extended period in summer. Seed pods, 2-5 cm long, contain 15-20 seeds attached to the ventral suture; the seeds are released by a sudden split of the pod along both sutures after one to two weeks of ripening, during which the pods change from green to brown. The seeds are round or oval form, greenish yellow or dark brown color. Bird's-foot trefoil is an excellent source of nectar and pollen for honeybees (Dzyubenko and Dzyubenko, 2008).

Bird's-foot-trefoil cultivation started at the beginning of the 19th century, and in the last decades, this species has been studied in many research centers, and thus over 100 cultivars have been created and implemented, including 4 cultivars that have state permission for use in Romania. Worldwide, more than 4 million hectares are planted with *Lotus corniculatus* cultivars. Romanian cultivars of bird's-foot trefoil reached green mass productivity 40-50 t/ha or 9-11 t/ha dry matter, seed yield 400-500 kg/ha (Maruşca et al., 2011). Bird's-foot trefoil is used as an excellent, non-bloating fodder and provides good forage, hay and silage. It is especially good on infertile, poorly drained soils and on soils that are otherwise difficult to cultivate. The overall forage quality under drought conditions is better than that of alfalfa due to a higher leaf-stem ratio, delayed maturity and better quality of all plant components. *Lotus corniculatus* contains condensed tannins and other secondary compounds that reduce protein degradation in the rumen and increase the flow of amino acids to the intestine for absorption, inhibit the formation of enteric methane, prevent bloat in the rumen and reduce internal parasites, has additional benefits for sheep production such as increased wool growth, live weight gain and fecundity (Doran-Browne et al., 2014). The condensed tannins have anthelminthic properties and decrease nematodes in wild and domestic ruminants (Molan et al., 2011).

The aim of this study was to determine the green mass productivity, the biochemical composition and the fodder value of prepared hay and haylage from bird's-foot-trefoil, *Lotus corniculatus.*

MATERIALS AND METHODS

The Romanian cultivar of bird's-foot-trefoil, *Lotus corniculatus,* 'Doru', created at the Research-Development Institute for Grasslands Braşov, Romania, and cultivated in monoculture in the experimental plot of the National Botanical Garden (Institute) "Alexandru Ciubotaru", Chişinău, latitude 46°58′25.7″N and longitude 28°52′57.8″E, served as subject of the research.

The green mass of 3-year-old bird's-foot-trefoil was harvested manually at 5-cm stubble height. The samples were collected in early flowering periods: the first cut (23.05.2019), the second cut (20.06.2019), the third cut (22.07. 2019) and the fourth cut (23.09.2019). The leaves/stems ratio was determined by separating the leaves, buds and flowers from the stem, weighing them separately. The prepared hay was dried directly in the field. The haylage was prepared from wilted green mass, shredded and compressed in well-sealed glass containers. The content of dry matter, protein, fat, crude cellulose, calcium, phosphorus, soluble sugars, starch, ash, lactic, acetic and butyric acids was appreciated in accordance with standard laboratory procedures (Petukhov et al., 1989).

RESULTS AND DISCUSSIONS

Analyzing the agro-biological features of bird's-foot trefoil, *Lotus corniculatus* 'Doru', in the third growing season, it was established that the revival of plants from dormant buds was uniform, generative shoots developed in early April, they were characterized by faster grow and development rates, the flower bud formation of plants started at the middle of May. At the time when the green mass was harvested for the first time, the *Lotus corniculatus* plants reached 55 cm, the yield was 2.50 kg/m^2 green mass or 0.55 kg/m^2 dry matter, characterized by high content of leaves and flowers in the harvested mass (Table 1). Due to the moderate air temperatures during late May and June, and the optimal moisture content of soil, the revival of plants was fast. It was established that during 28 days, *Lotus corniculatus* 'Doru' plants developed shoots that grew about 33-35 cm, and the plants were cut for the second time, obtaining 1.21 kg/m^2 green mass or 0.25 kg/m^2 dry matter, the harvested mass was richer in leaves (71%). In spite of the favorable weather conditions in June-July 2019, with moderate amount of rainfall and optimal temperatures, the bird's-foot trefoil plants recovered well after the harvest, thus, several new shoots developed and, at the end of July, the length of the shoots was 23-25 cm and 0.41 kg/m^2 natural fodder were harvested. The unfavorable meteorological conditions, the lack of rainfall and the very high air temperatures (35-41ºC) during the second half of the summer affected the regeneration and development rate of *Lotus corniculatus* 'Doru' plants. A better growth and development was observed after the rain that fell at the end of August, the formed shoots were prostrate, thin, and over 17-21 cm long. The yield at the fourth harvest decreased in comparison with the previous harvests, and reached 0.33 kg/m^2 green mass, but with higher proportion of leaves (68%) and dry matter content (29%). The annual productivity of *Lotus corniculatus* 'Doru' in the third growing season reached 44.5 t/ha green mass or 9.7 t/ha dry matter.

Table 1. Some agrobiological peculiarities and the structure of the harvested mass depending on the harvest time

Harvest time	Plant height, cm	Stem, g		Leaf + flower, g		Productivity, t/ha		Content of leaves and flowers in fodder, %
		green mass	dry matter	green mass	dry matter	green mass	dry matter	
First cut	56	1.08	0.25	1.70	0.34	25.03	5.33	63.0
Second cut	34	0.52	0.12	0.79	0.15	12.07	2.47	55.6
Third cut	24	0.54	0.14	0.83	0.18	4.13	0.96	56.0
Fourth cut	18	-	-	-	-	3.27	0.94	68.0

Table 2. Biochemical composition and nutritive value of the green mass of depending on the harvest time

Indices	First cut	Second cut	Third cut	Fourth cut
Crude protein, % DM	16.35	19.45	17.87	14.35
Crude fats, % DM	3.91	4.48	4.16	3.01
Crude cellulose, % DM	35.70	31.90	26.17	27.85
Nitrogen free extract, % DM	33.83	33.92	41.14	45.61
Soluble sugars, % DM	3.95	3.75	4.03	6.10
Starch, % DM	1.81	2.00	2.89	3.78
Ash,% DM	10.21	10.26	10.66	9.14
Nutritive units/ kg GM	0.16	0.16	0.21	0.24
Metabolizable energy, MJ/kg GM	1.69	1.69	2.24	2.56
Calcium, %	1.06	1.06	1.49	1.33
Phosphorus, %	0.26	0.33	0.26	0.22
Carotene mg/ kg GM	60.67	48.45	57.00	77.00

The productivity, the quality and the seasonal distribution of forage may be of great importance to the livestock farmers. The quality of the green mass of *Lotus corniculatus* 'Doru' is presented in Table 2. It was determined that the biochemical composition of the dry matter varied depending on the harvest time: crude protein varied from 143.5 to 194.5 g/kg, crude fats – from 30.1 to 44.8 g/kg, crude cellulose – from 261.7 to 357.0 g/kg, nitrogen free extract – from 338.3 to 456.1 g/kg, soluble sugars – from 37.5 to 61.0 g/kg, starch from 18.1 to 37.8 g/kg, ash – from 91.4 to 106.6 g/kg, calcium – from 10.6 to 14.9 g/kg, phosphorus – from 2.2 to 3.3 g/kg. The concentrations of crude protein and fats were high in the green mass obtained after the second harvest and very low – after the fourth harvest. The significantly higher content

of crude cellulose in the first and second harvests, but nitrogen free extract – in the third and fourth harvests. The level of soluble sugars and starch increased substantially in the third and fourth harvests.

Plant carotenoids are precursors of retinol – vitamin A, together with vitamin E and polyphenols, are natural antioxidants in ruminant diets. Higher carotene concentrations in feed contribute to an improvement in the nutritional value of milk products. It was found that, during the third growing season of *Lotus corniculatus* 'Doru' plants, the amount of carotene decreased from 60.67 mg/kg fodder at the first harvest to 48.45 mg/kg fodder at the second harvest, and increased substantially to 77.00 mg/kg in the green mass obtained at the fourth harvest.

The content of nutrients and their digestibility influence the feed and energy value of natural fodder. Therefore, 100 kg of green mass obtained at the first and second harvests contained 16.0 nutritive units and 169 MJ metabolizable energy, at the third harvest – 21 nutritive units and 224 MJ metabolizable energy and at the fourth harvest – 24 nutritive units and 256 MJ metabolizable energy for cattle. The calculated annual feed productivity achieved 7600 nutritive units and 1200 kg digestible protein.

Some authors have mentioned similar findings about the productivity and quality of *Lotus corniculatus*. So, as a result of a research conducted by Nelyubina and Kasatkina (2017) has been revealed that the yield of green mass from two cuts was 20.3-27.2 t/ha, dry mass 4.2-5.6 t/ha, including 18.9-21.4% crude protein, 2.6-2.9% crude fats, 24.9-29.1% crude cellulose, 7.8-8.3 % minerals, 40.6-42.4 % nitrogen free extract, which provided a high concentration of metabolizable energy (9.49-10.35 MJ/kg) and nutritive units (0.76-0.80 kg DM), the concentration of digestible protein 147-154 g/kg; Kshnikatkina et al. (2005) remarked the chemical composition of dry matter bird's-foot trefoil were: 21.0% protein, 3.31% fat, 25.68% cellulose, 7.31% ash; Shlapunov and Karpei (2014), found that in bird's-foot trefoil pure plantations, the annual productivity was 31.4 t/ha green mass, 5.3 t/ha dry mass, 4.9 t/ha fodder units and 1.01 t/ha crude protein, but in mixture with perennial grasses – 29.2-31.3 t/ha green mass, 5.5-6.1 t/ha dry mass, 4.7-5.3 t/ha fodder units and 0.78-0.93 t/ha crude protein.

The wilted silage, or haylage, from legume plants play an important role in the nutrition, wellbeing and productivity of animals. It can help solving some problems in the livestock sector by providing a balanced diet for animals with an appropriate amount of protein and fiber. As for the organoleptic properties, the haylage prepared from *Lotus corniculatus* 'Doru' consists of green leaves and yellowish-green stems; has a pleasant smell of pickled vegetables; the texture of the plants stored as haylage was preserved well, without mold and mucus. The fermentation quality and fodder values of haylage prepared from *Lotus corniculatus* 'Doru' are shown in Table 3. It has been determined that the amounts of organic acids reached 34.1- 37.1 g/kg and pH values 4.46-4.70, most organic acids were in fixed form.

Table 3. The fermentation quality of the investigated haylage from *Lotus corniculatus* 'Doru'

Indices	First cut	Second cut
pH index	4.70	4.46
Total organic acids, g/kg	37.1	34.1
Free acetic acid, g/kg	1.7	4.0
Free butyric acid, g/kg	0.2	0.0
Free lactic acid, g/kg	7.6	6.1
Fixed acetic acid, g/kg	4.0	3.9
Fixed butyric acid, g/kg	0.0	0.0
Fixed lactic acid, g/kg	23.6	18.6
Total acetic acid, g/kg	5.7	9.4
Total butyric acid, g/kg	0.2	0.0
Total lactic acid, g/kg	31.2	24.7
Acetic acid, % total acids	15.36	27.57
Butyric acid, % total acids	0.54	0.0
Lactic acid, % total acids	84.10	72.43

The haylage prepared from green mass obtained at the first cut was characterized by optimal content of lactic acid and low content of acetic acid, in comparison with the haylage prepared from the green mass obtained at the second cut. The butyric acid was detected in fixed form (0.2 g/kg DM) in the haylage prepared after the first cut.

The prepared haylages (Table 4) contained 170.9-188.8 g/kg DM crude protein, 41.6-44.6 g/kg DM fats, 332.2-358.9 g/kg DM crude cellulose, 298.6-357.5 g/kg DM nitrogen free extract, 8.7-18.6 g/kg DM soluble sugars and 9.1-19.1 g/kg DM starch, 97.8-109.1 g/kg DM ash, 10.0-10.8 DM calcium and 2.7-3.1 g/kg

DM phosphorus. In comparison with the initial mass, in the prepared haylages level of crude protein, crude fats, calcium and phosphorus did not change essentially. The amount of carotene decreased substantially to 14-24 mg/kg fodder.
It has been calculated that 100 kg of haylage prepared from green mass obtained at the first cut contained 22 nutritive units, 4.18 kg digestible protein and 265 MJ metabolizable energy, but – at the second cut – 25 nutritive units, 4.93 kg digestible protein and 296 MJ metabolizable energy.

Table 4. Biochemical composition and nutritive value of the investigated haylage from *Lotus corniculatus* 'Doru'

Indices	First cut	Second cut
Dry matter, g/kg	33.89	36.44
Crude protein, %	17.09	18.88
Crude fats, %	4.16	4.46
Crude cellulose, %	33.22	35.89
Nitrogen free extract, %	35.75	29.86
Sugars, %	1.86	0.87
Starch, %	1.79	0.91
Ash, %	9.78	10.91
Nutritive units/ kg	0.22	0.25
Metabolizable energy, MJ/kg	2.65	2.96
Calcium, %	1.00	1.08
Phosphorus, %	0.27	0.31
Carotene mg/ kg	14.00	24.00

Some authors mentioned various findings about the ensilage fodder. Heuzé al. (2015) revealed that the bird's-foot trefoil silage contained 34.7% dry matter with 20.2% protein, 3.8% fats, 38.5% NDF, 30.9% ADF, 9.7% lignin, 5.0% WSC, 9.3% ash, 14.3 g/kg tannins, 65.0% digestible organic matter, 19.2 MJ/kg gross energy, 11.7 MJ/kg digestible energy. Coblentz and Grabber (2013) mentioned that, in the USA, the silages made from bird's-foot trefoil green mass obtained at the first harvest, contained 18.8-20.9% protein, 38.1-41.5% NDF, 31.7-35.7% ADF, 9.3-9.7% lignin, 5.7-8.1% hemicellulose, and the silage made at the second harvest contained 20.2-21.4% protein, 38.5-39.3% NDF, 33.0-33.6% ADF, 8.2-10.0% lignin, 5.5-6.9% hemicellulose.
Hay is valuable feed for farm animals, a rich source of protein, vitamins and minerals, both in winter and throughout the year. The quality of the hay from *Lotus corniculatus* 'Doru' is presented in Table 5. The prepared hay contained 18.89-20.76 % crude protein, 2.56-2.74 % crude fats, 31.62-32.42 % crude cellulose, 33.86-36.33 % nitrogen free extract, 10.49-11.01 % ash. The nutritive value of 100 kg of hay was 51-56 nutritive units, 11.6-13.3 kg digestible protein and 758-764 MJ metabolizable energy.

Table 5. The biochemical composition and the nutritive value of the investigated hay from *Lotus corniculatus* 'Doru'

Indices	First cut	Second cut
Crude protein, %	18.19	20.76
Crude fats, %	2.56	2.74
Crude cellulose, %	32.42	31.62
Nitrogen free extract, %	36.33	33.86
Ash, %	10.49	11.01
Nutritive units/ kg	0.56	0.51
Metabolizable energy, MJ/kg	7.58	7.64
Calcium, %	1.16	1.16
Phosphorus, %	0.29	0.34

Several literature sources describe the bird's-foot trefoil hay quality. According to Medvedev and Smetannikova (1981), the chemical composition of hay was: 14.0-22.3% protein, 1.5-3.6% fat, 6.9-11.2% ash, 22.4-26.0% crude cellulose, 39-51% nitrogen free extract. Kaplan et al. (2009) remarked that hay contained 17.16-20.94% CP, 38.00-43.95% NDF, 30.6238.78% ADF, 7.17-10.17% ash, 2.87 g/kg condensed tannins, 58.69-65.04% digestible dry matter, 9.25-9.59 MJ/kg metabolizable energy, RFV 119.6-159.3. Coblentz and Grabber (2013) found that the concentrations of nutrients in the hay prepared from the green mass first cut ranged from 18.2 to 19.6% protein, 37.6 to 38.7% NDF, 30.7-32.2% ADF, 8.7 to 9.0% lignin, 6.2 to 6.2% hemicellulose and 1.16 to 2.43% condensed tannins, but in the hay prepared after the second cut – from 18.2 to 19.6% protein, 37.2 to 40.1% NDF, 31.8-33.6% ADF, 9.4 to 9.7% lignin, 5.4 to 6.5% hemicellulose and 1.23 to 2.77% condensed tannins.

CONCLUSIONS

The Romanian cultivar 'Doru' of *Lotus corniculatus*, in the third growing season, was characterised by high growth rate and regenerative capacity after being mowed, making it possible to mow it four times per season, reaching a productivity of 44.5 t/ha green mass or 9.7 t/ha dry matter.
The quality of the green mass varied depending on the harvest time: crude protein 143.5-194.5 g/kg DM, fats 30.1-44.8 g/kg, crude cellulose 261.7-357.0 g/kg, nitrogen free extract 338.3-

456.1 g/kg, sugars 37.5-61.0 g/kg, starch 18.1-37.8 g/kg, carotene 48.45-77.00 mg/kg, calcium 10.6-14.9 g/kg and phosphorus 2.2-3.3 g/kg.

The biochemical and fodder value of the prepared haylages was: pH 4.46-4.70, lactic acid 34.1-37.2 g/kg, acetic acid 5.7-9.4 g/kg, butyric acid 0.2 g/kg, organic matter 891.8-902.2 g/kg, crude protein 170.9-188.8 g/kg, fats 41.6-44.6 g/kg, crude cellulose 332.2-358.9 g/kg, nitrogen free extract 298.6-357.5 g/kg, sugars 0.87-1.86%, starch 0.91-1.76%, carotene 14.0-24.0 mg/kg, calcium 10.0-10.8 g/kg and phosphorus 2.7-3.1 g/kg.

The hay prepared after the first and second harvests contained 18.89-20.76% crude protein, 2.56-2.74% crude fats, 31.62-32.42% crude cellulose, 33.86-36.33% nitrogen free extract and 10.49-11.01% ash.

The Romanian cultivar 'Doru' of *Lotus corniculatus* can be used in the Republic of Moldova to restore degraded permanent grasslands, as a component of the mixtures of plants sown to create temporary grasslands, grass-legume strips in vineyards and orchards, and the harvested biomass can be used as natural and conserved fodder for animals.

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