Original paper

PRIMARY ACTIVE SEED SUBSTANCES FROM MEDICINAL PLANTS AS A POSSIBLE SUPPLEMENT TO LIVESTOCK NUTRITION

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Abstract

Some of the active substances from the seeds of medicinal plants have a beneficial effect on human and animal health. The representatives of this group of plants, in addition to the secondary (biologically) active substances significant for their therapeutic properties and the flavours in the content of their seeds, have a high percentage of primary active substances (carbohydrates, oils, proteins and the like). These components are essential for plant growth and reproduction, but they are also very important as constituent substances.

The objective of this study was to determine the effect of storage duration (from one to five years) on oil and protein content of the seeds of various medicinal plants. The seeds of 9 cultivated plant species namely: white mustard (*Sinapis alba* L.), flax (*Linum usitatissimum* L.), fenugreek (*Trigonella foenum graecum* L.), evening primrose (*Oenothera biennis* L.), marigold (*Calendula officinalis* L.), milk thistle (*Silybum marianum* Gaertn.), black cumin (*Nigella sativa* L.), borage (*Borago officinalis* L.) and castor oil plant (*Ricinus communis* L.) were analyzed. Laboratory studies have been performed in three replications in the course of 2014 according to accredited methods.

On average, the highest oil content was obtained from castor seed (50.44%), whereas the lowest average oil content was recorded in fenugreek seed (5.35%). The greatest statistically significant variation of the oil content was observed in the seeds of white mustard. In spite of that, the highest number of medicinal plants had a statistically considerable reduction in oil contents between seeds of different ages. The highest protein content was recorded in the seeds of white mustard (31.96%), which was by about 2.3 times higher than the lowest protein content in seeds of evening primrose (13.80%). The largest statistically significant variation in protein content was observed with the seeds of white mustard, marigold and flax.

The high content of oil and protein, with their beneficial effects, can positively affect the proper functioning of the immune system of bred animals, and can also work as an

antioxidant, as a blood purifier, it destroys intestinal parasites, stops diarrhea, and cures foot-and-mouth disease, and the like.

Key words: *medicinal plants, oil and protein content, seed storage duration*

Introduction

In the modern approach to livestock breeding, practical and research experiences with medicinal plants are also used for the treatment of animals. The possibilities of the application of selected medicinal plants and spice plants in the prophylaxis and treatment of various diseases of farm animals, particularly, their use in the production of fodderphytoadditives, as a safe alternative to synthetic antibiotics, growth promoters and other xenobiotics, can be used as an inexhaustible resource of animal health for the purpose of the production of safe food for humans (Kostadinović, 2013). In addition to the medicinal and the protective function in the utilisation of this group of plants, animals receive the necessary proteins, oils and carbohydrates for their growth and development.

In the production of fodder, plants provide a real opportunity to increase the value of food using a variety of functional accessories. In developed European countries, there is a very strong trend of replacing synthetic antibiotic preparations with medicinal herbal preparations. These compositions have antibacterial activity, antiinflammatory activity, they stimulate digestion, have laxative activity, antidiarrhoeal activity, choleretic activity, and the like, which are already used in human medicine, and which can be added to animal feed in the treatment of various disorders of animal health (Runjaić-Antić et al., 2010). A complete ban on antibiotics in poultry feeds was brought into force on 1st January 2011 by the European Union (EU); thus, all of the antibiotics used at sub-therapeutic doses for growth promotion were withdrawn (Toghyani et al., 2010).

The aim of this study was to determine the effect of storage duration (from one to five years) on the content of oil and protein in the seeds of several medicinal plants.

Material and methods

As the material, the seeds of plant varieties and domesticated plant populations were used: white mustard (*Sinapis alba* L.), flax (*Linum usitatissimum* L.), fenugreek (*Trigonella foenum graecum* L.), evening primrose (*Oenothera biennis* L.), marigold (*Calendula officinalis* L.), milk thistle (*Silybum marianum* Gaertn.), black cumin (*Nigella sativa* L.), borage (*Borago officinalis* L.) and castor oil plant (*Ricinus communis* L.), which had been produced in the production plots of the Institute for Medicinal Plant Research "Dr Josif Pančić" in Pančevo. The processing and preparation for the analysis of oil and protein content was performed in the seed testing laboratory of the Institute in Pančevo. The research was conducted with seeds ranging from five years of age (the seeds from the year 2009) to one year of age (the seeds from the year 2013).

The content of oil and protein was determined in the chemical laboratory of the Institute "Tamiš"–"Tamiš Agrolab" in Pančevo according to the methods prescribed by the Ordinance on the methods of sampling and the methods of physical, chemical and microbiological analysis of animal feed (the Official Gazette of the Socialist Federal Republic of Yugoslavia, Number 15/87). For the determination of protein and oil content was used by 1 g, and 10 g of seed in three replications.

The obtained experimental data were statistically analyzed using the method of variation statistics: the mean value (I) and the coefficient of variation (Cv). The statistical significance of the difference between the calculated mean values was obtained by applying the analysis of variance models (ANOVA), using the statistical software package "Statistica 10 for Windows".

Results and discussion

The highest protein content was recorded in three-year-old seeds (22.25%), and the lowest content was in the oldest ones – five-year old seeds (19.50%). The highest protein content was achieved with the seed of white mustard (31.96%), fenugreek (29.06%) and flax (25.74%). Twice as low protein content was recorded in the seeds of evening primrose, marigold and milk thistle (tables 1 and 2). Both tested factors, as well as their interactions, had a statistically significant variation between the obtained values of protein content ($p < 0.01$).

Table 1. *Protein content (%) in medicinal plant seeds*

Seed maturity	Year					Average
	V	IV	III	II	I	
White Mustard (*Sinapis alba* L.)	22.97	29.13	39.00	33.39	35.31	31.96
Flax (*Linum usitatissimum* L.)	24.45	27.17	26.75	24.71	25.66	25.74
Fenugreek (*Trigonella foenum graecum* L.)	27.79	27.78	28.66	30.53	30.53	29.06
Evening Primrose (*Oenothera biennis* L.)	13.18	13.91	14.66	14.95	14.66	14.27
Marigold (*Calendula officinalis* L.)	11.88	15.06	13.39	14.60	14.07	13.80
Milk Thistle (*Silybum marianum* Gaertn.)	15.25	15.88	15.22	15.53	15.97	15.57
Black Cumin (*Nigella sativa* L.)	22.66	23.41	24.54	24.34	24.50	23.89
Borage (*Borago officinalis* L.)	17.41	18.22	17.19	17.75	17.92	17.70
Castor Oil plant (*Ricinus communis* L.)	19.94	20.95	20.90	22.00	19.83	20.72
Average	19.50	21.28	22.25	21.98	22.05	21.41

Table 2. *Analysis of variance of protein content (%)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant species	8	5301.8230	662.7279	3208.18**	<.001
Years of age	4	129.6687	32.4172	156.93**	<.001
Interaction	32	429.8970	13.4343	65.03**	<.001
Residual	89	18.3851	0.2066		
Total	133	5824.2179			
Cv= 2,1%, * P≤5%, ** P≤1%					
	Plant species		Years of age		Interaction
LSD 5%	0.3298		0.2458		0.7374
LSD 1%	0.4368		0.3256		0.9768

The highest content of oil and protein was recorded in two-year-old seeds (23.17%), and the lowest content was in the oldest ones – five-year-old seeds (19.68%). The highest oil content was achieved with the seeds of castor oil plant (50.44%), flax (35.88%) and black

cumin (33.47%), whereas the oil content in white mustard was slightly lower than expected, amounting to 19.20% (tables 3 and 4). The obtained values of oil content were less than the significance level, 1 and 5%, so it can be concluded that the F-ratio was highly significant ($p<0.01$), or, in other words, that there was a significant effect of plant age and species on oil content.

Table 3. *Oil content (%) in medicinal plant seeds*

Seed maturity	Year					Average
	V	IV	III	II	I	
White Mustard (*Sinapis alba* L.)	14.53	23.91	21.00	17.67	18.91	19.20
Flax (*Linum usitatissimum* L.)	32.73	34.65	36.23	41.96	33.83	35.88
Fenugreek (*Trigonella foenum graecum* L.)	5.57	5.01	5.38	5.11	5.72	5.35
Evening Primrose (*Oenothera biennis* L.)	5.10	5.57	7.42	5.91	5.25	5.85
Marigold (*Calendula officinalis* L.)	12.59	12.20	11.25	11.29	11.29	11.72
Milk Thistle (*Silybum marianum* Gaertn.)	24.04	24.25	24.12	24.29	25.06	24.35
Black Cumin (*Nigella sativa* L.)	28.10	30.53	35.77	36.11	36.88	33.47
Borage (*Borago officinalis* L.)	10.17	12.23	10.49	10.89	11.33	11.02
Castor Oil plant (*Ricinus communis* L.)	44.33	47.14	48.86	55.34	56.54	50.44
Average	19.68	21.72	22.28	23.17	22.75	21.92

Table 4. *Analysis of variance of oil content (%)*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Plant species	8	28636.2660	3579.5333	22204.73	<.001
Years of age	4	203.6588	50.9147	315.84	<.001
Interaction	32	658.9649	20.5927	127.74	<.001
Residual	90	14.5085	0.1612		
Total	134	29513.3983			
Cv= 1,8%, * P≤5%, ** P≤1%					
	Plant species		Years of age		Interaction
LSD 5%	0.2913		0.2171		0.6513
LSD 1%	0.3858		0.2876		0.8627

The following section of the paper presents the results of some former research. Depending on the cultivated variety, better results than our results of the seeds of certain field genotypes of white mustard contain from 25.6% to 28.1% of oil (Piętka et al., 2004). Some of the selected genotypes of white mustard contain even up to 52.8% of oil. An increase in the protein content results in the reduction of the content of p-hydroxybenzyl glucosinolate and erucic acid (Katepa-Mupondwa et al., 1999). According to the statements by Carter (2002), the seeds of flax oil plant contain from 26.9 to 31.6% of protein, and from 31.9 to 37.8% of oil. Similar results in the conditions of South Banat, depending on the variety and agro-ecological conditions of cultivation, the average oil content of about 41.0% was achieved (Filipović et al., 2014). A higher content of oil in flax was achieved on chernozem soils with the signs of gleisation in relation to chernozem on sandy loess (Filipović et al., 2010). The content of protein and oil in the seeds of evening primrose varied depending on the mineral nutrition with nitrogen nutrients and the time of sowing.

The several times higher values than our values of oil content had an interval from 23.5 to 30.1%, whereas the content of protein ranged from 12.5 to 16.2% received Ghasemnezhad and Honermeier (2008). According to Hulan et al. (1987) and Christie (1999), the oil content of evening primrose seeds varies with such factors as the age of the seeds, the cultivar and growth conditions, and typically varies between 18 and 25%, with occasional outliers to 12 or 28%. The seeds of pot marigold have a significant around 20% oil content (Saleem et al., 1986), which is better in comparison with our results. Similar results of the oil content of pot marigold were reported by Dulf et al. (2013). The lipid seeds varied between 13.6 and 21.7 g oil per 100 g of seeds. Also, similar results with our results had the seeds of milk thistle, in addition to secondary metabolites, which are rich in vegetable oil, in the amount from 26.67 to 27.16%, and in protein, in the amount from 23.4 to 24.2% (Khan et al., 2007). Black cumin, depending on agro-ecological conditions, achieves higher or lower oil content. The above results also point to the high content of two primary metabolites. For example, in the conditions of Italy (D'Antuono et al., 2002), from 13 to 23% of oil was achieved, whereas, in the climate of Iran (Cheikh-Rouhou et al., 2007), the seeds of black cumin had as much as 40% of oil. The level of synthesized protein in the seeds of black cumin depends on the variety cultivated, so, in the pieces of research by Cheikh-Rouhou et al. (2007), an interval was recorded from 22.6% to 26.7%.

Conclusion

The age of the seeds of the tested medicinal plant species significantly influenced the content of the two primary metabolites: protein and oil. The highest protein content was recorded in the seeds three years of age (22.25%), and the lowest in five-year-old seeds (19.50%). The highest protein content was achieved in the seeds of white mustard (31.96%), fenugreek (29.06%) and flax (25.74%). The highest oil content was achieved, as expected, in oil plant species (castor oil plant (50.44%), flax (35.88%) and black cumin (33.47%)), whereas the oil content in the seeds of white mustard was somewhat lower than expected, totaling 19.20%.

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