

GENOTYPE-BIOSTIMULATIONS INTERACTIONS IN SOME HIGH QUALITY ACTIVE PRINCIPLES APPEARANCE FOR ALFALFA

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Abstract: The target of this paper was to demonstrate the biostimulators influence on chemical composition of Alfalfa. Alfalfa is appreciated because of its use as animal feed, having a very good production capacity and perennity. It is also resistant to frost and drying, but response promptly at irrigation. Alfalfa has high capacity of poisoning and in exceptional crop conditions it can be harvest 5 to 6 time in a year (MOISUC A and DUKIC D., 2002). Alfalfa can be used as green fodder, hay, silage (in combination with graminaceae), hay flour, granules, or for preparation of combined feed. Also, Alfalfa is a principal component of temporary grassland used after mowed. In the biological crop system, Alfalfa flour has essential role in bovine feeding. *Medicago sativa* is one of the ameliorator plants of soil because of its high content in nitrogen that remain in the soil, and thus improve the physical state of soil. Alfalfa is a very good source for good protein quality, with appreciable content of high biological value amino acids. Researchers try to find the secrets of genotip-biostimulators interactions, having the results increasing fodder yield and quality of Alfalfa plants. Quality of a crop used as animal feed has in view the increasing of total protein content, the digestibility, amino acids content and profile, beta-carotene content and composition in other vitamins, and also to decrease the quantity of hemolytic saponins content (BABINEC et al., 2003; OLESZEK and MARSTON, 2000). In this paper, from different crops of Alfalfa treated with various biostimulators, we analyzed some vitamins concentration, such as: liposoluble vitamins – vitamin A, and vitamin E; and water soluble vitamins – vitamin B₁, B₂, B₆, B₁₂ and vitamin C. The methods for quantitative analysis were specified for every vitamin. The results of our study demonstrate that biostimulators can positively influence the content of vitamins – especially liposoluble vitamins, in case of using Magafol as lucerne crop biostimulator. Alfalfa, usually used as feed, is used for a long time in traditional medicine for its therapeutically effects. Also, the Alfalfa leaf contains high quantities of vitamins (liposoluble vitamins A, D, E, K; and hydrosoluble vitamins B, C).

Key words: Alfalfa, biostimulators, liposoluble and water soluble vitamins.

INTRODUCTION

Alfalfa, also known as Lucerne, or “*Medicago sativa*”, is used as animal fodder with excellent nutritive value. Utilization of different biostimulators on Alfalfa crops influences the chemical composition of plants.

It is a plant with very good nutritional properties. It is used as feed fresh plant, forage, hay, or silage, granules or hay flour. It is resistant of frost and dry weather, and response very good to irrigation. Based on research data, it is better to manage the crop cultivation using the right biostimulator on Alfalfa, finally to obtain the best vitamins and protein composition of plants (BEKOVIĆ D. et al., 2010). Also, researchers reported that mechanical drying of Alfalfa tends to preserve the vitamins. The harvest procedure, drying way, the composition of the plan (presence of different enzymes), and many other factors are involved in preservation or destruction of more than one nutritional factors, such as vitamins.

Alfalfa is used as animal feed, having a very good production capacity and perennity. It is also resistant to frost and drying, but response promptly at irrigation. Alfalfa having high capacity of poisoning, and in exceptional crop conditions it can be harvest 5 to 6 time in a year (MOISUC A and DUKIC D., 2002; COJOCARIU L., MOISUC A., 2006).

Researchers try to find the secrets of genotip-biostimulators interactions, having the results increasing fodder yield and quality of Alfalfa plants. Quality of a crop used as animal feed has in view the increasing of total protein content, the digestibility, amino acids content and profile, beta-carotene content and composition in other vitamins, and also to decrease the quantity of hemolytic saponins content (BABINEC et al., 2003; OLESZEK and MARSTON, 2000).

Biostimulators interact with plant's genotype and can modify the nutritional quality of Alfalfa. Vitamins profile is very important, and because of this it is necessary to detect the type of vitamins and also the concentration. Also, Alfalfa is used in herbal traditional medicine for a very long time (over 1500 years) and has high concentration in proteins, minerals – as calcium, vitamins – as vitamin E and K (liposoluble vitamins) and vitamin from B group and vitamin C.

MATERIALS AND METHODS

The methods for quantitative analysis were specified for every vitamin.

So, vitamin E (as α -tocopherol) was analyzed with HPLC method. The samples were saponified with an ethanol solution of potassium hydroxide, and vitamin E was extracted in petroleum ether. Then, the extraction solvent was evaporated, and the residue was dissolved in methanol. Finally, the quantification of vitamin E, as alfa-tocopherol, we used the Reverse Phase High Performance Liquid Chromatography (HPLC) and with fluorescence detection.

For analysis the concentration of thiamine we used "VitaFast Vitamin B₁ (thiamin)" product (Product Code P1006). Thus, vitamin B₁ is extracted from the sample and the extract is diluted. The diluted extract and the vitamin B₁-assay-medium are pipetted into the wells of a microtiter plate which is coated with *Lactobacillus fermentum*. The growth of *Lactobacillus fermentum* is dependent on the supply of vitamin B₁. Following the addition of vitamin B₁ as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation is done in the dark at 37°C for 44-48 h. The intensity of metabolism or growth in relation to the extracted vitamin B₁ is measured as turbidity and compared to a standard curve. The measurement is done using a microtiter plate spectrophotometer at 610-630 nm (alternatively at 540-550 nm).

The analysis for concentration of vitamin B₂ (riboflavine) used "VitaFast® Vitamin B₂" protocol, with Product Code P1007. Vitamin B₂ was extracted from the sample and the extract was diluted. The diluted extract and the vitamin B₂ assay-medium were pipetted into the wells of a microtiter plate which was coated with *Lactobacillus rhamnosus*. The growth of *Lactobacillus rhamnosus* was dependent on the supply of vitamin B₂. Following the addition of vitamin B₂ as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation was done in the dark at 37°C for 44-48 h. The intensity of metabolism or growth in relation to the extracted vitamin B₂ was measured as turbidity and compared to a standard curve. The measurement was done using a microtiter plate spectrophotometer at 610-630 nm (alternatively at 540 - 550 nm).

Piridoxine or vitamin B₆ was analyzed with „VitaFast® Vitamin B₆” product (Product Code P1008). Vitamin B₆ was extracted from the sample and the extract was diluted. The diluted extract and the vitamin B₆ assay-medium were pipetted into the wells of a microtiter plate which was coated with *Saccharomyces cerevisiae*. The growth of *Saccharomyces cerevisiae* was dependent on the supply of vitamin B₆. Following the addition of vitamin B₆ as a standard or as a

compound of the sample, the bacteria grow until the vitamin is consumed. The incubation was done in the dark at 30°C for 44-48 h. The intensity of metabolism or growth in relation to the extracted vitamin B₆ was measured as turbidity and compared to a standard curve. The measurement was done using a microtiter plate spectrophotometer at 610-630 nm (alternatively at 540-550 nm).

For analyze of vitamin B₁₂ (cyanocobalamin) concentration we used „VitaFast® Vitamin B₁₂ » protocol, having the Product Code P1002. Thus, vitamin B₁₂ was extracted from the sample and the extract was diluted. The diluted extract and the vitamin B₁₂ assay-medium were pipetted into the wells of a microtiter plate which was coated with *Lactobacillus delbrueckii* subsp. lactis (leichmannii). The growth of *Lactobacillus delbrueckii* was dependent on the supply of vitamin B₁₂. Following the addition of vitamin B₁₂ as a standard or as a compound of the sample, the bacteria grow until the vitamin is consumed. The incubation was done in the dark at 37°C for 44-48 h. The intensity of metabolism or growth in relation to the extracted vitamin B₁₂ was measured as turbidity and compared to a standard curve. The measurement was done using a microtiter plate spectrophotometer at 610-630 nm (alternatively at 540-550 nm).

Concentration on vitamin C (ascorbic acid) was analyzed with 2,6-dichlorophenol-indophenol method. This method is based on the ability of ascorbic acid to reduce the dye 2,6-dichlorophenol-indophenol to a colorless compound, by titrimetry (Tillmans method).

The results were statistically evaluated and the mean and standard deviation were calculated with Excel program for statistics.

RESULTS AND DISSCUTIONS

For every result of vitamin concentration we made three independent analysis and the data were statistically evaluated. Thus, in the table 1 we present the result as mean (X) and standard deviation (SD). As we present in the introduction part, we analyzed the concentration of vitamin E (as alfa-tocopherol) – as liposoluble vitamin and the concentration of thiamine, riboflavine, pyridoxine, cyanocobalamin and ascorbic acid – as water soluble vitamins from Alfalfa dry plant. The data presented in table were expressed as milligrams of vitamin per 100 grams of whole dry plant of Alfalfa.

Vitamin E, as alfa-tocopherol, had very good concentration in Alfalfa dry plants. González-Martin and his collaborators (2006) found out a large variation of vitamin E content in Alfalfa plants. In theirs study, alfa-tocopherol varied from 0.55 to 5.16 mg/ 100g plant, and beta-tocopherol together with gamma-tocopherol varied from 0.07 to 0.48mg/100g.

Also, HASSAN and his collaborates (1983) present a study that demonstrate the importance of a good quality of Alfalfa and evaluating the biopotency of vitamin E in Lucerne meal for chicken. That explains the correlation between the quality of Alfalfa crop and the utilization as feed for different animals, having the final target a higher and better quality of production.

Table 1

Vitamins content from Alfalfa (whole dry plant) expressed as mg/100g dry plant

Alfalfa	Liposoluble vitamin	Water soluble vitamins				
	X ± SD	X ± SD				
	Vit. E	Vit. B ₁	Vit. B ₂	Vit B ₆	Vit. B ₁₂	Vit. C
Mt.	2.41±0.060	0.11±0.008	0.12±0.005	0.11±0.005	0.15±0.005	0.20±0.010
Megafol	2.70±0.049	0.11±0.008	0.12±0.012	0.15±0.005	0.15±0.010	0.15±0.005
Folibor	2.74±0.033	0.11±0.009	0.13±0.012	0.13±0.012	0.10±0.005	0.16±0.005
Cropmax	2.73±0.025	0.13±0.012	0.13±0.005	0.12±0.005	0.10±0.005	0.19±0.005

Note: vit. E – vitamin E (α-tocopherol), vit. B₁– thiamine; vit. B₂ – riboflavine; B₆ – pyridoxine; vit. C – ascorbic acid.

Data we obtained are very valuable for qualitative characterization of Alfalfa crops. For biological and nutritional value of Alfalfa plants we are interesting to evaluate the protein and vitamin content. Literature research data showed that Alfalfa is a very good source of vitamins. Thus, JAMES A. DUKE (in 1983), made a very good compilation of vitamins concentration analyzed from Alfalfa, where he observed that Alfalfa is a very good source of vitamins A and vitamins E, because it contains 6.24mg/100g beta-carotene, 15.23mg/100g alfa-tocopherol, 0.15 mg/100g thiamine, 0.46 mg/100g riboflavine, 1.81 mg/100g niacin, and small concentration of panthothenic acid, biotin, folic acid, pyridoxine, cyanocobalamin, and vitamins K. Also, fresh Alfalfa plant contains high concentration of ascorbic acid, but it losses about 80% on drying.

Our data are comparable with other studies, but in this evaluation we have to have in view a lot of aspects as: the variety; the soil composition; the time of cultivation and harvest; the way and time of drying; the storage conditions, and others, including the weather conditions. All these together can modify the chemical composition of Alfalfa and of course, the biological and nutritional value.

Compared to literature data, the concentration of riboflavine (0.12-0.13mg/100g) is very high in Alfalfa, and the concentration of pyridoxine is also important.

Our target was to observe the influence of genotip-biostimulators interactions, using some different biostimulators, having the final results increasing fodder yield and quality of Alfalfa plants. Thus, as we can observe from the table 1, most of the vitamin concentrations were greater after we treated the crop with biostimulators, excepting the vitamin B₁₂ and vitamin C.

CONCLUSIONS

Our results regarding the utilization of different biostimulators in Alfalfa crops followed the influence of genotip-biostimulators interactions, having the final results increasing quality of Alfalfa plants. Thus, we analyzed the concentration of some vitamins and we conclude the following aspects:

- The concentration of liposoluble vitamin – alfa-tocopherol was higher in case of using biostimulators for Alfalfa plans, especially after Folibor treatment.
- Concentration of all water soluble vitamins was positively influenced in case of using biostimulators for Alfalfa crops, with two exceptions: for vitamin B₁₂ and vitamin C.
- Vitamin B₁ (thiamine) has a very small increasing concentration after biostimulators, but Cropmax treatment was followed by the most increased quantum.
- Analyzing the most vitamin concentrations, we observe that the best positively variation was observed after using biostimulator treatment for Alfalfa crops.

Looking at the results of our study we can conclude that using the biostimulators for Alfalfa crops positively influence the concentration of vitamins in plants, and also the quality of Alfalfa. But, because the Alfalfa is a very important plant for animal feed, the quality is established meaning the protein and vitamin qualitative and quantitative composition that challenge us in more thoroughly study.

Finally, for the best results, we have to find the equilibrium between the production of Alfalfa using biostimulators, and the quality of crops that are used in animal feeding, and having some data of vitamins composition in Alfalfa after biostimulators applications is an appreciable progress.

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