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Structural fiber components of cell walls after herbicide treatment of bird's foot trefoil stand

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Abstract

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In the period 2020 – 2023, in the experimental field of Research Institute of Mountain Stockbreeding and Agriculture – Troyan, on light grey pseudopodzolic soil, a scientific experiment with bird's-foot trefoil (*Lotus corniculatus* L.), variety Targovishte 1, was conducted with the following treatments: 1. Control – untreated; 2. Kalam (20 g/da); 3. Kalam (40 g/da); 4. Focus ultra (100 ml/da); 5. Focus ultra – (200 ml/da) was conducted. The aim was to determine the structural fiber components of the cell walls and to calculate the nutritive value of the forage as a function of herbicide treatment. It was found that herbicides applied in the 2–4 leaf stage of bird's foot trefoil did not significantly affect the fiber composition of cell walls and the nutritional value of forage. Relatively favorable in terms of forage quality stood out those treated with Kalam herbicide at 20 g/da, due to the lowest values obtained for neutral detergent fiber (NDF), acid detergent fiber (ADF) and cellulose, and the highest for hemicellulose, as well as the highest feed units for growth. Correlation analysis revealed the highest correlations between dry matter yield and the relative proportion of leaves (r = 0.833); between the relative proportion of stems in the stand and acid detergent lignin content (r = 0.829) and degree of lignification (r = 0.837); and between neutral detergent and acid detergent fiber (r = 0.940), allowing regression equations to be derived for predicting forage quality.

Keywords: bird's foot trefoil; herbicide treatment; fiber components; nutritional value; correlations; regressions

Introduction

The bird's foot trefoil is a forage legume crop suitable for livestock feed due to its high productivity and forage quality (Naydenova et al., 2013). It is also applicable from an economic point of view due to the supply of nitrogen to the soil during the years of its cultivation. It shows good tolerance to acidic, poorly drained, dry and low-fertility soils (Ayala and Carámbula, 2009). Its main disadvantage is slow growth and plant development in the year of sowing (Formoso, 2011). During this period, the crop behaves as a weak competitor, and is highly susceptible to weed interference, which limits its development (Rodrigues-Costa et al., 2011).

The chemical method of weed control is the most efficient and inexpensive way to overcome this disadvantage of bird's foot trefoil. This method of weed control allows effective control in crops of large areas, has high productivity and lack of labor (Oliveira and Inoue, 2011). Weed-free bird's foot trefoil crops affect not only productivity, but also forage quality (Radovic et al., 2003). Herbicides determine the grassland density, botanical and morphological composition, which in turn determines forage quality (Chourkova, 2013).

The content of cell wall components (NDF, ADF, ADL) increases until the full bloom stage, after which it decreases (Naydenova et al., 2013). Mathematical models with linear regression predict *in vitro* dry matter digestibility of different cell wall fibers with high accuracy (coefficient of determination R = 0.847–0.937). The percentage content of the fractions of structural fiber components influences the digestibility of animal feed and participates as a factor in the

evaluation of its quality (Bozhanska et al., 2015). Forage quality is determined by the growth and development phase, soil-climatic conditions, leaf : stem ratio, harvest phase of forage, (Arzani et al., 2001), and largely depends on the basic chemical composition, energy value, digestibility, fiber, mineral and vitamin content (Newman et al., 2009).

The introduction into practice of the chemical method of weed control in bird's foot trefoil is advisable, but not enough herbicides have been studied for their management, making it necessary to search for suitable ones.

This study aimed to determine the content of structural fiber cell wall components in bird's foot trefoil forage mass after herbicide treatment.

Material and Methods

The research experiment was carried out in the period 2020–2023, in the experimental field of RIMSA-Troyan, on light grey pseudopodzolic soil under non-irrigated conditions with bird's foot trefoil (*Lotus corniculatus* L.), variety Targovishte 1. The trial was laid out in the block method in 4 replications with a plot size of 5 m^2 with the following treatments: 1. Control – untreated; 2. Kalam (dicamba 600 g/kg and tritosulfuron 125 g/kg) – 20 g/da; 3. Kalam (dicamba 600 g/kg and tritosulfuron 125 g/kg) – 40 g/da; 4. Focus ultra – 100 ml/da (active substance cycloxydim 100 g/l); 5. Focus ultra – 200 ml/da (active substance cycloxydim 100 g/l). The sowing was carried out in spring, at a seeding rate of 1.2 kg/da, at a row spacing of 12.5 cm, and the area was rainfed before and after. We applied the herbicides at 2–4 leaf stage of the bird's foot trefoil with a backpack sprayer at a working solution rate of 50 l/da. In the budding-early flowering phenophase, one tiller was harvested in the first year and two tillers in the remaining years of the experimental period. Average samples weighing 500 g were taken from each variant of the research experiment, and then dried to a constant dry weight.

The fiber composition of cell walls was determined in $g.kg^{-1}$ dry matter and included:

Neutral-detergent fiber (NDF); Acid-detergent fiber (ADF) and Acid-detergent lignin (ADL) were determined by the detergent analysis of Van Soest et al. (1991); **Degree of lignification** (expressed as a percentage of ADL/NDF); **Hemicellulose** (calculated by NDF – ADF); **Cellulose** = ADF – ADL.

Nutritional value of the forage was estimated using the Bulgarian system as Feed Units for Milk (FUM) and Feed Units for Growth (FUG), and calculated on the basis of equations according to the experimental values of crude protein (CP), crude fiber (CF), crude fat (FC) and nitrogen-free extractable substances (NFE), recalculated using digestibility coefficients proposed by Todorov et al. (2010) as follows: **Gross energy (GE**, MJ/kg DM) = 0.0242*CP + 0.0366*FC + 0.0209*CF + 0.017*NFE – 0.0007*Zx.; **Exchangeable energy (EE,** MJ/kg DM) = 0.0152*CmP + 0.0342*Cm + 0.0128*CmVl + 0.0159*CmBev – 0.0007*Zx.

Feed Units for Milk (FUM) = EE*(0.075 + 0.039*q).

Feed Units for Growth ((FUG) in kg DM) = EE*(0.04 + 0.1*q).

Statistical treatment of data is represented by mean (X), standard deviation (SD), coefficient of variation (VC), minimum (min) and maximum (max) values. The software products used were Analysis Toolpak for Microsoft Excel 2010 and Statgraphics Plus v.2.1.

Results and Discussion

Herbicide treatments affected the structural fiber compo-

Table 1. Content of fiber components of dry matter cell walls of bird's foot trefoil after herbicide treatment averaged over the period 2020–2023

Variants	NDF ($g\ kg^{-1}$DM)	ADF ($g\ kg^{-1}$DM)	ADL ($g\ kg^{-1}$DM)	Hemicellulose ($g\ kg^{-1}$DM)	Cellulose ($g\ kg^{-1}$DM)	Degree of lignification (Coefficient)
1. Control – untreated	408.0	328.1	98.4	79.9	229.7	24.2
2.Kalam 20 g/da	383.9	297.2	108.5	86.7	188.7	28.2
3. Kalam 40 g/da	396.3	313.7	97.0	82.7	216.6	24.6
4. Focus ultra 100 ml/da	404.3	319.4	101.4	84.8	218.0	25.1
5. Focus ultra 200 ml/da	403.1	330.3	122.3	72.8	208.0	30.6
X	399.1	317.7	105.5	81.4	212.2	26.5
SD	0.95	1.33	1.04	0.54	1.52	2.78
VC	2.38	4.18	9.82	6.67	7.19	10.47
Min	383.9	297.2	97.0	72.8	188.7	24.2
Max	408.0	330.3	122.3	86.7	229.7	30.6

*NDF – Neutral-detergent fiber; ADF – Acid-detergent fiber; ADL – Acid-detergent lignin; Hemicellulose; Cellulose; Degree of lignification.
Last column "Degree of lignification" is coefficient, not $g\ kg^{-1}$DM, but smaller number.
Source: Authors' own elaboration

nents of the cell walls in the forage mass of bird's foot trefoil differently, as indicated by their different contents (Table 1).

The results of the study showed that their forage mass had the lowest content of neutral and acid detergent fibers after treatment with Kalam 20 g/da herbicide, which positively affected the dry matter digestibility. The mean values of these parameters were lower than those of the control by 24.1 and 30.9 g kg^{-1} DM. Neutral and acid detergent fiber contents showed little variation among the different herbicide treatments, which is evident from their coefficients of variation, VC= 2.38 (NDF) and 4.18% (ADF), respectively. The very low degree of variability proves that the two herbicides and their doses do not affect the content of the two indicators. The cell wall is composed mainly of hemicellulose, cellulose and lignin. The presence of the lower fiber concentration in the cell wall improves feed intake, and its utilization improves energy availability (Nikolova et al., 2016). Hemicellulose values were lowest and ranged in a narrow range from 72.8 (Min) g kg^{-1} DM to 86.7 (Max) g kg^{-1} DM, with an average value 81.4 g kg^{-1} DM.

The lowest values of NDF, ADF and cellulose and the highest of hemicellulose were found after treatment with Kalam 20 g/da. Acid detergent lignin was affected to a higher extent compared to the neutral and acid detergent fiber fractions, with differences in the control being of higher values. The bird's foot trefoil as a legume has a higher content of lignin, which is necessary for plants but completely indigestible and not digestible by animals. Therefore, it is a limiting factor for the digestibility of forage plants. Higher lignin content implies lower digestibility of forage, which is confirmed by the research of Naydenova et al. (2015). An exception was observed after treatment with the herbicide Kalam at a dose of 40 g/da, which occupied lower values than the control, but the differences were unproven. Lignin serves to bind cellulose fibers and to strengthen plant cell walls. Acid detergent lignin ranged from 97.0 to 122.3 g kg^{-1} DM, depending on which the mean degree of variability was determined according to the coefficient of variation VC = 9.82%. The forage mass with reduced lignin content was digested faster in the rumen of the animal and utilization increased (Srinivasa Reddy et al., 2005).

The degree of lignification increased after herbicide application and from 24.2 in the control variant reached 30.6 in the forage mass, treated with the herbicide Focus Ultra at a dose of 200 ml/da. This proves that the degree of lignification was affected by the type and dose of herbicides used, with an increasing trend prevailing. The treatment with Focus Ultra at a dose of 200 ml/da has the most favorable and proven effect on this process. The lower doses of the two herbicides were more favorable to the lignification process, the differences being insignificant. At a later stage of plant development and as plants grow taller, lignification increases and leads to a strengthening of the plant stems (Niwińska et al., 2005). Treatment with this herbicide (Var. 5) adversely affected forage quality due to high values of acid detergent fiber (330.8 g kg^{-1} DM), acid detergent lignin (122.3 g kg^{-1} DM) and degree of lignification (30.6).

Table 2. Nutritional energy value of forage mass after herbicide treatment on average for the period 2020–2023

Variants	GE MJ/kg DM	EE MJ/kg DM	FUM kg DM	FUG kg DM
1. Control – untreated (C)	17.54	7.60	0.70	0.63
2. Kalam 20 g/da	17.20	7.62	0.70	0.64
3. Kalam 40 g/da	17.32	7.57	0.70	0.63
4. Focus ultra 100 ml/da	17.33	7.50	0.69	0.63
5. Focus ultra 200 ml/da	17.54	7.32	0.67	0.60

Source: Authors' own elaboration

Forage quality is determined by the energetic nutritional value expressed in feed units for milk and growth (Petkova and Pavlov, 2008; Nikolova et al., 2016). The obtained results (Table 2) showed that the grass biomass from bird's foot trefoil treated with herbicides has lower values of gross and exchange energy, which determines the low levels of feed units and thus lower potential milk and meat productivity. Only the grassland treated with Kalam at 20 g/da had a marginally higher, but unproven value relative to the control of exchange energy (0.62 MJ/kg DM). Herbicide treatment did not affect energy nutritional status in the variants compared to the control. The obtained values of forage energy nutritive value after herbicide application were almost similar or with insignificant differences compared to the control on average over the study period, which were statistically unproven (Marinov-Serafimov et al., 2016). This gives a reason to state that the applied herbicides Kalam and Focus ultra in two doses each, on stand from bird's foot trefoil, do not affect the nutritive value of the forage. The exception was the treatment with Kalam at a dose of 20 g/da, where the forage mass had a FUM value of 0.70, which was similar to those of the control, and the growth FUG were 0.64, and marginally exceeded that of the control, and those obtained from the forage mass of the other treatments. Application of the herbicide Focus Ultra at a dose of 200 ml/da reduced the amount of feed units for milk by 0.3% and feed units for growth by 0.4% relative to the control.

The treatment of the bird's foot trefoil with herbicides influenced to the highest degree the variation of the mean yield values, the morphological composition of the stand, the

Table 3. Correlation coefficients of yield, weed density, weed morphological composition and quality indicators after herbicide treatment of grassland from bird's foot trefoil

	Yield	Leaves	Stems	Weeds	NDF	ADF	ADL	Hemicel-lulose	Cellulose	Degree of lignifi-cation
Yield	1									
Leaves	0.833	1								
Stems	-0.371	-0.589	1							
Flowers	-0.811	-0.895	0.167							
Weeds	-0.681	-0.862	0.657	1						
NDF	0.494	0.478	-0.267	-0.811	1					
ADF	0.307	0.428	-0.123	-0.757	0.940	1				
ADL	-0.421	-0.325	0.829	0.345	-0.099	0.187	1			
Hemicellulose	0.110	-0.208	-0.173	0.430	-0.555	-0.805	-0.635	1		
Cellulose	0.553	0.592	-0.669	-0.893	0.887	0.745	-0.516	-0.270	1	
Degree of lignification	-0.515	-0.407	0.837	0.499	-0.313	-0.023	0.976	-0.495	-0.683	1

Source: Authors' own elaboration

density and the content of the fibrous components of the cell walls. The dry matter yield of forage production recorded the highest positive correlation (Table 3) with the relative proportion of leaves (r = 0.833) in the herbage, and in contrast a negative correlation with flowers (r = 0.811), percentage of stems (r = -0.371), and weed density (r = -0.681). Higher percent leaf participation in grassland determines higher forage quality and forage uptake, which has been proven by many authors (Cohen, 2001; Soster et al., 2004; Ducati et al., 2015). The theoretical regression line and the regression equation between dry matter yield and the relative proportion of leaves in stand from bird's foot trefoil treated with herbicide are depicted in Fig. 1, where y = 0.0117x + 36.298 with a high coefficient of determination, R^2 = 0.6935. The obtained yield showed a positive but relatively weak correlation with cellulose content (r = 0.553), which proved the correlation between these two parameters.

The effectiveness of the applied herbicide treatment is a numerical expression of the obtained high value of the correlation coefficient between the relative proportion of stems in the stand with the acid detergent lignin content (r = 0.829), and the degree of lignification (r = 0.837). Fig. 2 shows the very high coefficient of determination (R^2 = 0.9425), and the regression equation of the relationship between the proportion of stems in the grassland and the mean values of acid detergent lignin (y = 1.5128x – 64.395).

The results of the analysis indicate a very high positive correlation (r = 0.940) between the neutral detergent fiber and acid detergent fiber parameters. The high correlation relationship allows the derivation of a regression equation (Fig. 3), which is, respectively, y = 1.3142x – 20.679, with an extremely high coefficient of determination R^2 = 0.8834.

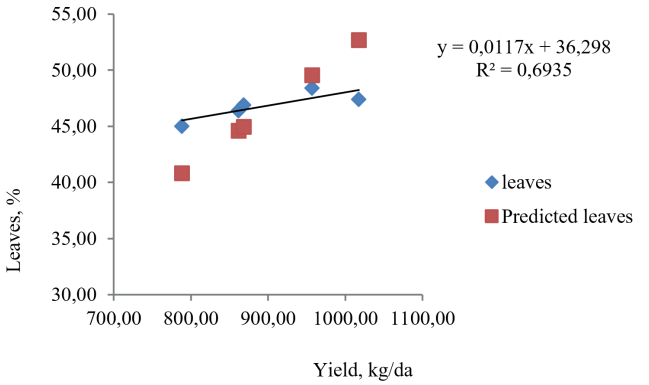


Fig. 1. Theoretical regression line and regression equation between yield and relative proportion of leaves in the grassland after treatment of bird's foot trefoil with herbicides

Source: Authors' own elaboration

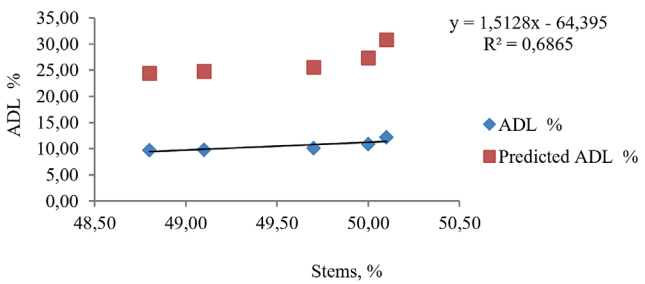


Fig. 2. Theoretical regression line and regression equation of the relationship between the relative proportion of stems in the grassland and the acid detergent lignin content after treatment of bird's foot trefoil with herbicides

Source: Authors' own elaboration

The amount of cellulose and that of neutrally detergent fibers also showed an extremely high degree of correlation, as evidenced by the high correlation coefficient, r = 0.887, respectively. The high correlation between the acid detergent lignin content and the degree of lignification is also evident, represented by the very high correlation coefficient r = 0.976, which allows the derivation of a regression equation (Figure 4), represented by y = 2.6152x – 1.0175, with a very high coefficient of determination $R^2 = 0.9251$.

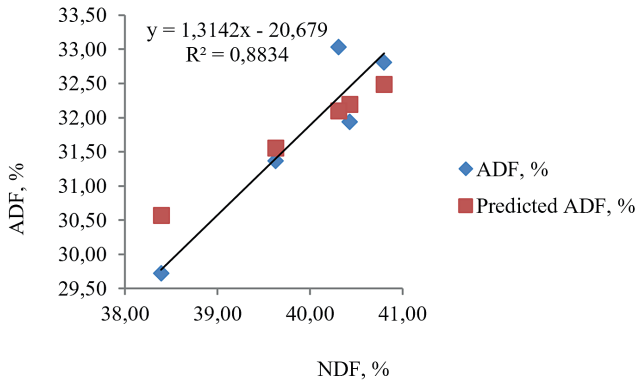


Fig. 3. Theoretical regression line and regression equation between neutral detergent fibers and acid detergent fibers content after herbicide treatment of bird's foot trefoil

Source: Authors' own elaboration

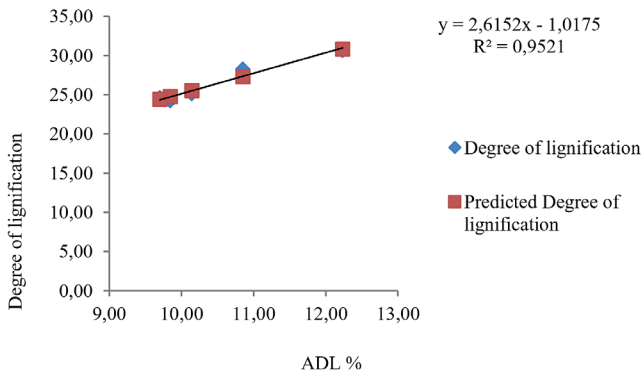


Fig. 4. Theoretical regression line and regression equation of the relationship between acid detergent lignin and the degree of lignification after treatment of bird's foot trefoil with herbicides

Source: Authors' own elaboration

Conclusions

The herbicides Kalam (20 and 40 g/da) and Focus ultra (100 and 200 ml/da) applied at 2–4 leaf stage of bird's foot trefoil had no significant effect on cell wall fiber composition and nutritive value of forage.

Crops of bird's foot trefoil treated with Kalam herbicide at 20 g/da recorded the lowest values of NDF, ADF and cellulose and the highest of hemicellulose, while the forage mass marginally exceeded the control in Feed Units for Growth.

High correlation coefficients were found between dry matter yield and the relative proportion of leaves (r = 0.833); between the relative proportion of stems in bird's foot trefoil and acid detergent lignin content (r = 0.829) and degree of lignification (r = 0.837), and neutral detergent and acid detergent fibers (r = 0.940), allowing the derivation of regression equations to predict forage quality.

References

Arzani, H., Torkan, J., Jafari, M. & Nikkhah, A. (2001). Investigation on effects of phenological stages and environmental factors (soil and climate) on forage quality of some important range species. *Journal of Agricultural Sciences, 32*, 385–397.

Ayala, W. & Carambula, M. (2009). The agronomic value of the genus *Lotus*. *Uruguay*: *INIA*, 424 (Es).

Bozhanska, T., Naydenova, G. & Naydenova, Y. (2015). Study on grazing ecotype of birds foot trefoil in terms of selection. *Plant science, LII*(5), 17–22.

Chourkova, B. (2013). Agro-ecological aspects of the weed infestation control in birds foot trefoil grown for forage. *Banat's Journal of Biotechnology, IV*(7), 29–34.

Cohen, D. C. (2001). Degradability of crude protein from clover herbages used in irrigated dairy production systems in northern Victoria. *Australian Journal of Agricultural Research, 52*(3), 415–425.

Ducati, C., Neres, M. A., Castagnara, D. D., Oliveira, P. S. R., Weirich, D., Silva, G. M. & Sunahara, S. M. M. (2015). Forage potential of oat Iapar 61 alone or in association with birds foot trefoil. *Cien. Inv. Agr., 42*(3), 341–351.

Formoso, F. (2011). Production of seeds of forage species. *Uruguay*: *INIA*, 234 (Es).

Marinov-Serafimov, P., Naydenova, Y., Golubinova, I., Enchev, S., Kikindonov, Tz. & Ilieva, A. (2016). Influence of some herbicides on forage quality of alfalfa. *Bulgarian Journal of Crop Science, 53*(5-6), 67–75.

Naydenova, G., Hristova, Ts. & Aleksiev, Y. (2013). Objectives and approaches in the breeding of perennial legumes for use in temporary pasturelands. *Biotechnology in Animal Husbandry, 29*(2), 233–250.

Naydenova, Y., Kyuchukova, A. & Pavlov, D. (2013). Plant cell walls fiber component analysis and digestibility of birds foot trefoil (*Lotus corniculatus* L.) in the vegetation. *Agricultural Science and Technology, 5*(2), 164–167.

Naydenova, Y., Vasilev, E. & Kirilov, A. (2015). Plant cell wall fiber components content and digestibility of orchard grass (*Dactylis glomerata* L.) and legume forage species in pure stands and mixtures. *Journal of Mountain Agriculture on the Balkans, 18*(1), 61–76.

Newman, Y. C., Adesogan, A. T., Vendramini, J. & Sollenberger, L. (2009). Defining Forage Quality: SS-AGR-322/AG332. *EDIS*, 5. https://edis.ifas.ufl.edu/pdffiles/AG/AG33200.pdf

Nikolova, I., Georgieva, N. & Naydenova, Y. (2016). Forage quality and energy feeding value estimation of alfalfa (*Medicago sativa* L.), treated by biological active compounds. *Journal of Mountain Agriculture on the Balkans, 19*(1), 78–95.

Niwińska, B., Strzetelski, J. A., Kowalczyk, J., Borowiec, F. & Domański, P. (2005). The effect of phenological stage and season on nutritive value, chemical composition and nutrient digestibility of lucerne (*Medicago sativa* L.) green forage in the alimentary tract of cattle. *Czech Journal of Animal Science, 50*(11), 511–518.

Oliveira, Jr. R. S. & Inoue, M. H. (2011). Selectivity of herbicides for crops and dangerous plants. In: *Biologia e manejo de plantas daninhas* (Oliveira Jr., R.S., Consttantin, J. & Inoue, M.H., Eds.). *Curitiba: Omnipax*, 243–262 (Es).

Petkova, R. & Pavlov, D. (2008). Nutritional value of varieties of winter fodder under irrigation. *Bulgarian Journal of Crop Science, 45*, 364–367.

Radovic, J., Dinic, B. & Pudio, V. (2003). Productivity and quality of some bird's foot trefoil (*Lotus corniculatus* L.) varieties. In: *Proceedings of the 12th Symposiumof the European Grassland Federation*, Pleven, Bulgaria, 26–28 May, 118–121.

Rodrigues-Costa, A. C. P., Martins, D., Costa, N. V., Campos, C. F., Martins, C. C., Pereira, M. R. R. & Silva, J. I. C. (2011). Selectivity of herbicides applied pre-emergence in forage grasses. *Planta Daninha, 29*(3), 625–633 (Es).

Soster, M. T. B., Scheffer-Basso, S. M., Dall'Agnol, M., Brustolin, R. & Fontaneli, R. S. (2004). Agronomic characterization of birdsfoot trefoil genotypes (*Lotus corniculatus* L.). *Revista Brasileira de Zootecnia, 33*, 1662–1671 (Pt).

Srinivasa Reddy, M. S., Chen, F., Shadle, G., Jackson, L., Aljoe, H. & Dixon, R. A. (2005). Targeted down-regulation of cytochrome P450 enzymes for forage quality improvement in alfalfa (*Medicago sativa* L.). *Proceedings of the National Academy of Sciences, 102*(46), 16573–16578.

Todorov, N., Atanassov, A., Ilchev, A., Ganchev, G., Mihailova, G., Girginov, D., Penkov, D., Shindarska, Z., Naydenova, Y., Nedjalkov, K. & Tshobanova, S. (2010). *Practice in Animal nutrition*. East-West Edition, Sofia, Bulgaria. ISBN 978-954-321-733-5.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*, 3583–3597.

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