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Evaluation of selected feed parameters of grapevine leaves using the near infrared spectroscopy method

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Abstract: In the face of increasing demand for food and limited natural resources, agriculture faces great challenges, including the need to find alternative sources of feed for livestock. One promising solution is the use of grapevine (*Vitis vinifera*) leaves, which are a by-product of the wine industry. The aim of this study was to determine the differences in the chemical composition of the leaves of four grape cultivars ('Regent', 'Rondo', 'Seyval Blanc', 'Solaris') and to assess their potential as feed for livestock. The research was carried out at the Nobilis vineyard in the Sandomierska Upland. Leaf biometric and quality parameters such as leaf area, mass of 1 leaf, number of leaves per shoot, number of lignified shoots per bush and content of moisture, protein, fibre, ash, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), organic matter (OM) and dry matter (DM) digestibility and total sugars were analysed. The results showed significant differences between cultivars. 'Solaris' had the highest area (1.27 m^2) and leaf mass (0.0086 kg), which may suggest its higher potential as a feedstock. 'Seyval Blanc' had the smallest leaves but the highest number of leaves per shoot and per 1 ha area, which may be beneficial for crop intensification. The 'Rondo' and 'Regent' cultivars stood out for their high protein content and digestibility, making them a valuable feed ingredient. 'Regent' also contained the highest soluble sugar content (7.59%). The use of vine leaves as feed can help reduce waste in wine production, support sustainable agriculture and improve the quality of feed for livestock.

Keywords: animal feed, by-products, feed quality, sustainable agriculture, *Vitis vinifera*

INTRODUCTION

In the face of increasingly scarce natural resources and growing demand for food, agriculture faces great challenges. The projected increase in consumption of animal products in the future will result in a huge demand for feed (Henchion *et al.*, 2021; Costa *et al.*, 2022; Erinle and Adewole, 2022). Forage must be included in ratios to ensure proper nutrition for ruminants. Consequently, the search for alternative sources of nutrition for livestock becomes a necessity (Wadhwa, Bakshi and Makkar, 2015). The ideal solution to this problem, it might seem, could be to recycle waste biomass and use them in animal feed. Well-optimised recycling in this form could reduce the waste of resources and lower the costs of raising and breeding animals (Dame-Korevaar *et al.*, 2021).

Agricultural production, including the processing of agricultural products, inevitably leads to the production of a variety of post-processing residues. The increase in waste generated by agricultural activities necessitates the development of an integrated technology for its management (Costa *et al.*, 2022; Erinle and Adewole, 2022). Efficient management of these wastes can significantly contribute to reducing the negative environmental impact of food production (Peng *et al.*, 2023; Rasool *et al.*, 2023). From an economic point of view, finding an outlet for waste biomass makes sense, and the agri-food industry is realising this. Much of what remains of agricultural crops contain valuable nutrients, even though it is currently treated as waste. Post-production waste and crop residues are not an intentional product of a process, but a by-product of its implementation. In a sustainable economy, it is important to reduce the amount of waste generated while using as many resources as possible. Waste biomass represents valuable raw materials that can serve many purposes, including food production. The use of biomass as animal feed can help to make up for the shortages found in most developing countries. Moreover, such a strategy can bring these ingredients back into the human food chain. This not only promotes innovation, but also cares for the environment. Transforming waste into new products for animal feed is an important step towards a sustainable future. Furthermore, it aims to add value to the by-product (Wadhwa, Bakshi and Makkar, 2015; UN, 2015; Partyka, 2020; Maia *et al.*, 2021). In the absence of high-quality roughage, tree and shrub leaves can provide an alternative source of nutrients. Their use for roughage is justified by the lack of need for grasses or other green crops, especially in winter (Simbaya, Chibinga and Salem, 2020; Karan and Basbag, 2022).

The grapevine (*Vitis vinifera*) is widely cultivated around the world for its fruit, which is used to produce wine, juices and other products (Brenes *et al.*, 2016; Spigno, Marinoni and Garrido, 2017). However, grapevine leaves, which are often treated as waste, one of the most abundant in the wine industry, while in countries such as the Mediterranean, they are considered a delicacy. Vine leaves prove to be a rich source of bioactive compounds in particular polyphenols and flavonoids, nutrients, and have numerous health-promoting properties (Pantelić *et al.*, 2017; Moldovan *et al.*, 2020; Goicoechea *et al.*, 2021; Maia *et al.*, 2021). Moreover, they are distinguished by their high protein and calorie content and increase the activity of digestive enzymes, which improves digestive function and promotes intestinal health (Peiretti and Tassone, 2019; Kazemi and Bezdi, 2022). Considering the above, it is worth appreciating grapevine leaves in terms of their use as a feed source in animal nutrition (Bordiga (ed.), 2016; Albergamo *et al.*, 2020; Maia *et al.*, 2021), in a balanced manner with other feed sources (Birkinshaw *et al.*, 2022).

The aim of the study was to assess the suitability of grapevine leaves as a potential feed or as a feed additive for farm animals. The differences in the chemical composition of leaves of four grapevine cultivars were analysed.

MATERIALS AND METHODS

The experiment was conducted in 2023, at the Nobilis vineyard (50°39'N; 21°34'E), located in the Sandomierska Upland, in south-eastern Poland. In spring 2010, self-rooted vines of four cultivars were planted: 'Regent', 'Rondo', 'Seyval Blanc' and 'Solaris'. The vines were grown on loess soil, at a spacing of 1.0–2.0 m, giving 5,000 vines per hectare. Cultivation was carried out in the form of a single fixed string with a shrub trunk 40 cm high and one immovable arm about 0.9 m long. Leaf area (m^2), mass per leaf and number of leaves per shoot were analysed and these parameters were then converted to area per ha and number of woody shoots per bush.

The leaves were collected in the autumn after the fruit harvest, separately for 50 selected bushes of each cultivar. Green, healthy, contaminant-free, disease- and pest-free leaves, as well as those in full vigour, were selected for analysis. Their mass, including petioles, was then determined by weighing them on an AXIS A250 electronic balance with an accuracy of 0.001 kg. After weighing, the leaves were dried in a laboratory dryer at 105°C to obtain a moisture level not exceeding 10%. Next, the dried material was ground. After grinding, the material was analysed using the near infrared spectroscopy method (NIRS) using the NIR Flex N-500 apparatus with a ready calibration for the evaluation of bulk feed by INGOT. The NIRS method uses the properties of near infrared radiation, which penetrates the sample, interacts with its molecules and causes characteristic absorptions. They are the result of oscillations of chemical bonds, especially those containing hydrogen. Analysis of the absorption spectrum obtained with this method allows for determining the chemical composition of the sample. Based on the degree of absorption of the beam in a given range, its percentage content in the tested sample is determined (Marchi de *et al.*, 2007; Workman and Weyer, 2012; Gąsior, 2013).

The experiment was designed in a randomised block design, with four combinations and five replicates. The replicates consisted of plots, each containing three plants. After the experiment was completed, the obtained results were statistically analysed using one-way analysis of variance (ANOVA). Additionally, the results were presented in graphical form. Statistical inferences were performed at a significance level of $p < 0.05$. Multivariate data analysis techniques were used, including cluster analysis, the aim of which was to group grape cultivars into homogeneous clusters, ensuring that objects in each cluster were more similar to each other than to objects in other clusters. The results of the cluster analysis were presented using a dendrogram. All analyses were performed using STATISTICA 13 software.

RESULTS AND DISCUSSION

The biometric parameters of the waste biomass for four vine cultivars, i.e. 'Regent', 'Rondo', 'Seyval Blanc' and 'Solaris' are presented in Table 1. The analysis was performed for leaf area (m^2), leaf area and mass per unit area 1 ha, mass of 1 leaf (kg), number of woody shoots per 1 bush, number of leaves per 1 shoot and number of leaves per unit area 1 ha. The study indicates that there are statistically significant differences between the cultivars tested.

The measured analysis of the area per leaf of the assessed grape cultivars ranged from 0.85 to 1.27 m^2 and varied significantly between the tested combinations. In the area per hectare, the variation ranged from 1,006,682.2 to 1,294,973.3. The significantly largest leaves were obtained for the cultivar 'Solaris', while the significantly smallest leaves were obtained for the cultivar 'Seyval Blanc'. Similarly, the leaves of the 'Seyval Blanc' cultivar had significantly the lowest leaf mass per 1 ha among the assessed grape cultivars, while the cultivar 'Solaris' had significantly the highest mass.

Table 1. Biometric analysis of the aboveground parts (leaves, shoots) of the vine in relation to the cultivar

Parameter	Value for				*p*-value
	'Regent'	'Rondo'	'Seyval Blanc'	'Solaris'	
Leaf surface (m^2)	0.92 ±0.01^{BC}	1.07 ±0.03^{B}	0.85 ±0.01^{C}	1.27 ±0.12^{A}	0.0001
Surface of leaves on the surface1 ha (m^2)	1,025,502.3 ±5,429.3^{B}	1,065,317.0 ±3,7128.0^{B}	1,006,682.2 ±13,246.3^{B}	1,294,973.3 ±127,202.2^{A}	0.0025
Mass of 1 leaf (kg)	0.0061 ±0.0002^{C}	0.0072 ±0.0002^{B}	0.0051 ±0.0003^{D}	0.0086 ±0.0006^{A}	0.0001
Mass of leaves on the surface 1 ha	6,763.4 ±215.2^{B}	7,167.7 ±211.0^{AB}	6,081.3 ±348.1^{B}	8,789.7 ±1,167.1^{A}	0.0043
Number of woody shoots on 1 bush	14.8 ±0.3^{A}	13.7 ±0.1^{BC}	13.2 ±0.2^{C}	14.3 ±0.4^{AB}	0.0005
Number of leaves on 1 shoot	15.1 ±0.2^{B}	14.5 ±0.2^{B}	17.9 ±0.1^{A}	14.3 ±0.5^{B}	0.0001
Number of leaves on an area of 1 ha	1,114,733.3 ±8,855.8^{A}	995,500.0 ±7,893.7^{B}	1,184,350.0 ±10,559.2^{A}	1,023,133.3 ±65,832.8^{B}	0.0005

Explanations: A–D = differences between averages shown with different letters in the same column are significant ($p < 0.05$).
Source: own study.

There were no significant differences in the evaluation of the analysed parameter between the cultivars 'Regent' and 'Rondo'. In a study carried out by Intrigliolo and Castel (2010) on the cultivar 'Tempranillo' and Buttaro *et al.* (2015) on nine grape varieties, significant differences were noted in the average area of a single leaf in the irrigation applied and cultivar dependence. In study of Intrigliolo and Castel (2010), the mean area per leaf was 0.789 and 0.816 m^2. The results obtained by Buttaro *et al.* (2015) showed significant differences in the mean area of a single leaf depending on the cultivar, where the highest mean area of a single leaf was characterised by the cultivar 'Victoria' and was 204.6 cm^2, while the lowest mean single leaf area, 116.5 cm^2, was characterised by the cultivar 'Crimson'.

The mass per leaf ranged from 0.0086 to 0.0051 kg and depended significantly on the cultivar. Shrubs of the cultivar 'Solaris' significantly formed the heaviest leaves, while 'Seyval Blanc' significantly formed the lightest leaves. A similar relationship was shown for leaf mass per ha, which ranged from 8,789.7 to 6,081.3 kg. The leaves of the 'Seyval Blanc' cultivar had significantly the lowest leaf mass per ha of the grape cultivars assessed, while the leaves of the 'Solaris' cultivar had the highest.

A further analysis was carried out to check for differences between the number of woody shoots per bush depending on the cultivar. The number of shoots oscillated between 13.2 and 14.8, and the parameters tested did not differ significantly within the tested combination. The highest number of lignified shoots was obtained for 'Regent', followed by 'Solaris'. In contrast, the least was obtained for the cultivar 'Seyval Blanc' followed by 'Rondo'. Analogous conclusions were obtained by Klimek, Kapłan and Maj (2023), where it was observed that the number of lignified shoots per single bush for the cultivars 'Rondo', 'Seyval Blanc' and 'Regent' did not differ significantly and oscillated between 12.5 and 15.5 shoots.

The parameters for assessing the number of leaves per shoot showed that the number of leaves ranged from 14.5 to 17.9. When analysing the trait, significant differences were noted between the combination used and the number of leaves per shoot. Shrubs of the 'Seyval Blanc' cultivar had significantly the highest number of leaves, while shrubs of the 'Rondo' cultivar had the lowest. Similar correlations to 'Rondo' were shown by the cultivar 'Solaris', followed by 'Regent'.

An analysis of the number of leaves per 1 ha was carried out to check for differences between the number of leaves produced per 1 ha and the cultivars. The evaluation parameters obtained showed that their number oscillated between 1,184,350.0 and 995,500.0, where the highest values were obtained for 'Seyval Blanc' and the lowest for the cultivar 'Rondo'. When analysing the trait, there was no significant difference between the combination used and the number of leaves per hectare.

The dendrogram shows a cluster analysis of leaf mass per unit area (1 $Mg \cdot ha^{-1}$) for four grape cultivars (Fig. 1).

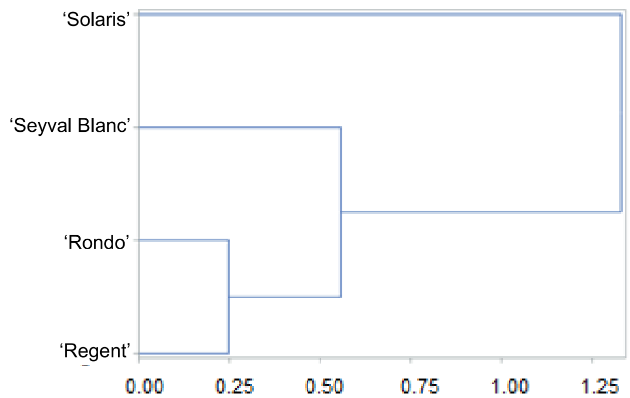

Fig. 1. Comparative analysis of leaf mass in relation to the cultivar used in viticulture; source: own study

This analysis illustrates the classification of these cultivars based on similarities in leaf biomass production. The dendrogram shows that there are three main clusters. The first cluster is formed by the cultivars 'Regent' and 'Rondo'. The proximity of these cultivars suggests that they have similar leaf mass per hectare, which may be beneficial in terms of homogeneity of cultivation and ease of management of these resources. In contrast, the second cluster is formed by the cultivar 'Seyval Blanc', which shows greater differences in leaf mass compared to the first cluster, but is still more similar to 'Regent' and 'Rondo' than 'Solaris'. 'Seyval Blanc' forms a separate cluster that joins the first cluster at a moderate distance. This indicates some differences, but also some similarities in leaf biomass production compared to the cultivars in the first cluster. In contrast, the third cluster is formed by the cultivar 'Solaris', which is furthest away from the other cultivars on the dendrogram. 'Solaris' only joins the rest at a much greater distance, indicating that it has a different leaf mass per hectare compared to the other cultivars. This difference suggests that 'Solaris' may have unique characteristics in terms of leaf biomass production, which may be beneficial in terms of specific production needs.

The division of varieties into three main clusters provides information on the potential use of different grape cultivars according to specific production and quality needs. Knowledge of leaf mass per hectare enables better planning and management of resources in viticulture in a sustainable manner. Cultivars in the first cluster may be more homogeneous and easier to manage, while cultivars in the second and third clusters may offer unique characteristics beneficial for specific production purposes.

The quality parameters of vine leaf forage according to cultivar are shown in Table 2. The study indicates that there are statistically significant differences between the cultivars tested, with the exception of fibre.

The moisture content of the test material ranged from 8.69% for 'Rondo' to 11.95% for 'Regent'. The difference between these values is 3.26%. The leaf moisture content of 'Regent' and 'Solaris' was more than 2.5 times lower than that reported for fresh leaves of other woody species in the study by Kazemi and Bezdi (2021), where the average leaf moisture exceeded 65% (wet basis – w.b.). In contrast, the values obtained in the present study (8.69–11.95% w.b.; 0.095–0.136 kg H_2O·kg^{-1} d.m.) are comparable to those reported for dried grapevine leaves, approximately 9% w.b. (≈0.10 kg·kg^{-1} d.m.) after microwave and convective drying (Alibaş, 2014), as well as to the final moisture range described in convective drying studies (Doymaz, 2012).

Similar correlations were observed in the determination of protein, where the protein level was highest for 'Rondo' (14.98%) and lowest for 'Solaris' vines (7.95%). Analysing grapevine leaves, Kocadayioğulları, Boğa and Ermetin (2024) found that they contained 12.83% total protein while in most of the analysed cultivars it was around 14%, and only a lower content was found in the leaves of the 'Solaris' cultivar.

In terms of leaf fibre content, the cultivar 'Regent' was distinguished by having the lowest fibre content at 3.37%, while the cultivar 'Solaris' had the highest fibre content at 7.88%. In a study by Kok *et al.* (2007), the fibre content of the leaves of the cultivars studied was about 37%, whereas in the analyses of the examined leaf material it was much lower.

In relation to ash, significant differences were found, ranging from 14.55 to 21.04%. The highest ash content was observed for the cultivar 'Regent', and the lowest for the cultivar 'Rondo'.

Fibre, also known as structural carbohydrates, plays a key role in animal diet. The detergent natural fibre (NDF) content of grapevine leaves varies depending on the cultivar. The cultivar 'Rondo' has the lowest content, at 19.09%, while the cultivar 'Solaris' has the highest NDF content, reaching 26.79%. In comparison, Kazemi and Bezdi (2022) found in their study that the average proportion of NDF in grape leaves is much higher at 43.5%. Kocadayioğulları, Boğa and Ermetin (2024) analysed grapevine leaves and found that they contained 47.84% NDF, whereas Birkinshaw *et al.* (2022) reported a 26% NDF content in vine leaf pellets.

For acid detergent fibre (ADF), significant differences were shown, ranging from 27.33% to 36.09%. With the highest ADF content shown for the 'Solaris' cultivar and the lowest for the 'Rondo' cultivar. In a study on the use of grape leaves as feed for sheep, Romero *et al.* (2000) found the proportion of ADF in grape leaf fibre to be around 0.6%, while in the material analysed these values were much higher. Birkinshaw *et al.* (2022) in their study showed for vine leaf pellets a 14.2% proportion of ADF. In contrast, Gürsoy (2023) obtained 24.43%, while Kocadayioğulları, Boğa and Ermetin (2024) showed an ADF of 32.04% for vine leaves regardless of cultivar.

Detergent-acid lignin (ADL) levels oscillated between 7.46 and 8.04%. As with the other fibre types, the highest values were observed for the 'Solaris' cultivar and the lowest for the 'Rondo' cultivar. In comparison, Birkinshaw *et al.* (2022) obtained a much lower value of 4.80%, while Kocadayioğulları, Boğa and Ermetin (2024) obtained a much higher value, which was 10.2% for this parameter.

Table 2. Forages of different grape cultivar leaves

Parameter	'Regent'	'Rondo'	'Seyval Blanc'	'Solaris'	*p*-value
Moisture (%)	11.95 ±0.84^{A}	8.69 ±0.66^{C}	10.15 ±0.99^{B}	11.6 ±0.76^{A}	0.0001
Protein (%)	14.64±0.57^{AB}	14.98 ±0.71^{AB}	14.1 ±0.43^{B}	7.95 ±0.25^{C}	0.0001
Fibre (%)	3.37±1.97^{B}	4.2 ±0.56^{AB}	5.3 ±2.92^{AB}	7.88 ±3.69^{C}	0.0036
Ash (%)	21.04 ±0.76^{A}	14.55 ±0.64^{B}	20.18 ±0.5^{A}	20.11 ±0.53^{A}	0.0001
NDF (%)	19.16 ±1.4^{C}	19.09 ±1.81^{C}	22.13 ±1.75^{B}	26.79 ±1.5^{A}	0.0001
ADF (%)	31.43±0.99^{C}	27.33 ±0.14^{D}	32.28 ±0.11^{B}	36.09 ±0.19^{A}	0.0001
ADL (%)	7.66 ±0.1^{BC}	7.46 ±0.27^{C}	8.04 ±0.11^{A}	7.72 ±0.01^{B}	0.0001
OM digestibility (%)	41.21±1.51^{D}	63.06 ±0.83^{A}	49.07 ±0.29^{B}	42.91 ±0.81^{C}	0.0001
DM digestibility (%)	45.61 ±3.63^{B}	59.76±0.78^{A}	45.82 ±1.34^{B}	45.57 ±2.03^{B}	0.0001
Total soluble sugars (%)	7.59 ±0.79^{A}	1.2±0.64^{D}	4.26 ±0.31^{C}	5.44±0.18^{B}	0.0001

Explanations: A–D = differences between averages shown with different letters in the same column are significant (*p*0.05); NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin, OM = organic matter, DM = dry matter.
Source: own study.

Digestibility parameters of OM ranged from 42.91 to 63.06%. The highest values were shown for the cultivar 'Rondo' and the lowest for the cultivar 'Solaris'. An analogous relationship was shown for DM digestibility parameters, where they oscillated between 45.57 and 59.76%, with the highest value recorded for the 'Rondo' cultivar and the lowest for the 'Solaris' cultivar. Analysing the vine leaves, Kocadayioğulları, Boğa and Ermetin (2024) recorded a DM of 89.72%, while Gürsoy (2023) recorded 93.28%.

The total sugar content of the leaves showed significant differences between cultivars. Their values ranged from 1.2 to 7.59%, where indeed the highest value was recorded for 'Regent' and the lowest for 'Rondo'.

The dendrogram shows the cluster analysis of leaf feed parameters for four grape cultivars (Fig. 2).

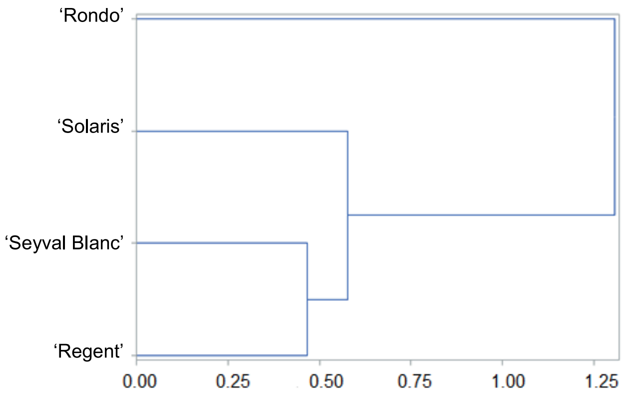

Fig. 2. Comparative analysis of forage parameters from leaves of different grape cultivars; source: own study

This analysis illustrates the classification of these varieties based on similarities in leaf feed parameters. The dendrogram shows three main clusters. The first cluster is formed by the cultivars 'Regent' and 'Seyval Blanc', suggesting that their leaves show very similar feed characteristics. The second cluster is represented by the cultivar 'Solaris', which shows some differences compared to the first cluster, but is still relatively close in terms of the analysed characteristics. The cultivar 'Rondo' is the most distinct from the others, indicating the greatest differences in the leaf feed parameters of this cultivar.

CONCLUSIONS

The results show that there are statistically significant differences in the chemical composition and nutritional properties of the leaves of the different vine cultivars.

The cultivar 'Solaris' stood out for having the largest leaf area (1.27 m^2) and leaf mass (0.0086 kg), which may suggest its potentially higher value as a roughage feedstock. In contrast, 'Seyval Blanc' had the smallest leaves in terms of area and mass, but the highest number of leaves per shoot and per hectare, which may be beneficial in the context of crop intensification.

Vine leaves, especially of the cultivars 'Rondo' and 'Regent', can be a valuable feed ingredient due to their high protein content (14.98% and 14.64%, respectively) and digestibility. However, the cultivar 'Solaris', despite its higher fibre content (NDF: 26.79%), may require additional consideration due to its lower digestibility (DM – 45.57%, OM – 42.91%).

The 'Regent' cultivar contains as much as 7.59% soluble sugars, which may be beneficial for forage energy value.

The use of vine leaves as feed can contribute to reducing waste in wine production and support sustainable agriculture. The nutrients they contain can improve forage quality and livestock health, while reducing the cost of forage production.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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