Characterization of polyphenol content and antioxidant activity of a network of French pasture grasses

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Abstract

Pasture grasses contain many secondary metabolites such polyphenols or vitamins that have different health-promoting abilities in animals, including antioxidant effects. Pastures would provide farmers the opportunity to contribute to animal health improvements with minimal investment. However, knowledge on the polyphenol content and antioxidant activity of pastures is limited. The objective of this study was to assess the variability of polyphenol content and antioxidant activity (ORAC and DPPH assays) of 45 pastures located in three different areas of France and chosen to represent a diversity of environmental conditions and botanical diversity. Important differences between pastures were observed: polyphenol content varied from 8.6 to 40.8 mg of gallic acid equivalent per g dry matter (DM). Antioxidant activity varied from 9.1 to 63.5 mg trolox equivalent per g DM with DPPH and from 50.9 to 280.3 mg trolox equivalent per g DM with ORAC. The present work provided a first valuable report on the potential variability in polyphenols and antioxidant activity of pastures. Next step will be to study the correlations between botanical diversity and pasture management and these values in order to improve pastures utilization by taking account health benefits for ruminants.

Keywords: grassland, antioxidants, polyphenols, animal health

Introduction

Animal health management is a key point of the agroecological transition of ruminant production. Plants contain many secondary metabolites such as vitamins or polyphenols that may have different health-promoting abilities in animals. Numerous studies have demonstrated the role of these metabolites in promoting animal health via antioxidant, anti-parasitic, bloat preventing and anti-microbial effects (Poutaraud *et al.*, 2017). In a survey on French dairy farms, Sulpice *et al*. (2019) observed that higher levels of grazing were associated with a reduction in veterinary intervention and medical drugs consumption by cows. Pastures would provide farmers the opportunity to contribute to the health improvements of animals with minimal investment. However, knowledge on the polyphenol content and antioxidant activity of pastures is scarce and do not provide support to demonstrate the real supplies of antioxidants and polyphenols by pastures. Moreover, potential differences on antioxidant activity and polyphenol content between pastures and the factors affecting these values remain unclear. Reynaud *et al.* (2010) suggested that botanical composition and vegetative stage could affected the polyphenol content and composition of pastures. Environmental conditions may also act on polyphenols in plants. Thus, the first objective of our project was to investigate the variability of antioxidant activity and polyphenol content within French pastures in order to objectify a potential difference in health value of pastures.

Materials and methods

Grass samples were taken in three different areas of France: Auvergne-Rhône-Alpes, East (mainly Haute-Saône), West (Pays de Loire and Indre). The pastures, 15 per area, were chosen to represent a diversity of environmental conditions and botanical diversity. Grass samples were collected twice during spring 2022: at a sum of temperature of about 432 ±80°C.D (P1) and at the sum of temperature of 950 ±107°C.D (P2). The sum of temperatures was calculated, as the cumulative sum of positive (> 0°C) daily temperature, from 1 February to the present day considered and measured at local level. The botanical composition of each pasture was determined using 10 quadrats distributed within the parcel. Biomass contained in each quadrat was collected and mixed to constitute a representative sample of each pasture. Samples were quickly stored at -20°C, then freeze-dried and ground before analysis. The antioxidant activity of pasture samples was estimated using two assays: the measurement of the free-radical scavenging activity of the DPPH* (2,2-diphenyl-1-picrylhydrazyl) and the measurements of the oxygen radical absorbance capacity (ORAC). Total polyphenol (TP) content (Folin–Ciocalteu method) and DPPH (Galmarini *et al.*, 2013) were analysed at GRAPPE. ORAC assay was realised by the Végépolys Valley lab (Angers, France, Ou *et al.*, 2001). ORAC and DPPH results were expressed as mg trolox equivalent per g dry matter (DM) and TP were expressed as mg of gallic acid equivalent per g DM. Nutritive values of grass samples (organic matter, nitrogen and crude fibers contents) were determined by Lano Lab (Saint-Lo, France). Comparisons between sampling dates for each parameter analysed were performed (*t* test for paired data). Pearson's correlation tests were also performed to assess the relationships between the TP content, antioxidant activity and nutritive values.

Results and discussion

Total polyphenol content varied from 8.6 to 40.8 mg of gallic acid equivalent per g DM (Table 1), which was similar to the range of values previously reported for grasses (Amrit *et al*., 2023) or for highly diversified natural grassland (Reynaud *et al*., 2010). Antioxidant activity assessed with DPPH method varied from 9.1 to 63.5 mg trolox equivalent per g DM and from 50.9 to 280.3 mg trolox equivalent per g DM with ORAC method. These results underlined an important difference in antioxidant activity between pastures.

Table 1. Total polyphenol content and antioxidant activity of the 45 French pastures studied

	Sampling date	n	Mean[1]	Min	Max	SD[2]
Dry matter, %	P1	45	25.7[a]	17.5	42.8	6.2
	P2	45	26.1[a]	15.7	42.7	7.1
Organic matter, g kg^{-1} DM	P1	45	890[a]	810	927	24.9
	P2	45	919[b]	884	948	16.4
Crude protein, g kg^{-1} DM	P1	45	173[a]	80	242	34.9
	P2	45	135[b]	69	249	45.4
Crude fibers, g kg^{-1} DM	P1	45	195[a]	134	312	34.8
	P2	45	246[b]	158	323	46.2
Total polyphenols, mg gallic acid equivalent per g DM	P1	45	23.3[a]	8.6	37.2	7.0
	P2	45	24.5[a]	11.8	40.8	6.6
DPPH, mg trolox equivalent per g DM	P1	45	26.3[a]	9.4	47.1	9.3
	P2	45	29.5[a]	9.1	63.5	11.1
ORAC, mg trolox equivalent per g DM	P1	45	144.8[a]	50.9	280.3	47.6
	P2	45	149.1[a]	67.6	277.8	42.9

[1]Significant differences ($P < 0.05$) between sampling date within pastures are indicated by different lowercase letter superscripts. [2]SD = standard deviation.

Total polyphenol content was significantly and positively correlated to DPPH (r = 0.91) and ORAC (r = 0.90) values, suggesting than polyphenols present in pastures have a significant antioxidant activity. These findings have been already reported by Amrit *et al*. (2023) and Rapisarda & Abu-Ghannam (2023). DPPH and ORAC values were significantly correlated (r = 0.83), but very different as both assays assess the antioxidant capacity differently. Sampling date had no effect on TP content, DPPH and ORAC values, probably due to the high variability obtained within a sampling date. Changes in polyphenols may be affected by many factors as weather, stress, botanical composition or phenological stage making challenging to explain these observed variations. Crude protein was significantly higher in P1 (173 g kg^{-1} DM) than P2 (134 g kg^{-1} DM, $P < 0.05$), whereas CF was significantly lower in P1 (195 g kg^{-1} DM) than P2 (247 g kg^{-1} DM, $P < 0.05$). These changes are commonly found with increase maturity of plants and values were consistent to references for these types of pasture (INRAE, 2018). No correlation was observed between CP or CF and the TP content, DPPH and ORAC values.

Conclusion

Results of this study showed important variations in polyphenol content and antioxidant activity in a network of French pastures, suggesting that pastures could have a different animal health potential. Next step of our study will be to assess the effects of botanical diversity and pasture management (fertilization, grazing intensity…) on these characteristics in order to improve pasture utilization by taking into account health benefits for ruminants.

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