Potential health value of alternative plant resources explored as feed for ruminants

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Abstract

To face current challenges, ruminant feeding systems have to adapt the use of common resources or develop the use of alternative ones. This study explored the potential of alternative plant resources that could be used on farms to provide nutrients with health-promoting abilities for ruminants, i.e. tree leaves (Lutèce elm, common ash, goat willow, white mulberry, Italian alder, black locust), duckweeds, reeds and grass from orchards. Samples were collected in summer 2022 and assayed for condensed tannin, tocopherol, carotenoid, total polyphenol contents and antioxidant activity (DPPH assay). Tree leaves except white mulberry had the highest total polyphenols (67.1 *vs* 10.9 mg eq gallic acid g^{-1} DM for other resources) and DPPH values (118 *vs* 25 mg eq trolox g^{-1} DM for others). Black locust was the richest in tannins and carotenoids whereas goat willow was the richest in tocopherols. DPPH values were positively correlated with total polyphenols (r = 0.93) and tocopherols (r = 0.66), in agreement with their significant antioxidant activity. To conclude, several tree leaves seem to be good sources of metabolites with health-promoting abilities for ruminants.

Keywords: tree leaves, reeds, duckweed, polyphenols, antioxidant, carotenoids, tocopherols

Introduction

Ruminant feeding is facing new and strong challenges due to climate change, environmental footprint of ruminant production and feed-food competition. To face these challenges, ruminant feeding systems have to adapt the use of common resources or develop the use of new resources. Increasing the proportion of pasture in the diet is limited by grassland availability. The use of alternative forages to replace or combine with main forages could fill this grass shortage. Unusual resources available on farms, such as tree leaves, grass from orchards or marsh could constitute good alternative resources for ruminants, having interesting nutritive value (Mahieu *et al.*, 2021), and may also present other benefits as they contain secondary metabolites that are likely to improve animal health and animal product quality (Poutaraud *et al.*, 2017). The aim of this study was to explore the potential of 10 unusual plant resources to be used to provide nutrients with health-promoting abilities for ruminants.

Materials and methods

Three representative samples of each resource were collected and analyzed. Tree leaves (Lutèce elm - *Ulmus cv. Nanguen*, common ash - *Farinas excelsior L.*, white mulberry - *Morus alba*, Italian alder - *Alnus cordata* (*Loisel*), black locus - *Robinia pseudoacacia L.* and goat willow - *Salix caprea L.*) were collected at Lusignan; grass from orchards at Saint-Marcel-Les-Valence;

reeds (*Phragmites australis* (Cav.) Trin. ex Steud.) and duckweeds (*Lemna minuta* Kunth and *Lemna gibba L.*) at Saint Laurent de la Prée. All samples were collected in July 2022 except for black locus and goat willow which were collected in August 2022. Plant samples were quickly stored at -20°C, freeze-dried and ground before analysis. Different indicators (Maxin *et al.*, 2018) of animal health potential were assayed in the samples: carotenoids, tocopherols, total polyphenols (TP) and condensed tannins (CT). Carotenoids and tocopherols were analysed as described in Maxin *et al.* (2020) and CT were determined by the colorimetric HCl–butanol method. A purified CT extract of sainfoin was used as a standard. TP content was determined by the Folin–Ciocalteu method. Results were expressed as mg of gallic acid equivalent per g dry matter (mg eq GA g^{-1} DM). The antioxidant activity of samples was estimated via the measurement of the free-radical scavenging activity of the DPPH* (2,2-diphenyl-1-picrylhydrazyl). The results were expressed as mg trolox (Tx) equivalent per g DM. Anova was performed to test differences between resources on the indicators assayed (resource as effect, Minitab® version 21). Pearson's correlation tests were also performed to assess the relationships between TP, CT, carotenoids and tocopherol contents and antioxidant activity.

Results and discussion

The TP content varied highly with resources and was significantly higher for the tree leaves than for other resources (Table 1). These values obtained for tree leaves were higher than values previously observed for highly diversified natural grassland (Graulet *et al.*, 2012). In agreement with Mahieu *et al.* (2021), black locus had considerably higher CT content (166 mg g^{-1} DM) than other resources (< 49.1 mg g^{-1} DM), these values being in the range of values previously observed for legume species (Maxin *et al.*, 2020). High dietary CT content (> 50 mg g^{-1}) is known to have detrimental effects on animal intake (Min *et al.*, 2003). Therefore, its inclusion in the diet should be limited to avoid adverse effects. Significant differences in total tocopherols among the 10 resources were observed. Goat willow and Italian alder had the greatest contents whereas duckweed and grass from orchards had the lowest contents. All resources were richer in α-tocopherol (> 86% of total tocopherols, data not shown) than γ-tocopherol.

Table 1. Total polyphenol, condensed tannins and total tocopherol contents, and antioxidant activity of the 10 alternative resources explored.

	TP[1], mg GA eq g^{-1} DM	CT[1], mg sainfoin CT eq g^{-1} DM	DPPH, mg Tx eq g^{-1} DM	Total tocopherols, mg g^{-1} DM
Black locus	61.2^{b}	166.0^{a}	116.6^{b}	0.28^{d}
Common ash	52.8^{b}	2.0^{d}	77.8^{c}	0.60^{bc}
Duckweed *L gibba*	10.4^{d}	1.4^{d}	26.4^{d}	0.10^{d}
Duckweed *L minuta*	9.7^{d}	2.3^{d}	30.2^{d}	0.20^{d}
Goat willow	84.2^{a}	32.6^{bc}	134.5^{b}	1.16^{a}
Grass Orchards	14.0^{cd}	2.6^{d}	28.7^{d}	0.13^{d}
Italian alder	90.9^{a}	11.3^{cd}	122.6^{b}	1.01^{a}
Lutèce elm	89.8^{a}	49.1^{b}	203.3^{a}	0.64^{b}
Reeds	9.9^{d}	1.7^{d}	15.2^{d}	0.32^{cd}
White mulberry	23.6^{c}	1.5^{d}	50.2^{cd}	0.31^{cd}
SEM	4.20	8.5	12.6	0.11
Pvalue (specie)	<0.001	<0.001	<0.001	<0.001

[1]TP: total polyphenols, CT: condensed tannins. Values in the same column with different superscript are different ($P < 0.05$)

A total of 9 carotenoid compounds were identified and quantified in all resources (Table 2). Lutein, violaxanthin and all-E-β-carotene were quantitatively the major carotenoids in all resources. These carotenoid profiles are consistent with those observed for natural pastures and legume species (Graulet *et al.*, 2012; Maxin *et al.*, 2020). Black locus was the richest whereas

Lutèce elm was the poorest in carotenoids. Antioxidant activity assayed with DPPH method varied from 15.2 to 203.3 mg Tx eq g^{-1} DM, underlining important difference in antioxidant supply between resources. DPPH values were positively correlated to TP (r = 0.93, P < 0.001), total tocopherols (r = 0.66, P < 0.001) and CT (r = 0.48, P = 0.008). This suggests that polyphenols and tocopherols present in these resources have a significant contribution in the whole antioxidant activity whereas CT would contribute to a lesser extent.

Table 2. Content in the 5 main carotenoids[1] of the 10 resources explored

µg g^{-1} DM	Antheraxanthin	Lutein	All E-β-carotene	Neoxanthin	Violaxanthin
Black locus	86.7^{ab}	544.7^{a}	163.9^{a}	112.0^{a}	228.5^{a}
Common ash	35.2^{cde}	213.3^{cd}	72.0^{cd}	38.6^{bc}	68.1^{d}
Duckweed *L gibba*	47.6^{bcde}	225.7^{cd}	47.2^{d}	44.7^{bc}	167.1^{abc}
Duckweed *L minuta*	39.0^{cde}	498.4^{ab}	81.1^{bcd}	86.6^{ab}	222.4^{a}
Goat willow	68.1^{abc}	392.5^{abc}	129.7^{ab}	76.7^{abc}	112.1^{bcd}
Grass Orchards	35.3^{cde}	262.3^{cd}	69.3^{cd}	50.0^{bc}	113.5^{bcd}
Italian alder	14.9^{e}	392.1^{abc}	119.8^{abc}	62.0^{acd}	63.5^{d}
Lutèce elm	26.2^{de}	170.3^{d}	66.3^{cd}	32.3^{c}	76.8^{cd}
Reeds	64.8^{abcd}	283.2^{bcd}	97.3^{bcd}	54.6^{bc}	164.7^{abc}
White mulberry	95.8^{a}	334.1^{abcd}	95.3^{bcd}	62.6^{abc}	194.7^{ab}
SEM	14.1	76.0	18.5	17.4	32.2
Pvalue (specie)	<0.001	<0.001	<0.001	<0.001	<0.001

[1]The 4 other minor carotenoids quantified were zeaxanthin, lutein epoxide, 13cisβ-carotene and 9cisβ-carotene. Values in the same column with different superscript are different (P < 0.05)

Conclusion

The contents in secondary metabolites and antioxidant activity greatly differed between the resources explored. Several tree leaves would be good sources of secondary metabolites and antioxidants for ruminants. However, *in vivo* trials are required to assess the quantities animals can ingest of these resources and confirm their positive interest for animal health.

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Context

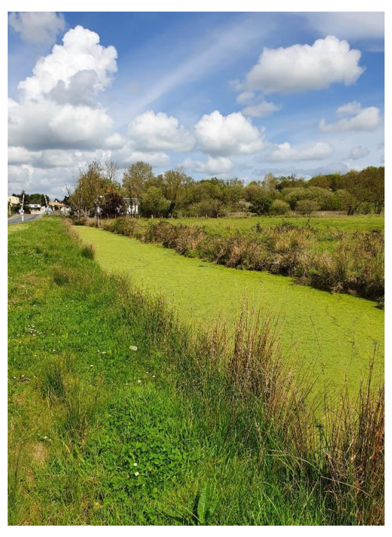
The use of alternative resources is a way to adapt ruminant feeding systems to current challenges they are facing (climate change, environmental footprint or feed-food competition). Unusual resources available on farms such as tree leaves, grass from orchards or mash could constitute good alternatives as they have interesting nutritive values. They may also contain secondary metabolites that are likely to improve animal health and animal product quality.

The aim of this study was to explore the potential of <u>10 unusual plant resources</u> to be used to <u>provide metabolites with health-promoting abilities</u>

Methods

Three samples from **10 unusual resources** were collected on INRAE experimental sites during summer 2022

- **Tree leaves** at Lusignan (46.40404, 0.07912): Lutèce elm (*Ulmus cv. Nanguen*), common ash (*Farinas excelsior L.*), white mulberry (*Morus alba*), Italian alder (*Alnus cordata*), black locus (*Robinia pseudoacacia L.*) and goat willow (*Salix caprea L.*)
- **Aquatic resources** at Saint-Laurent de la Prée (45.99238, -1.02829): reeds (*Phragmites australis* (Cav.) Trin. ex Steud.) and duckweeds (*Lemna minuta* Kunth and *Lemna gibba L.*)
- **Grass from orchards** at Saint-Marcel-les-Valences (44.97740, 4.93055)



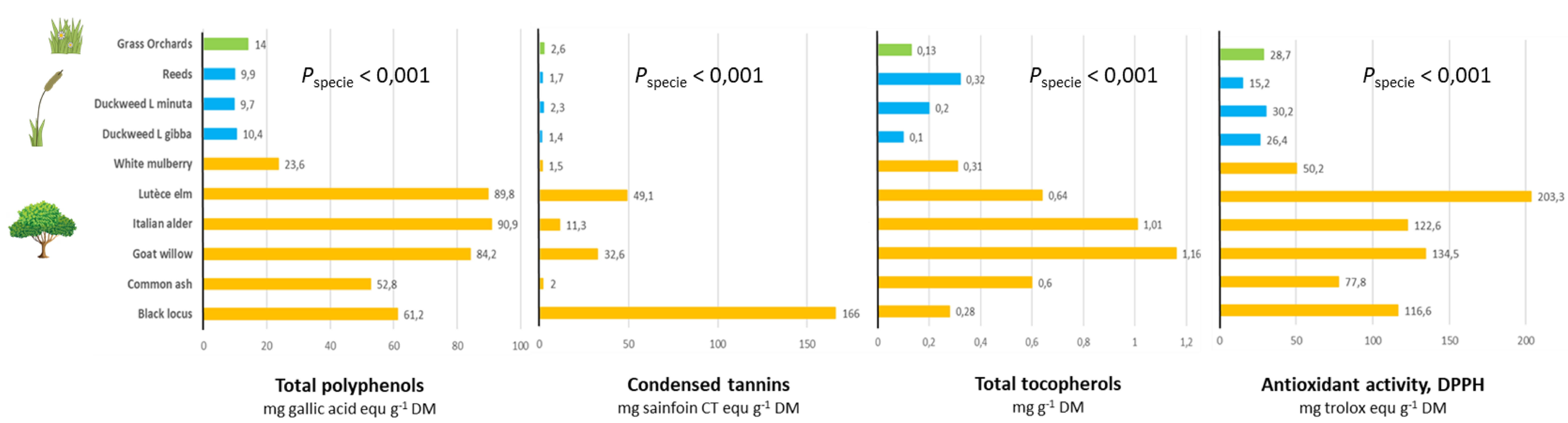
Samples were stored at -20°C, then freeze-dried and ground before analyses to determine:

- Condensed tannins (**CT**, HCl-butanol method)
- Total polyphenols (**TP**, Folin-Ciocalteu method)
- Tocopherols and carotenoids content and composition (as described in Maxin *et al*., 2020)
- Antioxidant (**AO**) activity: DDPH method

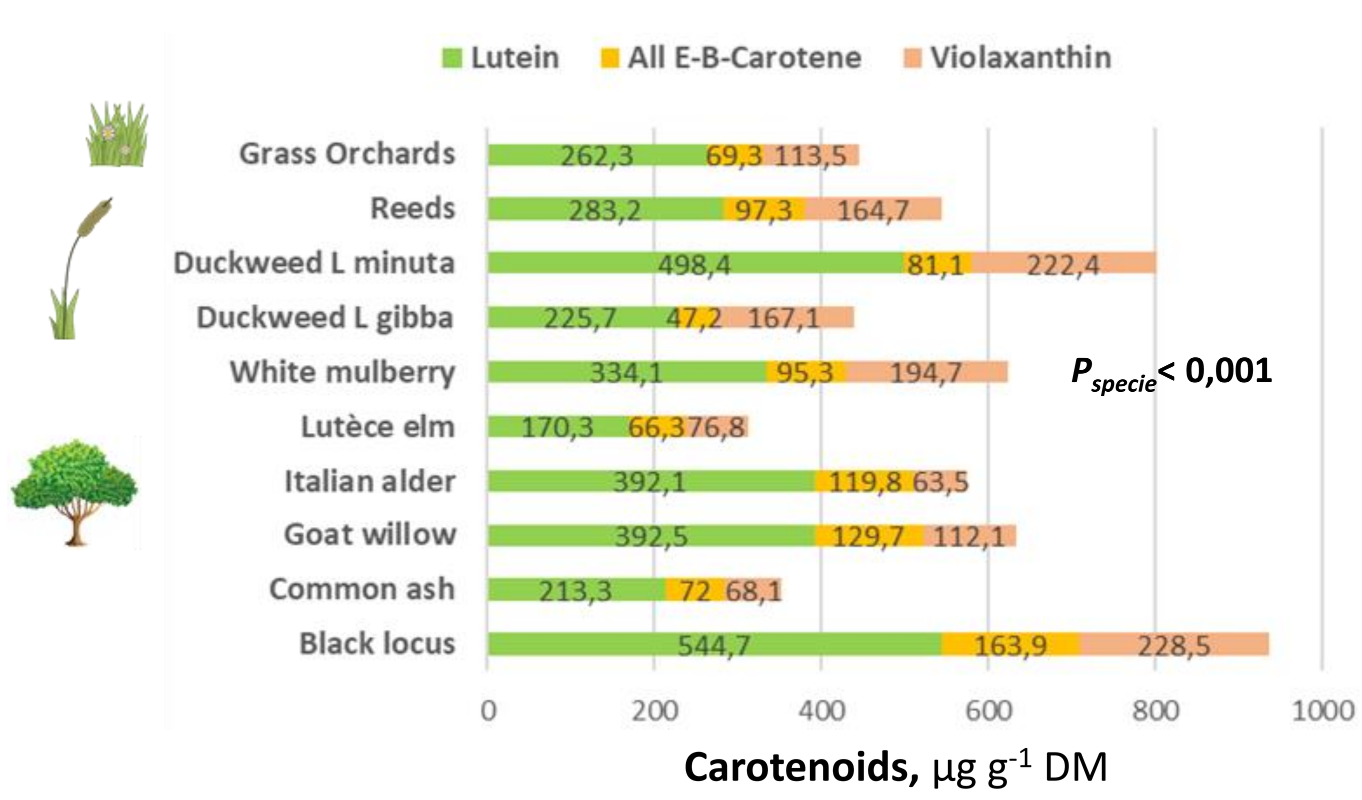
ANOVA was performed to test differences between resources on the metabolites assayed

Results

- TP, CT and total tocopherols varied with resources. TP content was higher for the tree leaves; black locus had considerably higher CT content; and goat willow and Italian alder had the greatest contents in tocopherols
- AO activity widely varied suggesting important differences in AO supply between resources
- AO activity was significantly and positively correlated with TP (r = 0.93), tocopherols (r = 0.66) and CT (r = 0.48) → polyphenols and tocopherols would have a significant contribution in the whole AO supply



- 9 carotenoids compounds were quantified in all resources
- Lutein, violaxanthin and all-E-β-carotene were the major carotenoids in agreement with previous results on pastures
- Black locus was the richest whereas Lutèce elm was the poorest in carotenoids



Conclusion & perspectives

- Contents in secondary metabolites and AO activity differed greatly between plant resources
- Tree leaves would be good sources of health-promoting metabolites
- *In vivo* trials are required to confirm these positive effects and assess quantities animal can ingest

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