# **BIOACTIVE COMPOUNDS OF LEGUME BASED SWARDS ARE** AFFECTED BY LIGHT INTENSITY IN A MEDITERRANEAN ISPAAM Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo L19.P.28







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# **INTRODUCTION**

In Mediterranean grazed woodlands, microclimate changes induced by trees influence growth and development of the understory but very little is known on its phenolic composition under full sunlight or shade. Phenolic acids, flavonoids and tannins are the most important compounds for biological activities and especially antioxidant properties and related implications in animal nutrition and welfare (Piluzza et al., 2013)

# **OBJECTIVE**

The research was carried out in a cork oak agrosilvopastoral ystem in Sardinia, where we investigated the bioactive compounds and antioxidant capacity of different legume-based swards subjected to variations in light intensity

### MATERIALS AND METHODS

A field experiment was carried out during 2015-16 in a private farm (40°37'99"N, 9°15'33"E, elevation 700 m a.s.l.) located in North East Sardinia (Italy). The climate is Mediterranean with hot dry summer. Long-term rainfall is 840 mm and average annual temperature is 12.7 °C

The area is characterized by extensive agro-silvopastoral systems, inside a representative Mediterranean evergreen cork oak woodland with tree density of 450 trees ha<sup>-1</sup>

Partial shade conditions P.A.R. 15-30% (PS)

**L100MIX** (Fertiprado: 100% legume species) **L80GMIX** (CNR-ISPAAM: 80% legume species and 20% grasses **<u>100BCLO</u>** (Trifolium spumosum 100%) L60SNPA (Unsown species: 60% legumes and 40% non legume species)

Legume based swards

unight conditions P.A.R. 100%

Plant extracts were screened in vitro for antioxidant capacity by ABTS<sup>+</sup> [2,2'-azino-bis(3ethylbenzthiazoline-6-sulfonic acid) diammonium salt and DPPH (1,1-diphenyl-2-picryl-hydrazyl) methods. The concentration of total phenolics (TotP) was estimated using spectrophotometric analysis with Folin Ciocalteau's phenol reagent and total flavonoids (TotF) by colorimetric assay with the AlCl<sub>3</sub> method. Chromatographic separation was carried out according to Karimi et al., (2013) with some modifications (Re et al., 2019)

# **RESULTS AND DISCUSSION**

Antioxidant capacity, TotP and TotF of forage were significantly affected by light intensity, as well as by the type of legume-based swards.

Compared to full sunlight, partial shade reduced antioxidant capacity values by 29 and 42%, and TotP content by 23 and 53% in Fertiprado (L100 mix) and semi natural pasture (L60SNPA) (Fig 1).

Twelve phenolic compounds were detected: neochlorogenic acid, chlorogenic acid, rutin, verbascoside, 3,5-di-O-E-caffeoylquinic acid (3,5-DCQ), naringenin, isorientin, p-cumaric acid, luteolin-7-O-glucoside, luteolin, quercetin, and gallic acid.

Verbascoside in bladder clover mixture was the most abundant in full sunlight and neochlorogenic acid and isorientin were abundant in partial shade in CNR ISPAAM mixture (L80GMIX) and in unsown semi-natural pasture (L60SNPA), respectively (Table 1).

The presence of verbascoside has not been reported in other HPLC studies on clover species so



analysis of polyphenolic compounds (g kg<sup>-1</sup>) from shoot of legume based swards growing in full sunlight (FS) and partial shade (PS)

	Neochlorogenic acid			Chlorogenic acid			Isorietin			Rutin			Verbascoside			3,5-DCQ			Narigenin		
Retention time	9.49			11.39			17.40			20.52			21.18			24.50			37.40		
1	FS	PS		FS	PS		FS	PS		FS	PS		FS	PS		FS	PS	2	FS	PS	
L80GMIX	0.09 <sup>a</sup>	0.35 <sup>b</sup>	***	2.36ª	2.71ª	ns	ND	ND		0.3 <sup>bc</sup>	<b>0.19</b> <sup>b</sup>	*	<b>0.3</b> ¢	Tr		0.1°	ND		0.32ª	<b>0.17</b> <sup>b</sup>	***
L100MIX	0.08ª	Tr		2.03 <sup>ab</sup>	0.44 <sup>c</sup>	***	0.17 <sup>ab</sup>	0.23 <sup>b</sup>	ns	<b>0.85</b> ª	0.21 <sup>ab</sup>	***	ND	ND	F,	0.31 <sup>b</sup>	0.23 <sup>ab</sup>	ns	0.186	<b>0.44</b> ª	•••
L60SNPA	0.05ª	0.05°	ns	1.449	1.649	ns	0.71ª	4.78*	***	0.33 <sup>b</sup>	ND		1.33 <sup>b</sup>	ND		0.47ª	0.58°	•	n.	Tr	
100BCLO	0.05 <sup>a</sup>	<b>0.46</b> ª	***	0.23°	0.45°	115	ND	ND		0.19°	<b>0.28</b> ª	*	<b>2.84</b> ª	2.06	***	ND	<b>0.13</b> ª		ND	ND	
	14.				5.		-					-14							100	100	-

Fig. 1. Antioxidant capacity (ABTS and DPPH methods), total phenolic (TotP) and total flavonoid (TotF) contents in shoot of legume based swards growing in full sunlight (FS) and partial shade (PS).



ABTS

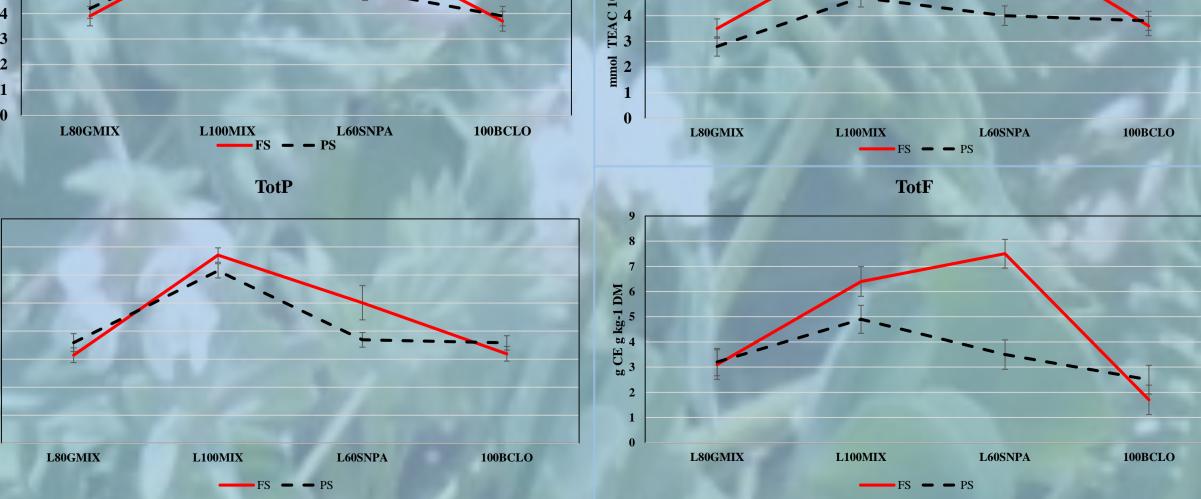




far. The effect of verbascoside administration on plasma oxidative status and specific blood and milk production parameters was evaluated in Lacaune ewes (Casamassima et al., 2012); verbascoside provided benefits in terms of blood parameters, oxidative status and milk production.

# **CONCLUSIONS:**

As antioxidant capacity and the content of bioactive compounds ascertained in the legumebased swards could potentially affect the nutritional properties of forage; their variations caused by contrasting light intensities thus represent a particular benefit, which must be exploited as an additional service from agroforestry.



#### References

Casamassima et al., 2012. Small Rum. Res. 105, 1-8. Karimi et al., 2013. J. Med. Plants Res. 7, 290-297. Piluzza et al., 2013. Grass For. Sci. 69, 32-48. Re et al., 2019. J. Sci Food Agric. 99, 191-198.



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