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Use of Essential Oils of *Prunus avium* in Sheep Feeding: Biological and *in vitro* Ruminal Fermentation

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Abstract

The chemical composition and antioxidant activity of *Prunus avium* and the effect of its essential oil on the parameters of ruminal fermentation in sheep have been studied. Chemical analysis of *Prunus avium* was determined and *in vitro* fermentation parameters were measured in 100 ml glass syringes for 48 hours to monitor gas production. *Prunus avium* contains high levels of total fiber (44.44%) and CP (13.85%). The extraction of HE by hydro distillation reveals a low yield. The study of the anti-radical activity shows that the antioxidant activity of the aqueous extract is greater than that of the essential oil. *Prunus avium* is rich in secondary metabolites (54.35 mg GAE/g DM). For the additive study, there is a significant difference between doses ($p<0.01$). Increasing the dose of HE can be toxic for the ruminal flora. However, the low dose of 10 µL and 30 µl of essential oil improve *in vitro* digestibility and therefore the possibility of applying it *in vivo*.

Keywords: Essential oil; *Prunus avium*; *in vitro* fermentation; Sheep; Antioxidant activity

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Introduction

Tunisia is characterized by four important breeds which are Barbarine, Fine Tail West, Black of Thibar and Sicilian-Sardinian with a total of 3736820 female units [1] and a number of breeders estimated at 274,000 which is more than twice the number of dairy cattle farmers in Tunisia [2]. For many years, antibiotics have been used as growth promoters for livestock [3]. In 2001 and according to the World Health Organization, this use was estimated at 50% of the antibiotics produced in the world. However, these substances appeared to have favoured the emergence of a large number of resistant bacterial strains and allergic reactions in consumers [3]. In 2006, the use of antibiotics to improve the growth and performance of animals was banned in the European Union. This has led to the reappearance of pathogens responsible for diseases and economic losses [4]. As a result, considerable efforts have been made to develop alternatives to antibiotic substitution. Among these alternatives, essential oils are receiving increasing attention as substitutes for natural antibiotics and also as additives for the manipulation of rumen fermentation [5]. Integrates our work that aims to enhance the essential oil of *Prunus avium* by adjusting the optimal dose for the increase of digestibility and improve ruminal fermentation parameters in sheep.

Materials and Methods

Animals material

The rumen content is taken from sheep at the TABARKA municipal slaughter house. It is transferred to the laboratory in a thermos preheated to 39°C.

Plant material

As part of this project, we will focus on the study of a new species that is *Prunus avium*, Sampling is carried out in spring in March and April, from two different regions. Tbeinia and Swiny et al. analyses were performed in triples within the laboratory of the Sylvo-Pastoral Institute of Tabarka.

Chemical and parietal composition

All samples were analysed for Dry Matter (DM), Ash, Organic Matter (OM), Crude Protein (CP), and fat content using the AOAC method [6]. The determination of the contents of ADF, NDF, ADL, crude cellulose and hemicelluloses are carried out according to the method [7].

Secondary metabolites and antioxidant activity

The determination of the total polyphenols, flavonoids and condensed tannins of the aqueous extracts are carried out respectively according to the methods of Singleton VL et al., Yi ZB et al., Sun B et al. [8-10]. The antioxidant activity of the aqueous extracts of different samples is evaluated by the DPPH test described by Ammar RB et al. [11]. The antioxidant activity of essential oils is evaluated according to the method described by Grzegorczyk I et al. [12].

In vitro fermentation

Monitoring the kinetics of gas production follows the addition of different doses of essential oil (0 µl; 10 µl; 30 µl; 50 µl; 100 µl) to rumen juice, a technique described by Spanghero M et al. [13] and based on the method of Menke KH et al. [14].

Statistical analysis

All data were subjected to statistical analysis by the variance according to the GLM procedure of the software [15] and compared by Duncan multiple rank tests. The model used was: $Y_{ij}=\mu+A_i+E_{ijk}$.

Where; Yij: dependent variable. µ: overall of Y; Ai: effect of the ith essential oil; Eijk: residual error

The characteristic parameters of the gas production kinetics are predicted according to the nonlinear regression by the use of the line procedure according to the model [15,16].

The equation of the model:

$Y=a+b\ (1-\exp-ct)$ where

Y: volume of gas produced after each incubation time (ml)

a: production of gas from the easily fermentable soluble fraction (ml)

b: production of gas from the insoluble, potentially fermentable fraction (ml)

c: gas production speed (h^{-1})

Results and Discussion

Chemical and parietal composition

Tables 1 and 2 present the chemical and parietal composition of *Prunus avium*. Indeed, the results show that the cherry tree is characterized by a low content of DM (29.66%) but a significant value in NDF and ADF respectively 44.4% and 32.75% of these results are in agreement with that presented by Ela hi MY et al. [17].

Table 1: Chemical composition of *Prunus avium*.

	DM%	Ash%	OM%	Fat%	CP%
Prunus avium	29.66 ± 1	7.03 ± 0.085	92.97 ± 0.0854	7.933 ± 4.54	13.85 ± 1.48

Table 2: Parietal composition of *Prunus avium.*

	ADF%	ADL%	CF%	NDF%	HC%
Prunus avium	32.75 ± 1	37.24 ± 10.95	11.36 ± 4.91	44.44 ± 1.42	11.69 ± 0.51

Secondary metabolites and antioxidant activity

The average content of total polyphenols, flavonoids and condensed tannins of *Prunus avium* illustrated in **Table 3** shows a slight difference between what is found by Bastos C et al. [18]. In fact, the total polyphenol content is important 54.35 mgGAE/g DM, however, the contents of flavonoids and condensed tannins are low.

Table 3: Concentration of *Prunus avium* as secondary metabolites.

	Polyphenols (mg GAE/g DM)	Flavonoids (mg QE/g DM)	Condensed tannins (mg CA/g DM)
Prunus avium	54.35 ± 3.69	6.79 ± 1.062	7.82 ± 0.955

Table 4 shows the existence of a significant difference (p<0.01) between the samples tested for antioxidant activity. Indeed, the one with the lowest IC_{50} value is characterized by the most important antioxidant activity. it has been deduced that the aqueous extract has antioxidant activity (IC_5=150.673 µg/ml) greater than that of essential oil (IC_{50}=271.54 µg/ml) for ascorbic acid has the highest antioxidant activity (IC_{50}=61.3 µg/ml). Our results are superior to those found by Bastos C et al. [18].

Table 4: Inhibition concentration of 50% of DPPH free radicals.

	IC_{50} (µg/ml)
Essential oil	$271.54^a \pm 1$
Aqueous extract	$150.673^b \pm 6.647$
Ascorbic acid	$61.3^c \pm 1$
p>F	<0.0001

a: production of gas from the easily fermentable soluble fraction (ml)
b: production of gas from the insoluble, potentially fermentable fraction (ml)
c: gas production speed (h^{-1})

In vitro fermentation

Figure 1 shows the gas productions recorded for oatmeal supplemented with EO at different concentrations (dose 10 µl, dose 30 µl, dose 50 µl, and dose 100 µl) while comparing the zero concentration. After 48 hours of incubation of *Prunus avium* HE decreases the production of gas with both concentrations 50 µl and 100 µl. While the use of low doses of 10 µl and 30 µl increases the production of gas. This can be explained by the fact that the

EO of *Prunus avium* at a low dose of less than 30 µl increases the fermentation of oat hay and the increase in the EO concentration of *Prunus avium* induces a strong reduction in the production of gas. This decrease does not vary substantially between the two doses 50 µl and 100 µl which have the same pace of gas kinetics curve. This can be explained by the fact that the addition of EO at a dose greater than or equal to 50 µl greatly reduces the fermentation of oat hay and this reduction of gas can be linked to selective inhibition of the flora which degrades the Parietal fraction of oat hay. This suggests that this EO has a very strong antimicrobial activity on rumen microorganisms. However, the mechanism of action of EO is difficult to define because this extract consists of a very complex mixture of chemical molecules where each type of molecule has its own mechanism of action. So each dose belongs to the interval 10 µl and 30 µl are optimal doses and if we exceed 50 µl it can cause toxicity to the animal. The kinetic parameters of the *in vitro* fermentation of the different doses are shown in **Table 5** deduced from the exponential model of Orsskov ER et al. [16]. They reveal, as for the biological model, that the highest value of the volume of gas is recorded for the dose 30 µl (47 ml), followed by 10 µl (46 ml), against the dose 50 µl displays the value the weaker (26.5 ml). The total gas production at 24 h of incubation expressed significant differences ($p<0.01$) between the different doses. Indeed, the averages are of the order of 46 ml, 47 ml, 26 ml and 29 ml respectively for the dose 10 µl, 30 µl, 50 µl and 10 µl. There is a significant difference between the different doses studied for parameter "c" (gas production rate (h^{-1}) where the 10 µl and 30 µl doses have the highest speed.

Table 5: Characteristic parameters of the gas production of the different doses.

	a	b	c	G_{24}	G_T
Control	$2.723^b \pm 0.005$	$58.83^a \pm 0.011$	0.02^c	$25.66^c \pm 5.03$	$41.33^b \pm 2.51$
10 µl	$1.64^c \pm 0.005$	$50.43^a \pm 0.005$	0.05^a	$37^a \pm 1$	$46^a \pm 1$
30 µl	$0.3^d \pm 0.598$	$30.64^b \pm 17.43$	$0.056^a \pm 0.028$	$22^c \pm 1$	$47^a \pm 0,5$
50 µl	6.46^a	24.56^b	0.04^b	33^b	$26.5^c \pm 5.50$
100 µl	6.34^a	$25.90^b \pm 0.005$	0.04^b	$22^c \pm 1$	$29^c \pm 1$
P<F	<0.0001	0.0002	<0.0001	<0.0001	<0.0001

a: production of gas from the easily fermentable soluble fraction (ml)
b: production of gas from the insoluble, potentially fermentable fraction (ml)
c: gas production speed (h^{-1})

The digestibility of the OM, the Metabolizable Energy (ME) and the concentration of volatile fatty acids (total VFA) are grouped in **Table 6**. In fact, the doses studied show a significant difference ($p<0.05$) between the values of the digestibility of organic matter, the averages are of the order of 54.76% 51.2% 41.42% 41.42% respectively for 10 µl, 30 µl, 50 µl, and 100 µl. The metabolizable energy and the total VFA concentration were statistically different ($p<0.01$). The doses of EO 10 µl and 30 µl induce greater production of metabolizable energy and VFA. The use of HE in high concentrations strongly inhibits rumen flora, which decreases the digestibility of oat hay and the production of VFA [19]. This can be explained by the toxicity of EO to high levels. Depending on its chemical composition, low doses increase the fermentation and digestibility of oat fiber.

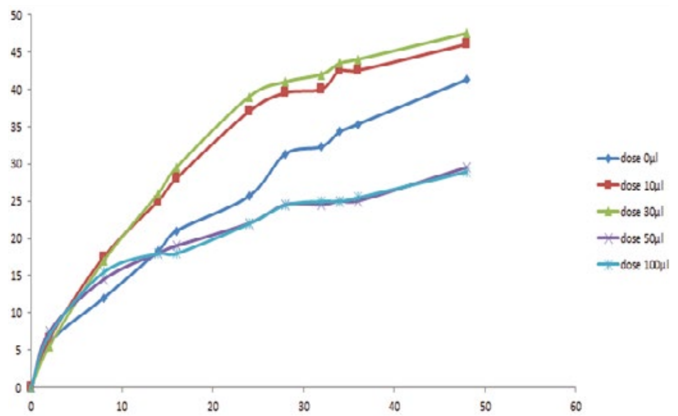

Figure 1 Gas kinetics curve for ovine species after using different doses of Prunus avium essential oil.

Table 6: Estimated digestibility parameters from the gas produced at 24 hours of incubation.

	ME (Kcal/Kg DM)	IVOMD%	VFA mmol/syringe
Control	$6.52^b \pm 0.2$	$44.68^a \pm 1.507$	$0.55^c \pm 0.12$
10 µl	$8.06^a \pm 0.757$	$54.76^a \pm 5.82$	$0.823^b \pm 0.025$
30 µl	$7.516^a \pm 0.63$	$51.20^b \pm 5.005$	0.73^b
50 µl	$6.02^c \pm 0.75$	$41.426^b \pm 5.83$	$0.466^b \pm 0.025$
100 µl	$6.02^c \pm 0.757$	$41.42^b \pm 5.831$	$0.466^b \pm 0.025$
p<F	0.00215	0.00081	0.00104

a: production of gas from the easily fermentable soluble fraction (ml)
b: production of gas from the insoluble, potentially fermentable fraction (ml)
c: gas production speed (h^{-1})

Conclusion

The analysis of the chemical composition and the prediction of the food value of *Prunus avium* has shown that this tree constitutes a natural resource in the northwestern areas of Tunisia which can contribute to the nutritional needs of small ruminants. *Prunus avium* essential oil represents a natural source of chemical molecules that at low doses can positively modify ruminal fermentation.

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