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in

López-Francos A. (ed.), Jouven M. (ed.), Porqueddu C. (ed.), Ben Salem H. (ed.), Keli A. (ed.), Araba A. (ed.), Chentouf M. (ed.).
Efficiency and resilience of forage resources and small ruminant production to cope with global challenges in Mediterranean areas

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 125

2021
pages 471-474

Effect of the drying process of sainfoin on *in vitro* fermentation parameters

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Abstract. Sainfoin (*Onobrychis viciifolia)* is an interesting legume due to its high production and its content of condensed tannins, which reduce methane production in the rumen. It is advisable to preserve this forage to optimize its use for animal feeding because two thirds of the annual production are obtained in the first spring cut. The objective of the present study was to evaluate the effects of different drying processes on the chemical composition and the *in vitro* fermentation parameters. Sainfoin was collected at the early flowering stage and was dried under the following conditions: freeze-dried, sun-dried and dried at 40, 60 and 80 ºC in an air-forced oven. After 24 h of incubation, contents of ammonia and volatile fatty acids were determined and *in vitro* organic matter degradability (IVOMD) was estimated. Freeze-dried sainfoin presented the lowest content of crude protein and fibres (neutral and acid detergent fibres) and the greatest condensed tannins (CT) content ($P<0.05$). In contrast, the 80ºC-oven dried sainfoin presented the highest fibre contents and the lowest CT content. Regarding the *in vitro* fermentation, the drying method had not effect on most of the assessed parameters, except for IVOMD ($P<0.001$), showing the lowest value the sun-dried sainfoin. In conclusion, the different drying conditions affected the chemical composition, but they had minor effect on the *in vitro* fermentation.

Keywords. Condensed tannins – IVOMD – Gas production – Methane production.

***Effet des conditions de séchage du sainfoin sur les paramètres de fermentation* in vitro**

Résumé. *Le sainfoin (*Onobrychis viciifolia*) est une légumineuse intéressante par son potentiel de rendement at par son contenu en tannins condensés, qui réduisent la production de méthane ruminale. Il est conseillé de conserver ce fourrage pour optimiser son utilisation en alimentation animale car les deux tiers de la production annuelle sont obtenus lors de la première coupe du printemps. L'objectif de la présente étude était d'évaluer les effets de différents processus de séchage sur la composition chimique et les paramètres de fermentation in vitro. Le sainfoin a été récolté au début de la phase de floraison et a été séché dans les conditions suivantes: lyophilisé, séché au soleil et séché à 40, 60 et 80 ºC dans un four à air forcé. Après 24 h d'incubation, les teneurs en ammoniac et en acides gras volatils ont été déterminées et la dégradation de la matière organique in vitro (IVOMD) a été estimée. Le sainfoin lyophilisé présentait la plus faible teneur en protéines brutes et en fibres (fibres au détergent neutre et acide) et la plus grande teneur en tanins condensés (CT) ($P<0,05$). En revanche, le sainfoin séché au four à 80 ºC présentait les teneurs en fibres les plus élevées et les teneurs en CT les plus faibles. Concernant la fermentation in vitro, la méthode de séchage n'a pas eu d'effet sur la plupart des paramètres évalués, à l'exception de l'IVOMD ($P<0,001$), indiquant la valeur la plus faible du sainfoin séché au soleil. En conclusion, les différentes conditions de séchage ont affecté la composition chimique, mais ont eu un moins d'effet sur la fermentation in vitro.*

Mots-clés. *Tanins condensé – IVOMD – Production de gaz – Production de méthane.*

I – Introduction

The interest in sainfoin (*Onobrychis viciifolia),* a perennial legume extensively used in the Mediterranean area, has increased due to its content of condensed tannins (CT; 50-80 g/kg DM), which reduce methane production in the rumen (Hatew *et al*., 2016). It is advisable to preserve the sainfoin

to optimize their use for animal feeding because two-thirds of the annual production of these forages are obtained in the first spring cut. Preservation can increase the fibrous fraction, reduce the crude protein (CP) and change the fractions of CT (Wang *et al*., 2015). Nevertheless, the extent of the effects is dependent on the method of preservation, silage or hay, and the legume species (Rufino-Moya *et al*., 2019). To the best of our knowledge, the effects of haymaking on ruminal fermentation have been scarcely studied in sainfoin and differed on the drying conditions (Scharenberg *et al*., 2007; Wang *et al*., 2015). The objective of the present study was to evaluate the effects of different controlled drying conditions on the chemical composition and the *in vitro* fermentation parameters of sainfoin, in order to assess how these conditions can affect to the nutritional value of this forage.

II – Materials and methods

Samples of sainfoin were collected at the early flowering stage. Fifty samples were obtained from 0.25 m^2 areas randomly allocated in the plot. The samples were randomly assigned to one of five treatments. The 10 samples of each treatment were mixed homogenously into 1 sample, and then divided into three sub-samples, to have three repetitions. The five treatments were: freeze-dried, sun-dried and dried at 40, 60 and 80 ºC in an air-forced oven. The sub-samples for the sun-dried treatment were extended on 10-cm elevated "mosquito" nets for 16 days, that were kept outdoors except during the night or when it rained. After the drying process, samples were ground to pass 1 mm screen (Rotary Mill, ZM200 Retsch, Germany) and used to determine ash and fibre fractions. One part of the sample was ground to pass a 0.2 mm screen and used to determine CP and CT content. Chemical analyses are described in Rufino-Moya *et al*. (2019). Briefly, the dry matter (DM), ash and crude protein (CP) contents were determined according to the AOAC methods (AOAC, 2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (lignin)) contents were determined according to the method described by Van Soest *et al*. (1991). Condensed tannins content was determined with the colorimetric HCl-butanol method described by Grabber *et al.* (2013), using the cyanidin as standard.

All the procedures used in the experiment were carried out in accordance with the Spanish guidelines for experimental animal protection (RD 53/2013) with the approval of the Institutional Animal Care and Use Committee of the Research Centre (Procedure number 2011-05). Four Rasa Aragonesa wethers (65 ± 2.1 kg body weight) fitted with rumen cannula were used as donors of ruminal contents. The animals were housed in individual pens (150 cm x 150 cm) with free access to water and a mineral-vitamin mixture. Wethers were fed 70% alfalfa hay and 30% barley grain. The rumen digesta was strained through 4 layers of cheesecloth and the fluid was mixed with the buffer solution, based on the protocol of Menke and Steingass (1988). Three 0.5 g DM sub-samples of each diet were incubated in bottles with 60 ml of incubation solution in a water bath (39 ºC) for 24 h, in three runs on three separate days. Blanks were included in each run and gas production was corrected with the blanks. After this period, 8 ml of gas were collected in 5 ml vacutainer tubes to quantify methane production. Then, the fermentation was stopped placing the bottles for 5-10 minutes in ice and the pH was measured immediately with a microPH 2002 (Crison Instruments S.A., Barcelona, Spain). To determine the ammonia (NH_3-N) content, 2.5 mL of liquid was mixed with HCl 0.1 N in a proportion of 1:1 (v/v). For volatile fatty acid (VFA) determination, 0.5 mL of liquid was added to 0.5 mL of deproteinising solution (5 mL of 85% (v/v) ortho-phosphoric acid and 0.125 mL of 4-methylvaleric acid (Sigma Aldrich, St.Louis, MO, USA) as internal standard, dissolved in 250 mL of distilled water) and 1 ml of distilled water. Tubes with samples of VFAs were stored at –20 °C until future analyses. Methane was analysed with gas chromatograph HP-4890, equipped with a capillary column TG-BOND Q+ (Thermo Scientific). Methane identification was based on the retention time as compared with the standard. *In vitro* organic matter digestibility (IVOMD) was estimated by filtering residues using pre-weighed bag (50 ! m; Ankom, NY, USA). The bags with sample were dried at 103 ºC for 48 h to obtain the dry matter content. After 48 h, bag content was weighed and

was placed at 550 ºC for to obtain the ashes. The organic matter of bag content was obtained as DM-ashes and the IVOMD was obtained as: (Incubated OM-bag content OM)/Incubated OM.

Data were analysed with a general lineal model with the forage conservation as fixed effect. Differences were significant for P<0.05 and tendencies for P<0.10.

III – Results and discussion

The drying method affected all the parameters of the chemical composition (Table 1). Freeze-dried sainfoin presented the lowest content of crude protein and fibres (neutral and acid detergent fibres) and the greatest CT content (P<0.05). In contrast, the 80 ºC-oven dried sainfoin presented the highest fibre contents and the lowest CT content. The greatest NDF content of 80ºC-oven dried sainfoin could be ascribed to an effect of temperature. Lignin also increased with the temperature whereas CT decreased. The presence of CTs led to an overestimation of the lignin content using conventional gravimetric methods such as the ADL (Marles *et al*., 2008). Limited information is available about the persistence of CT-effects after the forages have been conserved. The artificial drying using high temperatures (65 ºC) can decrease the chemical activity of CT (Scharenberg *et al*., 2007). As in the present study, the hay had greater fibre contents than the fresh forage (Rufino-Moya *et al*., 2019), however, the CP total CT contents were not affected.

Table 1. Chemical composition of the sainfoin hay according to the drying process

			Air forced oven				
% Dry matter	**Freeze-dried**	**Sundried**	**40ºC**	**60ºC**	**80ºC**	**S.E.**	**[1]P-value**
Organic matter, %	91.7^{a}	91.3^{c}	91.2^{d}	91.3^{cd}	91.5^{b}	0.03	0.001
Crude protein, %	17.1^{b}	18.3^{a}	18.8^{a}	18.7^{a}	18.1^{ab}	0.30	0.04
Neutral detergent fibre, %	45.3^{c}	47.3^{b}	47.4^{b}	47.9^{b}	51.0^{a}	0.60	0.001
Acid detergent fibre, %	31.5^{d}	33.6^{c}	34.5^{b}	33.9^{bc}	36.1^{a}	0.23	0.001
Acid detergent lignin, %	8.6^{d}	10.1^{c}	11.9^{ab}	10.9^{bc}	12.7^{a}	0.47	0.001
Condensed Tannins[2]	29.4^{a}	14.6^{b}	10.6^{cd}	12.9^{bc}	8.9^{d}	1.14	0.001

[1] Standard error. [2] Expressed as g of equivalent of cyanidine/kg DM.

Regarding the *in vitro* fermentation, the drying process had not effect on most of the parameters studied, except for pH and IVOMD (P<0.001, Table 2). Although differences were observed in the pH, they ranged into the optimal fermentation conditions (6.5-6.8; Amanzougarene *et al.,* 2017*).* The gas and methane production was numerically greater in freeze-dried sainfoin, but not significantly, whereas the rest of the drying methods presented similar productions (Table 2). The sun-dried sainfoin presented the lowest IVOMD whereas the freeze-dried sainfoin presented the greatest value (P<0.05), reflecting that CT and fibre contents determine the degradability. Ammonia, total VFA production and the ratio C_2:C_3 were similar among treatments (P>0.05, Table 2).

IV – Conclusions

In conclusion, the different drying conditions affected the chemical composition, but had minor effect on the *in vitro* fermentation of sainfoin. Studies of the CT fractions and composition should be carried out to unmask the importance of CT on the ruminal fermentation.

Table 2. Effect of the drying process of sainfoin on the in vitro fermentation parameters after 24 h of incubation

Parameters	Freeze-dried	Sundried	Air forced oven			S.E.	P-value
			40ºC	60ºC	80ºC		
pH	6.58[c]	6.68[b]	6.75[a]	6.7[ab]	6.71[ab]	0.04	<.0001
Gas production (mL/g MO)	170	144	149	144	149	17.86	0.13
CH4 production (mL/g MO)	5.84	4.29	5.10	4.83	4.63	1.36	0.46
IVOMD	71.8[a]	62.9[c]	68.6[b]	68.7[b]	70.1[ab]	2.34	<.0001
NH_3-N (mg/L)	114	123	128	122	115	27.21	0.91
Total VFA (mmol/L)	57.02	55.87	59.04	56.36	55.82	4.73	0.81
Acetic acid, %	65.9	66.0	65.4	65.8	66.1	0.69	0.47
Propionic acid, %	15.3	15.3	15.1	15.1	15.1	0.56	0.96
Butyric acid, %	12.7	12.7	13.0	12.9	12.8	0.87	0.97
Iso-butyric acid, %	1.6	1.5	1.7	1.5	1.5	0.14	0.37
Valeric acid, %	1.6	1.6	1.7	1.6	1.6	0.11	0.42
Iso-valeric, %	2.5	2.5	2.7	2.5	2.5	0.20	0.23
Acetic: Propionic	4.32	4.34	4.33	4.36	4.38	0.17	0.98

Acknowledgments

The authors gratefully acknowledge the staff of the CITA Research Centre for technical support. Projects INIA RTA2012-080-00, INIA RZP2017-00001) and (A14_17R). INIA and ESF funded M. Blanco and P.J. Rufino contract.

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