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in

Acar Z. (ed.), López-Francos A. (ed.), Porqueddu C. (ed.).
New approaches for grassland research in a context of climate and socio-economic changes

Zaragoza : CIHEAM

Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 102

2012

pages 251-255

Article available on line / Article disponible en ligne à l'adresse :

<http://om.ciheam.org/article.php?IDPDF=6890>

To cite this article / Pour citer cet article

Pereira-Crespo S., Valladares J., Flores G., Díaz N., Fernández B., Resch C., González-Arráez A., Bande-Castro M.J., Rodríguez-Diz X., Piñeiro J. **New annual legumes as winter crops for intensive forage rotations in Galicia (NW Spain). II – Nutritive Value.** In : Acar Z. (ed.), López-Francos A. (ed.), Porqueddu C. (ed.). *New approaches for grassland research in a context of climate and socio-economic changes*. Zaragoza : CIHEAM, 2012. p. 251-255 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 102)



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New annual legumes as winter crops for intensive forage rotations in Galicia (NW Spain).

II – Nutritive Value

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Abstract. The digestibility and chemical composition values of six species of annual legumes (Crimson clover, Balansa clover, Persian clover –ssp. *resupinatum* and ssp. *majus*–, Arrowleaf clover and French serradella) sown in small plots as a winter monoculture crop in the CIAM research station farm (coastal zone of Galicia, NW Spain) and harvested in the next spring following a silage-cutting strategy is presented in this paper. Forage was given a first cut at two weeks intervals in six harvest dates beginning at mid-March, and a second cut was taken after six weeks of regrowth. Nutritive value, estimated by NIRS, was high in average, and was affected by species and harvest date. For the first and second growth cycles the mean values of *in vitro* organic matter digestibility (IVOMD, %) and crude protein content (CP, % dry matter) were, respectively, 73.0±3.7% and 71.7±2.9% for IVOMD and 17.8±3.8% and 18.4±2.7% for CP. Forage quality, whilst reducing from the first harvest date, showed a more marked decline after the third harvest date (mid-April). This date could be adequate for ensiling, providing a high-digestibility, protein-rich forage and would fit into an intensive two crops per year rotation with maize as the summer crop.

Keywords. Digestibility – Crude protein – Serradella – Clovers.

Les nouvelles légumineuses annuelles utilisées comme des cultures d'hiver pour les rotations fourragères intensives en Galice (NO Espagne). II – Valeur nutritive

Résumé. L'objectif de ce travail a été de comparer l'évolution de la composition chimique et la digestibilité de six espèces de légumineuses fourragères annuelles (*Trifolium incarnatum*, *T. michelianum*, *T. resupinatum* –ssp. *resupinatum* et *majus*–, *T. vesiculosum* et *Ornithopus sativus*) cultivés en cultures d'hiver et récoltés à des dates différentes au printemps suivant d'une stratégie pour l'ensilage. Les résultats, obtenus par SPIR, montrent en moyenne une haute valeur nutritive, qui est significativement affectée par les espèces, les dates de récolte et leur interaction. Pour les premier et deuxième cycles, respectivement, la valeur moyenne de la digestibilité *in vitro* de la matière organique était de 73,0 ± 3,7% et 71,7 ± 2,9, tandis que la protéine brute (% matière sèche) était de 17,8 ± 3,8% et 18,4 ± 2,7%. Dans le premier cycle a montré une baisse de la qualité plus marquée à partir de la coupe de la mi-Avril, par conséquent cette date pourrait être indiquée pour l'ensilage, en fournissant un fourrage hautement digestible, riche en protéines, qui s'intègre bien dans la rotation de deux récoltes par an avec le maïs comme culture d'été.

Mots-clés. Digestibilité – Protéine brute – Serradelle – Trèfles.

I – Introduction

In recent years different cultivars of annual legumes adapted to areas with mild winters have appeared in the market. These new pasture legumes have been selected, among other characters, based on the depth of their root system, large growing season, tolerance to pests and diseases and symbiotic association adapted to acid soils (Loi *et al.*, 2008). Some of these forage species were of

common use, no many years ago, in Galician farms, as it is the case of crimson clover and serradella (Lloveras, 1987) and in a recent study about the improvement of pasture systems in suckler-cow hill farms in the SE of Galicia (NW Spain) Piñeiro *et al.* (2010) reports about the good performance, in terms of sward establishment and yield of Crimson clover, Balansa clover, Persian clover, Arrowleaf clover and French Serradella in a grazing-summer hay cut system.

There is no detailed information, at the present, about the comparative nutritive value of the cited species when grown for silage in lowland situations. In order to assess their utility in the Galician dairy farms as winter crops in intensive forage rotation systems with maize, it is the objective of this study to assess the evolution of the *in vitro* organic matter digestibility and chemical composition of the first cycle of these annual legumes under a strategy of different cutting dates for silage in spring.

II – Materials and methods

This work was carried out with six annual forage legume species sown in monoculture in the autumn of year 2009, which were harvested at six different dates the next spring during the first growth cycle (15 March-24 May) at two weeks intervals. The species studied were Crimson clover (*Trifolium incarnatum* L. cv Viterbo), Balansa clover (*T. michelianum* Savi. cv. Bolta), Persian clover (*T. resupinatum* L. ssp. *resupinatum* cv. Maral and ssp. *majus* cv. Kyambro), Arrowleaf clover (*T. vesiculosum* Savi. cv. Zulu) and French serradella (*Ornithopus sativus* Brot. cv. Margurita). The experiment was carried out at the Centro de Investigacións Agrarias de Mabegondo (CIAM, A Coruña, Galicia, NW Spain, 43° 15' N, 8° 18' W, 100 m altitude) following a split-plot design with species as the main plot and harvest date as the subplot, with ten blocks. Other details of the experiment can be found in Valladares *et al.* (2012).

Dry matter (DM) content of fresh samples (n=360) was determined by oven-drying (80 °C, 16 hours) and dry samples were ground in a Christy-Norris hammer mill to pass a 1 mm screen. The chemical composition and digestibility of ground samples was estimated by NIRS using a calibration equation obtained at the CIAM for fresh annual legume species (Pereira-Crespo *et al.*, 2012). Estimated quality parameters were: organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), water soluble carbohydrates (WSC), and *in vitro* organic matter digestibility (IVOMD). Data were subjected to ANOVA and multiple comparison of means by Fisher's Least Significant Difference procedure using Proc GLM of SAS (SAS Institute, 2000).

III – Results and discussion

Species and harvest dates significantly affected ($p < 0.001$) all the parameters of chemical composition and the *in vitro* organic matter digestibility. Mean values of all samples in the first cycle of growth (n=360) were: IVOMD (%) 73.0 ± 3.7 (range 61.9 to 80.9), OM (% DM) 90.0 ± 1.4 (range 87.6 to 94.3), CP (% DM) 17.8 ± 3.8 (range 8.0 to 26.2), WSC (% DM) 12.1 ± 2.2 (range 6.8 to 18.1) and NDF (% DM) 35.8 ± 6.0 (range 24.2 to 52.4), showing a good average nutritive value.

As can be seen in Table 1, where the average values for the main effects are shown, Crimson clover, Persian clover ssp. *resupinatum* and Balansa clover were the most digestible species, with IVOMD (%) values of 74.9, 74.6 and 74.0% respectively, whilst the French serradella showed the lowest value (70.4%). The variation in OM (%DM) content among species was quantitatively small, ranging between 91.3 and 89.1 % for Persian clover *majus* and French serradella, respectively. Crude protein content (%DM) of Persian clover *majus* was superior, on average, compared with the rest of species (19.1 %DM), whilst Arrowleaf clover showed the lowest CP value (16.6 %). Regarding to WSC (%DM) content, Crimson clover showed the highest value (14.1 %) followed by Arrowleaf clover (13.7 %), being observed the lowest values for the Persian clovers (ssp. *resupinatum* 10.8% and ssp. *majus* 10.5%). Cell-wall content of French serradella

and Arrowleaf clover were, respectively, the highest and lowest values compared with the rest of species, with NDF values of 41.8% and 33.6%DM for both species.

Table 1. Effect of species and harvest date on chemical composition (%DM) and *in vitro* organic matter digestibility (%) of annual legumes

Species [†]	OM	CP	WSC	NDF	IVOMD	Harvest date ^{††}	OM	CP	WSC	NDF	IVOMD
French Serradella	91.3	18.2	11.5	41.8	70.4	15 Mar.	89.2	20.6	14.5	31.6	77.5
Crimson clover	90.5	18.1	14.1	35.3	74.9	29 Mar.	89.1	22.7	10.7	31.6	74.3
Balansa clover	89.8	17.0	12.4	35.6	74.0	12 Apr.	89.7	19.6	13.1	31.1	74.6
Persian clover <i>resup.</i>	89.3	17.9	10.5	34.8	74.6	26 Apr.	89.9	17.0	11.7	36.7	72.2
Persian clover <i>majus</i>	89.1	19.1	10.8	33.9	72.1	10 May	90.0	14.6	11.5	40.5	71.0
Arrowleaf clover	90.1	16.8	13.7	33.6	71.8	24 May	92.1	12.5	11.5	43.5	68.3
<i>l.s.d.</i>	0.19	0.22	0.14	0.29	0.21	<i>l.s.d.</i>	0.20	0.41	0.29	0.32	0.16

[†] Mean values for each species across harvest dates. ^{††} Mean values for each harvest date across species. *l.s.d.*: least significant difference between two means in the same column at $p < 0.05$.

Species digestibility, crude protein and sugar content declined with advancing maturity, with mean values in first (mid-March) and last cutting dates (late May) for IVOMD (%), CP (%DM) and WSC (%DM) of, respectively 77.5 and 68.3%, 20.6 and 12.5% and 14.5 and 11.5%. As expected, NDF (%DM) increased clearly from 31.6 to 43.5% between these dates, and OM content (%DM) also increased slightly from 89.2 to 92.1%. As indicated by Valladares *et al.*, (2012), legume DM content remained low along the spring, with overall mean values of 12.1 ± 2.8 (range 5.2 to 21.3). The ratio WSC/N remained fairly low, on average, all along the season, ranging from 2.9 to 5.8. It has been suggested that WSC/N ratio values less than 6 are indicative of a risk of deficient fermentation in the silo (Braithwaite, 1987). This fact, together with the very low DM content of these species in the spring, which ranged from 9.8 to 15.8 % (Valladares *et al.*, 2012) highlights the need of wilting in order to succeed in the ensiling of these annual legumes.

Table 2 reflects the interaction effect ($p < 0.001$) of species x harvest date for the digestibility of annual legumes. Comparing the first and the last harvest dates, Crimson clover and the two cultivars of Persian clovers (*resupinatum* and *majus*) showed a lower rate of IVOMD decrease (-0.79, -0.69 and -0.80 percent units per week, respectively) whilst the two early-flowering species (French serradella and Balansa clover) and Arrowleaf clover showed a faster rate (IVOMD decrease of -1.15, -1.05 and -1.02 percent units per week, respectively). With regard to the reduction of protein content with advancing maturity of forage, there is also significant differences among species although this effect is less marked ($p < 0.05$). To this regard, the Persian (ssp. *resupinatum*), Balansa, Arrowleaf and Crimson clovers showed the highest average rate of decrease between mid-March and late-May, with values of -0.96, -0.95, -0.93 and -0.92 units of CP (DM basis) whilst Persian clover (ssp. *majus*) and Serradella values were -0.67 and -0.43 units. It is to be noted that, for all species in general, both IVOMD and CP of legumes remains fairly high up to mid-April, and from this date on a more marked decline in quality was observed in the experiment.

These results are in agreement with previous works cited in the literature, indicating the good nutritional quality of annual legumes and its decline with advancing maturity, although we found differences in the behaviour of different species. For example, Lloveras and Iglesias (2001) observed a rapid drop in quality of Crimson clover after flowering; in contrast, our results showed that this specie was one of the highest quality, according IVOMD and CP contents. Also in contrast to our findings, Akin and Robinson (1982) reported a lower digestibility for Crimson clover

Table 2. Interaction of Species x Harvest date on crude protein (CP, %DM) and *in vitro* organic matter digestibility (IVOMD, %) of annual legumes

Species	Crude protein (%DM)					<i>In vitro</i> OM digestibility (%)						
	15 Mar.	29 Mar.	12 Apr.	26 Apr.	10 May	24 May	15 Mar.	29 Mar.	12 Apr.	26 Apr.	10 May	24 May
French Serradella	19.5	21.6	19.2	17.2	16.3	15.2	76.1	72.9	72.8	69.5	66.8	64.6
Crimson clover	21.2	23.6	20.1	17.2	14.7	12.0	78.2	75.3	76.7	75.5	73.3	70.3
Balansa clover	20.6	21.9	19.3	15.7	13.5	11.1	79.0	76.5	75.9	72.8	71.5	68.2
Persian clover <i>resup.</i>	21.6	23.3	19.6	16.9	13.8	12.0	78.7	75.5	76.1	74.0	72.7	70.7
Persian clover <i>majus</i>	20.6	23.6	21.0	19.2	16.1	13.9	76.7	71.9	71.3	70.3	73.0	69.8
Arrowleaf clover	20.0	22.5	18.4	16.0	13.4	10.7	76.4	73.6	74.7	71.1	68.8	66.2

l.s.d.: least significant difference between two means in the same column at $p < 0.05$ CP 0.72, IVOMD 1.05. Flowering date: Serradella 29 Mar., Balansa clover: 12 April, Persian clover *resupinatum*: 23 April, Crimson clover 26 April; Arrowleaf clover 5 May, Persian clover *majus*, 22 May.

that Arrowleaf clover, at similar stages of maturity, which is not observed in our study. The use of different varieties and their interaction with environmental conditions of each case may be one reason to explain these differences, thus reinforces the need for evaluation of different species and varieties locally and keep them updated in time.

IV – Conclusions

It is confirmed the good overall nutritional quality of the annual legumes evaluated, and Crimson clover, Balansa clover and Persian clover ssp. *resupinatum* showed advantages, in terms of IVOMD and CP content, for a harvest in the last half of April, compatible with the usual maize seeding time in Galician dairy farms conditions. Since silage fermentation can be compromised by the low dry matter and WSC/N ratio of these species, it seems obligated and adequate wilting of forage before ensiling.

Acknowledgments

The authors wish to acknowledge the financial support received from the INCITE of Xunta de Galicia (Project PGIDIT09MRU012E) and the dairy company Leyma Central Lechera. S.A.

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