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Nutritive value for ruminants of winter oats–legume intercrops in organic cultivation

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Abstract. Winter oats were grown according to European organic farming regulations in monoculture (oats) and in intercropping with bard vetch (BAV), bitter vetch (BIV) or both legumes (MIX) to evaluate the effects of intercropping on forage yield and nutritive value for ruminants. The experiment was carried out as a randomised complete block design with four replications, and whole forage samples were obtained at two harvest dates (June and July). For both harvest times, all intercrops increased ($P < 0.05$) forage yield compared with oats, but forage crude protein content was only increased ($P < 0.05$) for BAV and MIX. Compared with oats, intercropping with BAV increased ($P < 0.05$) *in vitro* rate of gas production and total volatile fatty acid production, indicating a higher rate and extent of rumen degradation of BAV forage. In contrast, BIV forage harvested in June had lower ($P < 0.05$) rate of gas production and total volatile fatty acid production than June oats, but in general no differences in the *in vitro* rumen fermentation were detected between oats and BIV samples harvested in July. The results indicate that forage yield and quality can be enhanced by intercropping oats with BAV; however, intercropping with BIV increased yield but decreased nutritive value of the forage.

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Introduction

In European countries organic livestock must be fed with organic feed to meet the EU regulations on organic farming (European Communities 2007). Moreover, the EU regulation establishes that rearing systems for herbivores have to be based on maximum utilisation of grazing pasture according to the availability of pastures in the different periods of the year, and that at least 60% of the dry matter (DM) in their daily rations shall consist of roughage. Organic farming in Spain has increased considerably over the past decade, and cereals and legumes are the main crops (MAGRAMA 2013). However, the productivity of crops in organic farming has frequently been shown to be lower compared with conventional farming (Kitchen *et al.* 2003; Tejido *et al*. 2010, 2011). In addition, previous results indicate that organic cultivation of winter cereals reduced forage crude protein (CP) content compared with a conventional cultivation system (Tejido *et al*. 2010, 2011).

Intercropping of winter cereals with legumes can provide higher forage yield and quality than a winter cereal monoculture, but the choice of legume species affects the yield and quality advantages (Mariotti *et al*. 2006). In addition, the features of intercropping systems differ with soil, local climate and preferences of the local farmers (Steiner 1982). Another major problem with intercropping is determining the optimal harvest time because the growth cycle of consociated species is often not synchronised (Mariotti *et al*. 2006). The objective of this study was to assess the potential of winter oats (*Avena sativa* L.) –legume intercropping to enhance forage yield and quality as compared with oats as a monoculture when crops were grown according to European organic farming regulations. The legumes chosen were bard vetch (*Vicia articulata* Hormen) and bitter vetch (*Vicia ervilia*) as they are widely cultivated in the study area, and two harvest times (June and July) were investigated.

Materials and methods

Experimental design and cultivars

The study was carried out in the province of Zamora, located in the north-west of Spain (41°25′N, 6°16′W). The climate is semiarid, with cold winters, hot summers and low rainfall. The experimental plots were established on a sandy loam, acid and low fertility soil that had been organically cultivated for 2 years and was fallow for 1 year before the commencement of the study. The study area was divided in 16 subplots (60 m^2 each) with four treatments and four replicates in a randomised complete block design. Winter oats was grown in monoculture (oats) and in intercropping with bard vetch (BAV), bitter vetch (BIV) or both legumes (MIX) to evaluate the effects of intercropping on forage yield and nutritive value for ruminants. On 8 October 2012 organic seeds were sown in all plots using a seed drill at a rate of 230 kg/ha. The proportions of oat seeds : BAV seeds : BIV

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seeds in the cultivars in intercropping were 85 : 15 : 0 for BAV, 90 : 0 : 10 for BIV and 80 : 10 : 10 for MIX. These proportions were selected to be representative of those used by local farmers. No herbicide or fertiliser treatment was applied to the cultivars in accordance with European organic farming regulations.

Whole-plant yield was determined on 7 June 2013 (vegetative state) and 10 July 2013 (reproductive stage) by manually cutting 4 × 1-m^2 areas in each plot at a height of 3 cm above ground level. Samples were dried in the laboratory to estimate forage DM production before being bulked and ground through a 1-mm sieve for determination of chemical composition and *in vitro* incubations.

In vitro *incubations*

Ruminal fluid was obtained from four rumen-cannulated Merino sheep fed 800 g of grass hay and 200 g of concentrate per day administered in two equal portions at 0800 hours and 2000 hours. Sheep were managed according to the protocols approved by the León University Institutional Animal Care and Use Committee. Ruminal contents of each sheep were obtained immediately before the morning feeding, mixed and strained through four layers of cheesecloth into an Erlenmeyer flask with an O_2-free headspace. Particle-free fluid was mixed with the buffer solution of Goering and Van Soest (1970; no trypticase added) in a proportion 1 : 4 (vol/vol) at 39°C under continuous flushing with CO_2.

To estimate the fermentation kinetic parameters, samples of 500 mg of DM of each forage were accurately weighed into 120-mL serum bottles. Bottles were pre-warmed (39°C) before the addition of 50 mL of buffered rumen fluid into each one under CO_2 flushing, sealed with butyl rubber stoppers and aluminium caps and incubated at 39°C. A total of 32 bottles with substrate (one bottle per forage) and two without substrate (blanks) were incubated for 144 h. Gas production (GP) was measured at 3, 6, 9, 12, 16, 21, 26, 31, 36, 48, 60, 72, 96, 120 and 144 h using a pressure transducer and a calibrated syringe and the gas produced was released after each measurement. After 144 h of incubation, the fermentation was stopped by swirling the bottles in ice and the bottles were opened and their content was transferred to previously weighed filter crucibles. The residue of incubation was washed with 50 mL of hot distilled water (50°C), dried at 50°C for 48 h and the apparent disappearance of substrate was calculated. The residue was then analysed for ash to calculate the organic matter (OM) apparent disappearance after 144 h of incubation (OMD_{144}). Incubations were repeated four times on different days.

In vitro fermentation characteristics of forages were assessed in 24-h incubations. A total of 32 bottles containing 400 mg of forage (one bottle per each forage) and two bottles without substrate (blanks) were filled with 40 mL of buffered rumen fluid, sealed and incubated at 39°C. After 24 h of incubation, bottles were uncapped, the pH was measured immediately with a pH-meter, 0.5 mL of fluid were added to 0.8 mL of deproteinising solution (100 g of metaphosphoric acid and 0.6 g of crotonic acid per L) for volatile fatty acid (VFA) analysis and 0.5 mL were added to 0.5 mL 0.5 M HCl for ammonia-N determination. Incubations were repeated four times on different days.

In order to determine *in vitro* digestibility, samples of each forage (400 mg) were weighed into artificial fibre bags (#F57 bags; 50 × 40 mm; 25 ± 10-µm pore size; ANKOM Technology Corporation, Fairport, NY, USA). Bags were heat sealed and incubated with buffered rumen fluid in the ANKOM *in vitro* fermentation system Daisy II (ANKOM Technology Corporation) for 48 h. Bags were then washed in cold tap water for 5 min and extracted with boiling neutral detergent solution for 1 h (Van Soest *et al.* 1991) using an $ANKOM^{220}$ Fibre Analyser unit. Finally, the bags were washed with distilled water and dried at 60°C to determine true *in vitro* dry matter degradability (TDMD; Van Soest *et al.* 1966) and neutral detergent fibre degradability (NDFD). For replication, the complete procedure was repeated three times ($n = 3$).

Calculations, analytical procedures and statistical analyses

Gas production values were corrected for the amount of gas produced in the blanks and the values were fitted with time to the exponential model $y = A\,[1 - e^{(-c\,(t\text{-}lag))}]$, where A is the asymptotic GP (mL/g), c is the fractional rate of GP (%/h), *lag* is the initial delay in the onset of GP (h) and t is the gas reading time. The parameters A, c and *lag* were estimated by an iterative least-squares procedure using the NLIN procedure of SAS (SAS Inst., Inc., Cary, NC, USA). The effective degradability of diet OM (OMED; %) was estimated assuming a rumen particulate outflow (Kp) of 0.035 per h, characteristic for sheep fed forages at maintenance level (Ranilla *et al.* 1998), according to the equation proposed by France *et al.* (2000): $OMED = [(OMD_{144}c)/(c + Kp)]\ e^{(-clag)}$. The average GP rate (AGPR; mL gas/h) was defined as the AGPR between the start of the incubation and the time at which the cumulative GP was half of its asymptotic value, and was calculated as $AGPR = A\,c/[2\,(\ln 2 + c\,lag)]$.

Dry matter, ash and N were determined according to the Association of Official Analytical Chemists (1999). Analyses of VFA and ammonia-N have been described by Carro and Miller (1999). NDF, acid detergent fibre (ADF) and acid detergent lignin analyses were carried out according to Van Soest *et al.* (1991) and Goering and Van Soest (1970), respectively.

When data were analysed across harvest times, cultivar × harvest time interactions ($P < 0.05$) were detected for some parameters. Therefore, data were analysed independently for each harvest time by ANOVA using the MIXED procedure of SAS. The effect of cultivar was considered fixed and the effect of rumen inocula (replicates) was considered random. When a significant ($P < 0.05$) difference between cultivars was detected, means were separated by Tukey's test.

Results

Intercropping of oats with BAV, BIV and MIX resulted in significantly greater forage DM yield at both harvest times, but differences between intercrops were not significant (Table 1). No significant differences ($P > 0.05$) between cultivars were detected in OM and lignin content, but intercropping of oats with BAV and MIX significantly increased the CP content of forage in both harvest times. Whereas no significant effects of intercropping on NDF and ADF content of the forage were detected in July, forage harvested in June from oats had greater NDF content than that harvested from BAV and lower NDF and ADF content than that from BIV. Compared with oats,

Table 1. Dry matter (DM) yield, chemical composition, true *in vitro* DM degradability (TDMD) and neutral detergent fibre degradability (NDFD) of forage from winter oats as sole crop (oats) and in intercropping with bard vetch (BAV), bitter vetch (BIV) or both legumes (MIX) and harvested in June or July

A, B, C; means in the same row not sharing a common letter differ ($P < 0.05$)

Harvest time	Item	Oats	BAV	BIV	MIX	s.e.m.	*P*-value
June	DM yield (t/ha)	1.80A	2.52B	2.61B	2.53B	0.153	0.009
	Chemical composition (g/100 g DM)						
	Organic matter	93.7	94.1	93.3	94.0	0.53	0.735
	Crude protein	3.69A	6.91B	3.78A	6.34B	0.331	<0.001
	Neutral detergent fibre	58.0B	52.2A	61.6C	57.5B	0.70	<0.001
	Acid detergent fibre	31.6A	30.3A	35.1B	32.4A	0.58	<0.001
	Lignin	5.04	5.91	6.59	6.67	0.587	0.230
	TDMD (%)	71.8C	72.1C	64.0A	68.1B	1.04	<0.001
	NDFD (%)	51.4C	46.6BC	41.6A	43.1AB	1.80	<0.001
July	DM yield (t/ha)	1.60A	2.27B	2.39B	2.53B	0.150	0.009
	Chemical composition (g/100 g DM)						
	Organic matter	93.8	94.4	94.3	95.4	0.52	0.264
	Crude protein	3.62A	6.57B	3.86A	5.18B	0.357	<0.001
	Neutral detergent fibre	59.3	56.3	60.3	57.9	1.19	0.146
	Acid detergent fibre	33.6	33.7	33.9	33.2	0.95	0.964
	Lignin	5.67	6.62	5.82	5.27	0.527	0.364
	TDMD (%)	68.6	69.5	66.9	67.2	1.37	0.207
	NDFD (%)	47.1	45.8	45.1	43.3	2.35	0.545

Table 2. Parameters of gas production kinetics and organic matter effective degradability (OMED) of winter oats as sole crop (oats) and in intercropping with bard vetch (BAV), bitter vetch (BIV) or both legumes (MIX), harvested in June or July, and incubated *in vitro* in batch cultures of rumen microorganisms

A, B, C; means in the same row not sharing a common letter differ ($P < 0.05$)

Harvest time	Item[A]	Oats	BAV	BIV	MIX	s.e.m.	*P*-value
June	*A* (mL/g DM)	210C	190A	205BC	198AB	5.91	<0.001
	c (%/h)	0.019B	0.025D	0.016A	0.022C	0.0011	<0.001
	Lag (h)	0.85AB	0.42A	1.51B	1.09AB	0.435	<0.001
	AGPR (mL/h)	2.75B	3.42D	2.28A	3.13C	0.152	<0.001
	OMED (%)	23.4B	27.9C	19.5A	24.5B	0.95	<0.001
July	*A* (mL/g DM)	211B	190A	207B	205B	6.38	<0.001
	c (%/h)	0.017A	0.021B	0.018A	0.018A	0.0011	<0.001
	Lag (h)	2.40	1.89	2.53	2.57	0.521	0.231
	AGPR (mL/h)	2.38A	2.75B	2.44A	2.43A	0.133	<0.001
	OMED (%)	19.9A	22.8B	19.9A	20.0A	0.95	<0.001

[A]See text for significance of parameters.

both BIV and MIX intercrops decreased TDMD and NDFD of the forage harvested in June. In contrast, no differences among cultivars in TDMD and NDFD were detected in July.

The parameters of GP kinetics for each cultivar are shown in Table 2. Intercropping of oats with BAV increased the rate of GP, AGPR and OMED in both harvest times and decreased lag time in June. Intercropping of oats with BIV decreased the rate of GP, AGPR and OMED in June, but had no effect on GP kinetics of the forage cut in July. Intercropping of oats with MIX showed little effects, as only increased the rate of GP and the AGPR in the forage harvested in June.

Table 3 shows the rumen fermentation parameters after *in vitro* incubation of samples from each cultivar with buffered rumen fluid from sheep. Final pH in the *in vitro* cultures ranged from 6.80 to 6.91 in June and from 6.89 to 6.94 in July (results not shown), and differences between oats and intercrops were only observed in June, when *in vitro* cultures with BIV showed greater pH values than those with oats. Total VFA production of BAV was 11% and 13% greater than that of oats for June and July samples, respectively. In addition, propionate and butyrate production for June BAV was greater than that for June oats, and acetate, butyrate and others (isobutyrate, isovalerate and valerate) production for July BAV was greater than that for July oats. Both acetate : propionate ratio and ammonia-N concentrations were greater for BAV than for oats in July. Compared with oats, BIV produced lower amounts of both total VFA and propionate in June, but no differences were detected in July. *In vitro* fermentation of MIX harvested in June resulted in lower amounts of total VFA, acetate and propionate compared with June oats, but fermentation of MIX harvested in July produced more butyrate and other VFA (sum of isobutyrate, isovalerate and valerate) than fermentation of July oats.

Table 3. Production of volatile fatty acid (VFA), acetate : propionate ratio (Ac/Pr) and ammonia-N concentrations of winter oats as sole crop (oats) and in intercropping with bard vetch (BAV), bitter vetch (BIV) or both legumes (MIX), harvested in June or July, and incubated *in vitro* in batch cultures of rumen microorganisms

A, B, C; means in the same row not sharing a common letter differ ($P < 0.05$)

Harvest time	Item	Oats	BAV	BIV	MIX	s.e.m.	*P*-value
June	VFA production (μmol)						
	Total VFA	1510B	1678C	1355A	1372A	79.5	<0.001
	Acetate	1020bC	1104c	939AB	917A	70.9	<0.001
	Propionate	362B	408C	288A	321A	20.6	0.002
	Butyrate	85.8AB	126.0c	81.8A	98.6B	8.12	<0.001
	Others[A]	42.1AB	39.9AB	45.6B	36.3A	4.69	0.049
	Ac/Pr (mol/mol)	2.86A	2.72A	3.33B	2.86A	0.155	<0.001
	Ammonia-N (mg/L)	181.8	177.8	169.5	171.9	8.51	0.171
July	VFA production (μmol)						
	Total VFA	1275A	1441B	1241AB	1348AB	55.2	<0.001
	Acetate	887AB	1007C	863A	935B	38.0	<0.001
	Propionate	272AB	291B	257A	273AB	11.6	<0.001
	Butyrate	81.4A	96.3C	85.8AB	96.0BC	5.61	<0.001
	Others[A]	35.3A	47.8B	35.1A	44.7B	2.35	<0.001
	Ac/Pr (mol/mol)	3.33A	3.51B	3.42AB	3.49B	0.065	<0.001
	Ammonia-N (mg/L)	201.6A	221.7B	197.7A	207.8A	6.07	<0.001

[A]Calculated as the sum of isobutyrate, isovalerate and valerate.

Discussion

Oat is a popular cereal forage in cool semiarid regions and the legumes chosen for the study are widely cultivated in the study area. Different seeding ratios of oats : legume were used for each cultivar (85 : 15 : 0 for BAV, 90 : 0 : 10 for BIV and 80 : 10 : 10 for MIX) to replicate the seeding ratios traditionally used by local farmers. The forage produced from these cultivars is being used in practical sheep feeding without having actual research-based information about its nutritive value. Therefore, this study was conducted to analyse the nutritive value of the forage produced under farmers' conditions at two harvest times. It should be noted, however, that seeding ratio may affect yield and quality of forage produced by cereal–legume mixtures (Caballero *et al.* 1995; Lithourgidis *et al.* 2006) and results of this study therefore need to be interpreted with caution.

Forage DM yield was increased by 39%, 45% and 41% in June for BAV, BIV and MIX, respectively, and by 42%, 49% and 58% in July, compared with oats as monoculture. These results confirm previous findings that intercropping winter cereals with legumes can increase forage yield (Moreira 1989; Carr *et al.* 2004; Mariotti *et al.* 2006). Carr *et al.* (2004) found that intercropping barley and oats with peas increased forage DM yield by 21% and 19%, respectively, compared with the corresponding cereal as monoculture, and Mariotti *et al.* (2006) reported that intercropping of wheat and barley with vetch increased forage DM yield by 85% and 60%, respectively.

However, other studies (Anil *et al.* 1998; Lauriault and Kirsey 2004) have reported a lack of effects of winter cereals : legume intercropping on forage yield, indicating that the effects of intercropping are influenced by many factors, with soil characteristics and cultivar selection being two of the most important. Moreira (1989) observed that under N-deficient conditions intercropping oats with vetch increased forage DM yield and CP and mineral content, but when crop N nutrition was adequate, there was only a small increase in yield and nutritive value of forage. In the present study there were no differences between the three intercropped cultivars in forage DM yield at any harvest date.

Forage CP content was greater in BAV and MIX than in BIV and oats at both harvest times. The greater oats : legume seeding ratio used in BIV (90 : 10) compared with BAV (85 : 15) and MIX (80 : 20) may have contributed to the low CP content of BIV forage, which was similar to that in oats. In addition, BIV forage in June had the greatest NDF and ADF content and the lowest TDMD, indicating low quality. The lower GP rate, AGDR and OMED observed for BIV compared with oats would indicate that June BIV forage may be degraded in the rumen at a lower rate than oats forage. This was confirmed by *in vitro* total VFA production, which was 10.3% lower for BIV compared with oats forage. In contrast, no differences between oats and BIV forage in chemical composition and *in vitro* degradability were observed in July. The lack of effects of intercropping oats with BIV on chemical composition of July-harvested forage agrees well with the similar parameters of GP kinetics and VFA production observed for both forages when they were incubated *in vitro* with buffered ruminal fluid. These results indicate that quality of BIV forage was higher in July than in June when compared with oats forage harvested at the same dates; other studies (Lauriault and Kirsey 2004; Dear *et al.* 2005) have also reported the influence of harvesting times on the comparison of forage quality in cereal monocultures and cereal : legume mixtures.

Concentrations of CP in oats forage in monoculture in our study were similar to the 37–54 g of CP/kg DM reported by Moreira (1989) for the same species under low N inputs and low available soil N. As expected, forage CP concentrations were greater in BAV and MIX than in oats forage. Many studies (Moreira 1989; Carr *et al.* 2004; Dear *et al.* 2005; Mariotti *et al.* 2006) have shown that CP content of forage is enhanced by intercropping cereals with legumes compared with cereal monocrops, which is attributed to the ability of legumes to fix atmospheric N. The effects of intercropping cereals with legumes are expected to be greater under N-deficient conditions (Moreira 1989), such as those in organic farming due to N fertilisation limitations imposed by EU regulations. In addition, the GP kinetics parameters measured in our study indicate that BAV forage from both harvest times had greater degradation rates than oats forage. This was confirmed by the greater production of total VFA compared with oats (11% and 13% greater in June and July, respectively), indicating a better quality of BAV forage. Moreover, the greater GP rate observed for BAV compared with oats would indicate a higher degradation rate in the rumen, which may result in higher intake rate for this forage as degradation rate is directly related to passage rate and voluntary DM intake (Carro *et al*. 1991). Chemical composition, parameters of GP kinetics and *in vitro* fermentation parameters of MIX forage were in general intermediate between those for BIV and BAV. The results indicate that MIX forage quality was similar or even slightly lower than that of BAV, despite a oats : legume seeding ratio greater than BAV (80 : 20 vs 85 : 15).

In conclusion, under the seeding ratios and cultivation conditions of our study and the restrictions imposed by the EU organic farming regulations, intercropping of oats with BAV was the best choice of the intercrops tested to increase forage yield and CP content. Furthermore, the results indicate that rate and extent of rumen degradation were highest for BAV forage at both harvest times. Our results also indicate that the effects of intercropping can be affected by harvest time, as quality was lower for forage harvested in June compared with July. These results should be confirmed in multi-year studies before drawing up guidelines for organic farmers. In addition, lower oats : legume seeding ratios should be investigated to find the optimal ratio to achieve both better forage yield and quality.

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