Preliminary estimation of forage yield and feeding value of *Lupinus* angustifolius varieties cultivated in Jalisco, México, during the cool season

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ABSTRACT

The effects of genotype and harvest date on lupin (*Lupinus angustifolius*) forage yield and quality were evaluated in Zapopan Jalisco, Mexico. Cultivars Haags Blaue, Boregine, Borlu, Probor, Sonate, and Boruta were grown during the 2013–2014 cool season and harvested twice, in January (early harvest) and February (late harvest). Fresh and dry matter (FM, DM) yield, crude protein(CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), minerals, and total alkaloids were measured at each harvest. FM yields varied from 9.1 to 12.2 t ha⁻¹ and from 23.4 t ha⁻¹ to 35.3 t ha⁻¹ at the first and late harvests, respectively. DM yields from early harvest plants varied from 1.2 to 1.7 t ha⁻¹; those from the second harvest (late harvest date) varied from 3.4 to 6.8 t ha⁻¹. The highest CP content for both (early and late harvests) was identified in the Probor cultivar, at 226 and 187 g/kg, respectively. At early harvest, ADF values varied from 291.2 in Sonate to 392.3 g/kg in Borlu; at late harvest, ADF values varied from 417.2 in Sonate to 488.3 g/kg in Borlu. Harvest date and varieties did not influence alkaloid and mineral content.

Key words: Chemical composition, Forage Legumes, Forage yield, Lupinus angustifolius.

Abbreviations: CP:crude protein, DM:dry matter, FM:fresh matter, ADF:acid detergent fiber NDF:neutral detergent fiber INTRODUCTION is a need to exploit new fodder legumes that are not only

Perennial and annual forage legumes are important components of sustainable cropping systems (Sheaffer and Seguin, 2003;), and forage legumes are important in ruminant nutrition in terms of providing energy, protein, minerals and fiber (Ahmad et al., 2000). They can be grazed or stored as hay or silage and also contribute biologically fixed N and sustain the soil by reducing erosion and increasing soil organic matter levels (Ahmad et al., 2000; Sheaffer and Seguin, 2003). In different agro-ecological zones, crop and livestock production are major components of the farming system. Where the climate is relatively cool, crop residues are the major and widely available feeds because the growth of wild herbage species is limited. However, crop residues, especially cereals, have poor feeding value with poor metabolisable energy and negligible available protein and are seriously deficient in mineral and vitamins (Lulseged and Jamal, 1999). On the other hand, commercial concentrates are mostly inaccessible or quite expensive for small holder farmers. Growing of annual coolseason forage legumes could be one option for cheap protein sources in livestock production in a mixed crop-livestock farming system. In Mexico, only a few fodder legumes like lucerne (*Medicago sativa*) and chickpea (*Cicer arietinum*) are grown in very small agro-ecological zones. Thus, there is a need to exploit new fodder legumes that are not only adequate sources of proteins but also provide higher forage yield than traditional fodder crops. Lupins offer an opportunity for production of high protein forage while simultaneously providing some fertility in the form of nitrogen fixation (Schulze et al., 2006). More than 300 Lupinus species have been described, but only four are important as food: L. albus, L. luteus, L. angustifolius, and L. mutabilis (Gladstones et al., 1998). Lupinus angustifolius and L. albus are gaining importance in different regions of world for their potential in sustainable crop production systems and are being investigated as potential forage and grain crops (Bhardwaj et al., 2010). Previous studies conducted in the UK reported narrow-leafed lupin (L. angustifolius) whole crop forage dry matter (DM) yields of 6.6 to 8.4 Mg ha⁻¹and crude protein (CP) concentrations of more than 190 g kg⁻¹DM (Fraser et al., 2005). Very little is known about the forage yield and nutritive value of this crop under Mexican climate conditions. We hypothesized that ${\cal L}$. angustifolius varieties with low alkaloids could be an alternative for the production of high-content protein forage in Mexico. Thus, the aim of this work was to evaluate the effects of genotype and harvest date on the yield and quality of narrow-leafed lupin (Lupinus angustifolius) in the agricultural region of Zapopan Jalisco, Mexico.

MATERIALS AND METHODS

The experiment was conducted from November 2012 to February 2013 at the Agricultural Experimental Farm located in the Centro Universitario de Ciencias Biólogicas y Agropecuarias (Universidad de Guadalajara) in Zapopan, Jalisco, Mexico. This site is located at latitude 20°43' N, longitude 103°23' W and altitude 1560 m above mean sea level. The soil type is classified as Regosol, which is characterized by high sand content and acid pH (5.0).

The cultivars evaluated were Haags Blaue, Boregine, Borlu, Probor, Sonate, and Boruta, all obtained from a private company (Saatzucht Steinach GmbH) in Bornhofen Germany. Cultivars were arranged and sown in a 2 × 6 factorial layout in a completely randomized design with four replicates. Plots were 3.20 (w) \times 8 m (l) (4 rows/ plot). Spacing was 10 cm between plants and 80 cm between rows. Sowing date was November 14, 2012. Sowing was done by hand into a well-prepared seed bed, and no fertilizer was applied. The target plant density was 12-13 plants/m². Irrigation by subsurface dripping was applied every 15 days during the trial, starting 2 days after the sowing and ending when 80% of the pods in the main stem were ripped. Weed control was done by hand just before the flowering stages. Plots were inspected regularly, but disease control was not necessary. Each plot was divided in half crosswise to give an effective sub-plot of 3.2 × 4 m. At 70 days after sowing one half of each plot was used for the first harvest (early harvest date) when plants were at around 50% flowering stage (flowers on the main stem inflorescence), and at 89 days later the other half for the second harvest period (late harvest date), when plants were at around 50% pod formation stage (pod growth on the main stem inflorescence).

In each sub-plot, all plants located in a linear meter were harvested by hand approximately 3–5 cm above ground level, and data on fresh matter (FM) were recorded for each plot. The harvested biomass was dried at 70°C in a forced draught oven for 48 h and weighed to determine DM. In addition, forage samples of each cultivar were ground using a Wiley mill to a particle size of 1 mm for subsequent analysis. All samples were analyzed for CP, nitrogen (N) concentration was measured by the Kjeldahl method, and

protein content was calculated by multiplying N by the factor 6.25 (A.O.A.C.,1990). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) content were determined using an ANKOM fiber analyzer (Van Soest, 1991). All chemical analyses were carried out in triplicate.

Total alkaloid and mineral content: Alkaloids were extracted as described in Wink et al. (1995). Each sample (0.5 g) was homogenized separately in 20 mL 1M HCl. The homogenate was adjusted to pH 12 with 3 M aqueous NH₂OH. Alkaloids were extracted by solid-phase extraction using Extrelut- columns® and dichloromethane as eluent. The alkaloid concentration was measured using gas chromatography, and the total amount was calculated as the sum of the peak areas of main alkaloids, identified as angustifoline, isolupanine, lupanine, and 13-hydroxilupanine, employing pure standards. For determination of mineral content (K, Ca, Mg, Mn, Fe, Zn), about 0.5 g dried and ground materials was placed into a burning cup and 15 ml of pure HNO, added. The sample was incinerated in a MARS 5 microwave oven at 200°C and the solution diluted to the desired volume with water (Özcan, 2006). Concentrations were determined using inductively coupled plasma atomic emission spectrometry (Skujins, 1998).

Statistical analysis: Data for DM, FM, CP, ADF, NDF, and minerals and total alkaloids were subjected to two-way ANOVA using SPSS 11.00 (2002) statistical package program. Significance among individual means was identified using Tukey's test, and mean differences were considered significant at P<0.05.

RESULTS AND DISCUSSION

Forage yield: Different harvest dates were a significant source of variation (P<0.05) for FM and DM yield, CP, ADF and NDF. Genotypes also contributed significantly to the variation for these traits. In general FM and DM yield increased with the progression of harvest time, similar observations had earlier made by Kaplan *et al.*, (2014) who determine the effects of harvest time on hay yield and quality of different bitter vetch (*Vicia ervilia* L.) lines. The FM yields obtained from the first harvest (early harvest date) varied from 9.1 to 12.2 t ha⁻¹, with the Boregine cultivar exhibiting the highest FM yield whereas the Boruta cultivar exhibited the lowest (Table 1). At the late harvest date, FM yields

Table 1. Average forage yields of six (Lupinus angustifolius) cultivars at two harvest dates

	Fresh matter t ha ⁻¹			Dry matter t ha ⁻¹			
	Early	Late	Average	Early	Late	Average	
Haags Blaue	9. 2	29.4bc	15.7	1.2	3.6c	2.2	
Boregine	12.2	35.3ab	16.5	1.6	6.2ab	2.4	
Borlu,	12.0	23.4c	18.5	1.7	4.9abc	2.9	
Probor	10.8	37.4a	20.3	1.5	6.8a	3.2	
Sonate	11.0	26.2c	24.5	1.5	4.3bc	3.8	
Boruta	9.1	23.4c	24.7	1.3	3.4c	4.2	
Average	10.6	29.18		1.4	4.8		

Means followed by the same letter within each column are not significant different at P<0.05.

Table 2. Crude protein, acid detergent and neutral detergent fiber of the six *Lupinus angustifolius* varieties samples at two harvest dates (whole forage)

	Crude Protein (g/kg ⁻¹)		Acid Detergent Fiber (g/kg ⁻¹)		Neutral Detergent Fiber (g/kg ⁻¹)	
	Early	Late	Early	Late	Early	Late
Haags Blaue	216.5a	166.8bc	341.2b	476.2ab	445.3a	602.1b
Boregine	201.4ab	150.4cd	336.1b	454.3bc	420.2b	648.2a
Borlu	218.9a	162.3ab	392.3a	488.3a	441.6a	573.4c
Probor	226.2a	187.9a	348.4b	477.5ab	419.4b	640.7a
Sonate,	215.3ab	149.5d	291.2c	417.2d	447.3a	626.2ab
Boruta	198.1b	164.7ab	387.5a	442.7cd	425.2b	549.1c
Average	212.73	163.5	349.5	459.3	433.1	606.6

Means followed by the same letter within each column are not significant different at P<0.05

increased and varied from 23.4 t ha⁻¹ in Boruta to 35.3 t ha⁻¹ in Boregine. On the other hand, DM yields in plants harvested early varied from 1.2 to 1.7 t ha⁻¹ whereas those obtained at the late harvest date varied from 3.4 to 6.8 t ha⁻¹ in Boruta and Boregine cultivars, respectively. As expected, after the first harvest, FM and DM yields were increased in all cultivars as age advanced. Higher yields from late harvest of FM and DM compared toearly harvest may be related to better root development and/or an increased number of potential sites from which growth can take place, such as axillary buds, which increase the number of side branches and subsequent production of leaves, flowers, and fruits (Adejumo, 1992).

The average FM and DM yields of all six lupin cultivars obtained at the late harvest (29.1 and 4.8 t ha⁻¹, respectively) are comparable with yields reported by Aniszewski (1988) in Finland, who found, at 2 years of evaluation, FM values of 27.4 t ha⁻¹ and DM values of 3.9 t ha⁻¹ as the average of three *L. angustifolius* cultivars (Mirela, Kazan, and Remik).

Yeheyis *et al.* (2012a) reported that the average DM yield of Boregine, Borlu, Boruta, Haags Blaue, and Probor cultivars evaluated in four locations from Ethiopia and harvested when plants were at around 50% flowering was 1.2 t ha⁻¹, relatively lower than the average DM yield registered with the same cultivars tested in this assay at the early harvest date (1.4 t ha⁻¹). In Ethiopia, the highest DM yield was registered in the Boregine cultivar at 1.6 t ha⁻¹ (average of four locations); in the present study, Boregine also registered the highest DM yield (1.7 t ha⁻¹). The Haags Blaue cultivar registered the lowest DM yield in both agro-

ecological zones (1.0 t ha⁻¹ in Ethiopia vs 1.2 in Zapopan, Mexico). At the late harvest date (pod-growing stage), FM and DM yields were increased, as expected.

Protein and fiber fraction analysis: CP, ADF and NDF data for the six cultivars at two harvest dates are presented in Table 2. Protein content varied significantly (P<0.05) among cultivars and ranged from 198 to 226 g/kg-1 in the first cut and from 149 to 187 g/kg⁻¹ in the second cut (Table 3). The highest CP content for both harvest dats was produced by the Probor cultivar, with 226 and 187 g/kg⁻¹at the early and late harvest dates, respectively. The forage mean CP content in the present study was lower than those identified by Yeheyis et al. (2012b) for Boregine, Borlu, Boruta, Haags Blaue, and Probor cultivars (262 g/kg⁻¹) harvested at flowering stage in the four Ethiopian locations. However, these were comparable to the values reported by Fraser et al. (2005) for Bordako and Borweta cultivars harvested at growth stage 4.5 (green pod). In comparison with other lupin species, in our study, L. angustifolius cultivars had a mean CP content (average at two harvest dates) comparable to those of Bhardwajet et al. (2010), who evaluated 20 lines of white lupin (L. albus) in Virginia. On the other hand, the PC results in the current work were considerably greater than those obtained by Kökten et al. (2014) in different soybean varieties to be used as forage in Turkey, which ranged between 108 and 132 g/kg⁻¹. Protein content from plants harvested at the early date in the current work was significantly greater than at the late harvest date at 212.7 and 163.5 g/kg⁻¹, respectively (averages over all cultivars). On average, the protein content at the late harvest date was 20% less than at the early harvest date. The most pronounced

Table 3. Content of alkaloids and minerals in six forage *Lupinus angustifolius* varieties (average of two harvest date in Zapopan Jalisco, Mexico).

	<u>%</u>	g kg ⁻¹ DM			mg kg ⁻¹		
	Alkaloids	K	Ca	Mg	Mn	Fe	Zn
Haags Blaue	0.00127	18.94	11.50	1.98	59.73	75.73	38.37
Boregine	0.00315	19.09	11.16	1.56	49.13	77.76	40.39
Borlu,	0.00241	17.16	14.29	2. 32	55.09	79.56	32.54
Probor	0.00468	17.63	13.59	1.59	47.09	74.07	37.25
Sonate	0.00240	16.00	12.51	1.75	46.98	68.99	35.97
Boruta	0.00221	18.54	12.79	2.14	52.67	72.87	43.09

harvest date effect was observed in the Sonate cultivar, in which late harvest date reduced CP content by more than 30%. This reduction could have been associated with increasing forage maturity (Kaplan *et al.*, 2014).

These results are in agreement with those reported for *L. albus* and *L. mutabilis*, forage peas (*Pisumsativum*), forage rape cultivars (*Brassicanapus*), and other forage legumes during the spring growth (Romero *et al.*, 1993; Fraser *et al.*, 2001; Kirchhof *et al.*, 2010; Sincik *et al.*, 2007). Those authors attributed the decrease in CP to an increasing DM accumulation rate and N dilution during this growth period. On the other hand, higher CP associated with lower DM and vice versa in early and late harvest respectively. So it is a case of N dilution.

In this study, cultivar and harvest date significantly affected ADF and NDF concentrations. ADF and NDF contents differed among the varieties, which varied from one harvest date to another. At early harvest, ADF values between varieties varied significantly (P<0.05) from 291.2 in Sonate to 392.3 g/kg-1 in Borlu; at the late harvest date, ADF values increased, ranging from 417.2 in Sonate to 488.3 g/kg⁻¹ in Borlu. According to Van Soest (1991), low content of non-digestible cell wall (estimated as ADF) and high protein content suggest good forage quality. In our study, ADF content was greater than that reported by Bhardwaj et al.(2010) for the 20 lines of white lupin (Lupinus albus) harvested at flower initiation (221 to 252 g/kg⁻¹). They are similar, however, to values reported by Köktenet et al. (2014), who determined the nutritive value of 12 different forages of soybean varieties harvested at the full seed stage.

At the early harvest date, the NDF content of the varieties varied from 419.4 in Probor to 447.3 g/kg-1 in Sonate. With the later maturity (late harvest date), though, NDF values for each variety increased as expected, ranging from 54.19 for Boruta to 648.2 g/kg⁻¹ for Boregine. On average, the NDF content was higher than that reported for narrow-leaved lupin (L. angustifolius), Zeus variety, at two harvesting dates (Cut 1 at the flat pod stage, Cut 2 at the stage of green ripe seeds; Faliwoska, 2014). However, ADF content for all six varieties in the current study was lower than that reported by Fraser (2005) for two varieties (Arthur and Nelly) of Lupinus albus harvested at 12.5, 14.5. 16.5, and 18.5 weeks after sowing. The ADF and NDF concentrations at both early and late harvest dates (average of six cultivars) varied from 349.5 to 459.3 g/kg⁻¹ and from 433.1to 606.6 g/kg⁻¹, respectively, indicating an increase with plant maturity. This trend, but with lower values for ADF and NDF, was also observed in different legumes (Matizha et al., 2001). Other studies on the forage nutritive value of different legumes additionally have reported increasing ADF and NDF with maturity stage at harvest (Adejomu, 1992; González-Andrés and Ortiz, 1996; Frasser et al., 2001; Matizha et al., 2001; Fulkerson et al., 2007). According to Matizha et al. (2001), phenological development influences the nutritive value because it is associated with changes in proportions and composition of plant fractions. On the other hand, Norton and Poppi (1995) and Evitayani et al. (2004) suggested that differences in nutritive value between plant species are largely attributable to differences in their anatomy, biochemistry, and morphology. However, the differences observed in this study in both quantity and quality of the forage among varieties at each cutting could be attributed to the plant stem/leaf ratio (Matizhaet et al., 2001).

Total alkaloid and mineral content: Total alkaloid and mineral content did not differ significantly by growth stage or varieties (P>0.05). Data on total alkaloid and mineral content of the six cultivars at two harvest dates are presented in Table 3. The alkaloid content in all six varieties did not differ statistically, but values ranged from 0.0022% in Boruta to 0.0046% in Probor, which is considered a very low alkaloid level. According to Maknickienë and Asakavièiûtë (2008), low alkaloid levels in fodder lupines vary within 0.025-0.99%. Differences in the alkaloid content in seeds of L. angustifolius varieties are well known (Jansen et al., 2012; Jansen et al., 2015); however, information is limited about the variations in alkaloid content in Lupinus angustifolius varieties used as forage. In Vilniai and N1702 L. angustifolius varieties harvested at four phenological growth stages, Maknickienë and Asakavièiûtë (2008) reported a variation in alkaloid levels from 0.043 to 0.095% and from 0.099 to 0.108%, respectively. These differences in alkaloid content were greater than those found in this study at two harvest dates.

Different leguminous forage species are a significant source of minerals for ruminants, but compared to grasses, they have been less studied. Although there are no studies conducted to evaluate the mineral composition in lupin forage species, the mean content of minerals such as K, Ca, Mg, Mn, Fe, and Zn in the forage of six lupin varieties tested in the present study (Table 3) was similar to values reported by Pirhofer-Walzl et al. (2011) and Tiemann et al. (2009), who analyzed the mineral concentrations of different leguminous forages and also legumes with forage potential in Denmark and Colombia, respectively. The few and small differences between Lupinus angustifolius varieties in terms of mineral concentrations found in this study agree with the findings by Forbes and Gelman (1981) and Lindström et al. (2013), who studied the perennial legumes white clover (Trifolium repens), Birdsfoot trefoil (Lotus corniculatus), and red clover (Trifolium pratense), and reported that the differences in minerals were generally few and negligible among varieties within species. In this study, the harvesting time did not affect mineral concentrations, a finding similar to that reported by Pirhofer-Walzl (2011), who analyzed the mineral concentration in other forage species and reported that from the first to the third cuts, the concentration of macrominerals increased in herbs and grasses but not in legumes.

CONCLUSION

Under the experimental conditions of this study, the results indicate that the yields and chemical composition of

Lupinus angustifolius forage were less influenced by the varieties than by the maturation stage at harvest. On average, the varieties harvested at the late date had higher forage yields but lower protein content and higher ADF content. Alkaloid and mineral content, however, were not influenced by harvest date or variety in this study. Finally, our results indicate that Lupinus angustifolius could be cultivated as a potential source of forage in Jalisco, Mexico, during the cool season.

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