Ciencia e Investigación AGRARIA

Cien. Inv. Agr. 41(1):5-12. 2014
www.rcia.uc.cl

ANIMAL PRODUCTION

DOI: 10.4067/S0718-16202014000100001

RESEARCH PAPER

Ruminal degradation of aerial biomass and seeds of wild species of *Lupinus*

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Abstract

M. Pablo-Pérez, L.C. Lagunes-Espinoza, J. Ramos-Juárez, J. López-Upton, E.M. Aranda-Ibáñez, and L. Vargas-Villamil. 2014. Ruminal degradation of aerial biomass and seeds of wild species of *Lupinus*. Cienc. Inv. Agr. 41(1): 5-12. Ruminal degradation of dry matter (DMD) and crude protein (CPD) for aerial biomass at the flowering stage and for the seeds of *L. campestris, L. exaltatus, L. hintonii* and *L. montanus* was determined using the nylon bag technique with two fistulated steers in five incubation times (3, 9, 12, 24 and 48 h), including a sample of soybean paste (SP). The data obtained were analyzed using PROC MIXED of SAS for repeated measures. The results showed interactions between species and incubation time for DMD and CPD. Among species of lupine, a high DMD and CPD of seeds were observed after three hours of incubation. At 48 h, DMD and CPD did not show significant variation between species: DMD ranged from 94.3% to 96.7% and CPD from 98.9 to 99.2%. The degradation rate of the insoluble fraction of the DM (kd) in lupine seeds ranged from 6.3 to 8.1% h^{-1}, while that of the soluble fraction (SF) ranged from 42.3 to 57.3%, lower value that observed in SP. The DMD and CPD of aerial biomass showed statistically significant differences between species at 48 h of incubation. *L. hintonii* showed the lowest DMD (69.6%) and CPD (88.9%). *L. montanus* and *L. campestris* showed a lower SF and kd (kd of 5.4% h^{-1} in both, and 35.4 and 37.2 for SF, respectively) and high CPD of aerial biomass. It is concluded that the DM and CP of seeds and biomass of wild lupine were highly degradable as of 3 h of incubation. At 48 h, there were statistically significant differences between DMD and CPD of aboveground biomass (AGB). *L. campestris* and *L. exaltatus* showed the highest DMD and CPD of AGB and similar DMD and CPD of seeds.

Key words: Dry matter, protein, ruminal incubation, legume, *Lupinus*.

Introduction

In tropical regions grasses are the most abundant and economical source of nutrition in livestock systems (Bouwman *et al.,* 2005). The inclusion of leguminous forages in these systems can be an economical and profitable strategy to supplement the low nutritive value of the native and cultivated grasses of these regions and contribute to the conservation of natural resources and sustainability of the system by supplying organic matter (OM)

Received October 30, 2013. Accepted March 8, 2014.
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and nitrogen fixed in the soil (Gierus *et al*., 2012). Animal diets can be enriched by supplementing grains or legume silages with high concentrations of protein (Heinritz *et al*., 2012).

Legumes are used in animal feed because of their high concentration and quality of protein, expressed as nitrogen concentration and protein digestibility, respectively. Protein from legumes generally degrades rapidly into ammonium and amino acids in the rumen. This degradation contributes to the growth of ruminal microorganisms, but they can be lost as ammonium when energy in the diet is not sufficient. Excessive degradation of protein in the rumen caused by the consumption of leguminous forage from temperate climates decreases the nutrient efficiency of the ruminant (Valderrama and Anrique, 2011). Feed is most beneficial for the animal when a large proportion of proteins reach the small intestine where they will be degraded into amino acids and absorbed into the blood stream (Kononoff *et al.,* 2007).

The concentration of protein in the seeds of the genus *Lupinus* oscillates between 30 and 45% (Jezierny *et al*., 2010), and *Lupinus* species have often been compared with soybean for their protein value (Froidmont and Barthiaux-Hill, 2004). Soybean seed contains 40% protein and 17% lipids, making it a major source of protein and energy (Yin *et al.,* 2011), and it is widely used for animal feed. Soybean seed protein is highly degradable in the rumen, so different methods of extrusion have been assessed to reduce this degradation rate (Valderrama and Manrique, 2011). Like soybean seeds, sweet *Lupinus* seeds from the Mediterranean region are highly degradable in the rumen (91.6% at 12 h), reducing the availability of amino acids in the intestine (Solanas *et al*., 2005) and their use as nutrients by the animal (Barchiessi-Ferrari *et al*., 2011; Zhang *et al*., 2012). No information about protein and dry matter degradation is available for *Lupinus* seeds collected in Mexico. As for the foliage, *in situ* dry matter digestibility of *L. exaltatus* has been observed to be similar to that of other forage legumes, such as alfalfa (Ruiz-López *et al*., 2006).

Several species of the genus *Lupinus* are distributed throughout Mexico, and nutritional studies conducted on some of them reveal a high protein value that is advantageous for human or animal nutrition (Rodríguez-Ambriz *et al*., 2005; Ruíz-López *et al*., 2006). Currently, the worldwide *per capita* intake of protein has increased. In Mexico, the 8.3 g person^{-1} d^{-1} in 1970 is today 20.3 g person^{-1} d^{-1} (SIAPa, 2013). This protein comes mainly from animal sources, whose production, however, requires plant protein, for which soybean is currently the principal source. In Mexico, consumption of this oil and high protein seeds is approximately 4.6 million t, but only 285,000 t (6% of that consumed) is produced domestically and 4.2 million t is imported (SIAPb, 2013). Moreover, soybeans do not adapt to all climate conditions or soil types. Because of the high demand, imports increase; consequently, the cost of feed that includes soybean also increases.

For these reasons, it is increasingly important to search for alternative sources of plant protein for use in ruminant diets. Studies on wild Mexican *Lupinus* have shown high protein content in the seeds (Ruiz-Ambriz *et al*., 2005). Nevertheless, more knowledge of the nutritive potential of these species is required for their use in animal nutrition by the communities where they grow. Regarding the feed use potential, a crucial point for domesticated or wild species is to understand the kinetics of the degradation of plant components; this would allow researchers to detect the site of digestion and estimate the amount of nutrients a given species can provide to the animal. The objective of this study was to determine the degradation rate of dry matter (DMD) and crude protein (CPD) of aerial biomass at the flowering stage and seeds of four wild *Lupinus* species from the state of Puebla, Mexico.

Materials and methods

Plant material at the flowering stage was collected in the region of the Serdán and Libres Valleys in the state of Puebla, Mexico, at an altitude of 2,486 to 3,442 m (Table 1) during the month of May 2011. Of each species, five plants growing close together were collected: *L. campestris*, *L. exaltatus, L. hintonii,* and *L. montanus*. The procedure was repeated three times at a distance of more than 5 m. Dry fruits (pods with seeds) were collected at the end of the fruiting period in July of the same year at the same sites where the plants were collected. The analysis was conducted in the Animal Science Laboratory of the Colegio de Postgraduados, Campus Tabasco, Cárdenas, Tabasco, Mexico. The seeds and foliage of lupine species were characterized by their high CP content (Table 2).

Preparation of samples and analysis. Aerial biomass and seeds were dehydrated at 65 ºC for 72 h in a forced air oven and ground in a Wiley mill through a 1 mm screen to determine crude protein (CP) (microKjedahl N×6.25, AOAC, 1995), neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al*., 1991). The material was preserved in plastic containers at 4 ºC until their use in *in situ* degradation tests.

Determination of in situ *degradation*. To determine *in situ* dry matter and crude protein (CPD) degradation, the nylon bag technique was used (Orskov *et al*., 1980) in two three-year-old *Bos taurus* × *Bos indicus* steers (550 kg live weight) with rumen cannula (silicone 100 mm i.d., KEHL® industry). The animals were housed, usually fed with *Pennisetum purpureum* grass (8.19% PC, 75.7% FDN and 43.4% FDA), and they had free access to water and a salt block. Ten days before and during incubation, the animals consumed a commercial supplement (ALBACA®, 16% crude protein and 34% FDN) at a ratio of 6 g kg^{-1} live weight dry basis. Later, 5 g of lupine was placed in nylon bags (10 × 20 cm^2, 45 µm pore size) and incubated in the rumen for 3, 9, 12 and 48 h with two replications per time and animal for a total of four observations per time. To eliminate the soluble fraction before incubation, all of the bags were washed for 5 min in warm water (38 ºC). The bags were introduced into the rumen in inverse order of incubation time, and all were withdrawn at the same time. After removal, they were washed several times for 5 min with cold water and dried for 48 h at 60 ºC to determine DM and CP (Van Soest *et al*., 1991). The soluble fraction at zero hour was estimated. A sample of commercial soybean paste was included in triplicate and subjected to the same procedure as that of *Lupinus* seeds for chemical and degradation analyses.

Rate of in situ degradation. The *in situ* degradation rate for each variable was calculated with the exponential model (Orskov and McDonald, 1979): , where "a" is the soluble fraction, "b" is the insoluble potentially degradable fraction, and "kd" is a constant that represents the rate of degradation of the "b" fraction per unit of time (t). Development of the model was performed with the software Stella VI (Hulbert *et al*., 2000). To obtain

Table 1. Geographic location of collection sites of wild *Lupinus* of the Valleys of Serdán and Libres, Puebla, Mexico.

Species	Municipality	Location	Altitude (masl)
L. campestris	Tlachichuca	19°03'51,2"LN 7°23'01,12"LO	2866
L. exaltatus	Chalchicomula de Sesma	19°00'21,8"LN 97°22'0,14"LO	3066
L. hintonii	Chalchicomula de Sesma	19°04'08,1"LN 7°19'11,66"LO	3424
L. montanus	Tlachichuca	19°04'42,0"LN 7°19'17,47"LO	3442

the fit and numerical solution, Berkeley Madona 8.01 software (Macey *et al*., 2000) was used.

Statistical analysis. The data on CP, NDF and ADF were analyzed with a completely random design; an analysis of variance and Tukey's test were performed. The data on degradation were processed using the PROC MIXED procedure for repeated measurements (SAS Institute. 2010. User`s Guide: Statistics, version 9.3. SAS Inst. Inc., Cary, North Caroline, USA). The model included the effect of species, incubation time, and their interaction as fixed effects. The animal was used as a random effect, and incubation time was the repeated factor. The model was $Yij = \mu + species_i + time_j + (species \times time)_k + e_{ijkl}$, where Y_{ij} is the response variable, μ is the general mean, e_{ijkl} is the random error, $time_j$ is the effect of the j^{th} treatment on the response variable (j= 0, 3, 9, 12, 24 and 48 h), and (species × incubation time) is the interaction. The means of the treatments were separated and fit to Tukey.

Results and discussion

The contents of CP, NDF and ADF in seeds and aerial biomass of the lupine species are shown in Table 2. Among lupine species, the range of protein contained in seeds was 32.5 to 43.4%. These values are high compared to those of peas and fababean (Jezierny *et al*., 2010) but similar to those of other European (Sujak *et al*., 2006) and Mexican (Ruiz-López *et al.,* 2006) lupine species. The lupine species had high protein content in the aerial biomass (22.2 to 25.5%), particularly *L. campestris* (25.5%) and *L. exaltatus* (25.3%), coinciding with the findings of Ruíz-López *et al.* (2006) in the foliage of *L. exaltatus* from Nevado de Colima, Mexico.

A highly significant (P≤0.001) interaction was observed between species and incubation time for DMD of both seeds and aerial biomass. The difference in incubation time did not have the same effect on DMD per species. In seeds, the highest rates of degradation were observed at 48 h: *L. montanus* (96.3%) and *L. hintonii* (96.7%) (Table 3). In general, higher DM degradation was observed during the first hours of incubation (55.6 to 66.5%) compared to soybean paste (22.1%). At three hours, DMD of lupine species varied from 55.6 to 66.5%, even when there was a higher fiber content (NDF and ADF) than in soybean paste (Table 2). Higher DM degradation rates (kd) were also observed in lupine species seeds than in soybean paste and a lower soluble fraction (42.4 to 57.4%) (Table 3). This higher degradation of lupine flours could be influenced by particle size because in lupine it has been reported that a fine particle size increases DM and protein ruminal degradation. It could, however, also be due to the greater degradation of sugars used in microbial metabolism than starches, because lupine seeds have higher concentrations of sugars than soybean paste (Niwińska and Andrzejewski, 2011)

Table 2. Chemical composition of wild *Lupinus* species.

Component	%	*L. campestris*	*L. exaltatus*	*L. hintonii*	*L. montanus*
Seed	DM	85.8 ab	72.8 b	90.5 a	78.5 ab
	CP	40.5 bc	38.5 bc	32.5 c	43.5 ab
	NDF	16.7 c	24.7 a	18.4 c	21.6 b
	ADF	5.0 b	4.4 b	7.9 a	4.8 b
Aerial biomass	DM	81.0 a	82.6 a	82.1 a	81.6 a
	CP	25.5 a	25.3 a	22.2 b	22.6 b
	NDF	38.2 c	43.2 b	42.9 b	44.1 a
	ADF	21.7 c	27.6 b	26.3 b	30.1 a

Different letters in a row indicate significant differences (Tukey P≤0.05). DM= Dry matter; CP= Crude Protein; NDF= Neutral Detergent Fibre; ADF= Acid Detergent Fibre.

or particle losses (Velásquez and Pichard, 2010). In contrast, the treatment applied for extracting oil from soybeans protects the paste from rapid degradation (Solanas *et al*., 2005). Lupine seed DMD at 48 h coincides with that observed by other authors in the flour of seeds from different herbaceous legumes used in feeding animals, such as *Canavalia ensiformis* L. (98.9%) and *Canavalia gladiata* L. (98.7%) (González, 2004). As for other species of *Lupinus*, *L. exaltatus* seeds from the state of Colima have shown 76.6% DMD at 72 h of incubation in rumen-fistulated sheep (Ruiz-López *et al*., 2006). This difference in degradation could be due to environmental differences because there is little or no difference in degradation of samples incubated in sheep or cattle.

In aerial biomass, DMD values were high for the first hours of incubation for all of the lupine species (Table 3). At 48 h of incubation, statistically significant differences were observed among species. *L. exaltatus* and *L. campestris* had the highest DMD (83.08 and 80.24%, respectively). This could be associated with the higher degradation rate (3.31 and 2.77%, respectively) and greater solubility of the fiber (60.4%), at least in *L. exaltatus* (Table 3). These DMD values are similar to those observed in forage legumes, such as *Clitoria ternatea* (80%), and higher than those of *Pueraria phaseoloides* (60 to 70%) and *L. exaltatus* (60.6 to 72.9%) (Ruiz-López *et al*., 2006).

As with DMD, the interaction between species and incubation time for CPD of seeds and aerial biomass was highly significant (Table 4). After the first hours of incubation, CPD of seeds was above 70% for all lupine species, and for aerial biomass, it was above 60%. Crude protein degradation continued slowly, reaching 98.9 to 99.2% at 48 h. Soybean paste had similar kinetics but a lower degradation speed. This slower degradation of soybean paste is attributable to the process of oil extraction that used a heat treatment that increases the insoluble fraction, making CP less digestible in the rumen (Solanas *et al*., 2005).

Table 3. Degradation of dry matter (DM), in aerial biomass during the flowering stage, in seed of wild lupine and in soybean paste.

	Hours of incubation (%)						%		
Species	0	3	9	12	24	48	kd	Soluble Fraction	RMSE
Seed									
L. montanus	50.7 cd	56.5 ef	65.2 h	75.7 ij	96.5 l	96.5 l	7.46	57.37	0.03
L. exaltatus	49.7 c	55.6 def	66.1 h	74.9 i	94.6 l	94.7 l	7.14	57.03	0.03
L. hintonii	62.3 gh	63.3 gh	73.0 i	84.2 k	94.5 l	96.7 l	8.16	47.67	0.02
L. campestris	59.3 fg	66.5 h	74.9 i	80.9 jk	94.1 l	94.3 l	6.37	42.37	0.02
Soybean paste	13.5 a	22.1 b	52.4	53.5 cde	81.1 k	97.0 l	6.61	95.2	0.02
Aerial biomass									
L. montanus	31.5 a	43.0 bc	57.5 h	60.4 i	66.0 k	75.8 m	1.96	54.75	0.03
L. exaltatus	43.7 c	45.9 d	47.8 e	63.4 j	77.6 n	83.0 p	3.31	60.45	0.04
L. hintonii	42.2 b	43.0 bc	46.8 de	55.4 g	75.4 k	69.6 l	1.61	57.88	0.03
L. campestris	44.2 c	49.6 f	55.0 g	65.5 k	78.5 n	80.2 o	2.77	53.04	0.04

a, b, c, d, e= Component (seed and aerial biomass) means with a common letter are not significantly different (Tukey P>0.05).
RMSE= Root mean square error.
kd= Rate of *in situ* degradation (% h^{-1}).

Leguminous CP is generally highly degradable in the rumen. Solanas *et al*. (2005) observed that *L. albus*, peas, and soybeans have a degradability of 91.6, 98.1 and 90.5%, respectively. Values similar to that of CPD of the lupine species of our study have been observed after 48 h of incubation in *C. ensiformis* and *C. gladiata* (98.8%) (González, 2004), but they are higher than the 70% observed in *L. exaltatus* seeds from another region of Mexico (Ruiz-López *et al*., 2006).

Aerial biomass of the lupine species exhibited high CPD during the first hours of incubation. *L. exaltatus* and *L. campestris* at 24 h had the highest CPD of the species assessed (94.5 and 93.7%, respectively). *L. exaltatus* also had the highest rate of degradation (6.5%) and solubility (41.5%), while *L. hintonii* had the lowest CPD of the species assessed (Table 4). The CPD values observed for the aerial biomass of *L. exaltatus* at flowering are higher than those for foliage (67.3%) of *L. exaltatus* from the state of Colima (Ruiz-López *et al*., 2006). Thus, the flours made from the seeds and aerial biomass of *L. campestris, L. exaltatus, L. hintonii* and *L. montanus* are easily degradable in the rumen, both DM and CP, as has been observed in *L. albus* (Solanas *et al*., 2005; Barchiessi-Ferrari and Anrique, 2011; Valderrama and Anrique, 2011). They can thus be considered a source of degradable protein for the microorganisms in the rumen and, therefore, for microbial protein synthesis, affecting positively animal productivity.

The results for DMD and CPD of seeds and aerial biomass of the wild lupine species under study showed differences among species for DMD and CPD only of aerial biomass, *L. campestris* and *L. exaltatus* being those of higher degradation. DMD and CPD of seeds are similar to those observed for other lupine and forage legume species (González, 2004; Solanas *et al*., 2005; Barchiessi-Ferrari and Anrique, 2011).

Table 4. Degradation of crude protein (CP) in aerial biomass during the flowering stage, in wild lupine seeds, and in soybean paste.

	Hours of incubation (%)						%		
Species	0	3	9	12	24	48	kd	Soluble fraction	RMSE
Seed									
L. montanus	50.7 cd	76.1 h	85.7 j	90.8 l	98.9 q	99.1 q	10.56	33.40	0.009
L. exaltatus	49.7 c	77.3 h	88.9 k	92.1 l	98.4 pq	99.2 q	11.8	32.24	0.003
L. hintonii	62.3 g	82.0 i	85.4 j	95.5 n	97.5 op	99.2 q	9.38	24.71	0.02
L. campestris	59.3 f	82.6 k	84.7 j	93.8 m	97.4 op	98.9 q	7.99	23.25	0.02
Soybean paste	14.3 a	22.1 b	51.3 d	53.0 e	84.5 j	97.0 o	6.95	96.35	0.02
Aerial biomass									
L. montanus	31.5 a	65.4 e	79.3 j	83.9 l	88.3 n	92.9 o	5.41	37.2	0.03
L. exaltatus	43.7 c	66.6 f	73.5 h	82.6 k	94.5 p	94.3 p	6.49	41.5	0.02
L. hintonii	42.2 b	60.8 d	71.3 g	77.4 i	92.9 o	88.9 o	5.73	46	0.04
L. campestris	44.2 c	71.9 g	79.4 j	85.0 m	93.7 p	94.3 p	5.44	35.4	0.04

a, b, c, d, e= Component (seed and aerial biomass) means with a common letter are not significantly different (Tukey P>0.05).
RMSE= Root mean square error.
kd= Rate of in situ degradation (% h-1).

Acknowledgements

The authors thank the Consejo de Ciencia y Tecnología (CONACYT), the Línea Prioritaria de Investigación 6 Conservación y Mejoramiento de Recursos Genéticos of the Colegio de Postgraduados (CP), Mexico, for funding this study and Dr. J.G. Herrera H. for his statistical advice.

Resumen

M. Pablo-Pérez, L. C. Lagunes-Espinoza, J. Ramos-Juárez, J. López-Upton, E. M. Aranda-Ibáñez y L. Vargas-Villamil. 2014. Degradación ruminal de semillas y biomasa aérea de especies silvestres de *Lupinus*. Cienc. Inv. Agr. 41(1): 5-12. La degradación ruminal de la materia seca (DMS) y de la proteína cruda (DPC) en biomasa aérea durante la etapa de floración, y en las semillas de *L. campestris*, *L. exaltatus*, *L. hintonii* y *L. montanus* fue determinada mediante la técnica de la bolsa de nylon, con dos bovinos machos fistulados, en cinco tiempos de incubación (3, 9, 12, 24 y 48 h), incluyendo una muestra de pasta de soya (PS). Los datos obtenidos se analizaron usando PROC MIXED de SAS para medidas repetidas. Los resultados mostraron interacción entre especie y tiempo de incubación para DMS y DPC de la biomasa aérea y semillas. Entre especies de lupino, alta DMS y DPC de las semillas se observó desde las 3 h de incubación. A las 48 h la DMS y DPC no mostró variación significativa entre especies. DMS fluctuó de 94,3 a 96,7% y la DPC de 98,9 a 99,2%. La tasa de degradación de la fracción insoluble de la MS (kd) en semillas de lupino varió de 6,3 a 8,1% h^{-1}; y la fracción soluble (FS) de 42,3 a 57,3%; valor inferior al de PS. La DMS y DPC de la biomasa aérea mostró diferencias estadísticas significativas entre especies a las 48 h de incubación. *L. hintonii* con la menor DMS (69,6%) y DPC (88,9%). Para la DPC, *L. campestris* y *L. montanus* mostraron las menores kd y FS en biomasa aérea (kd de 5,4% h^{-1} en ambas y 35,4 y 37,2 para FS, respectivamente) y una alta DPC. Se concluye que las semillas y la biomasa aérea de las especies silvestres de lupino presentan alta degradabilidad de la MS y PC desde las 3 h de incubación, mostrando solo diferencias estadísticas significativas entre ellas a las 48 h para la DMS y DPC de la biomasa aérea (BA). *L. campestris* y *L. exaltatus* fueron las especies que mostraron la más alta DMS y DPC de la BA y la más baja de las semillas.

Palabras clave: Incubación ruminal, leguminosa, *Lupinus,* materia seca, proteína.

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