In Sacco Evaluation of Rumen Protein Degradation Characteristics and *In vitro* Enzyme Digestibility of Dry Roasted Whole Lupin Seeds (*Lupinus albus*)

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ABSTRACT : The effects of dry roasting whole lupin seeds (*lupinus albus*, WLS) at 110, 130 or 150°C for 15, 30 or 45 minutes on the *in sacco* rumen degradation characteristics, optimal heating conditions of time and temperature and *in vitro* enzyme digestibility were determined. Ruminant degradation characteristics (RDC) of crude protein (CP) of WLS were determined by *in sacco* technique in dairy cows. Measure RDC were soluble (S), undegradable (U), potentially degradable (D) fractions, lag time (T0) and rate of degradation (Kd) of insoluble but degradable fraction. Based on measured RDC, percentage bypass CP (%BCP) and bypass CP (BCP in g/kg, DM) were calculated. Degradability of CP was significantly reduced by dry roasting ($p<0.001$). The interaction of dry roasting temperature and time had significant effects on D ($p<0.05$), Kd ($p<0.01$), U ($p<0.01$), %BCP ($p<0.001$) and BCP ($p<0.001$) but not on S ($p=0.923>0.05$). With increasing time and temperature, S, D, Kd and U varied from 31.8%, 67.4%, 10.3%/h and 0.8% in the raw WLS (RWLS) to 27.1%, 35.8%, 3.6%/h, 38.4% in 150°C/45 min, respectively. All these effects resulted in increasing %BCP from 25.9 in RWLS to 61.0% in the 150°C/45 min. Therefore BCP increased form 111.2 to 261.2 g/kg DM, respectively. Both %BCP and BCP at 150°C/45 min increased nearly 2.5 times over the RWLS. The effects of dry roasting on %BCP and BCP seemed to be linear up to the highest value tested. Although RDC had been altered by dry roasting, the *In vitro* perpsin-cellulase digestibility was generally unchanged. It was concluded that dry roasting was effective in shifting CP degradation from rumen to the lower gastrointestinal tract to potential reduce unnecessary N loss in the rumen. It might be of great value in successfully synchronizing the rhythms of release of nitrogen and energy in the rumen, thus achieving a more efficient fermentation of diets with high proportions of lignocellulosic resources. To determine the optimal dry roasting conditions, the digestibility of each treatment in the cows will be measured in the next trial using mobile bags technique. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 3 : 358-365*)

Key Words : *Lupinus Albus*, Dry Roasting, Rumen Degradation Characteristics, Bypass Crude Protein, Enzyme Digestibility, Cows

INTRODUCTION

Whole lupin seeds (*Lupinus albus*, WLS) have attracted attention in recent year and appear to be the protein source best suited to the ecological and climatic conditions of many countries. High protein and lipid contents in WLS indicate a potential use as a protein supplement in ruminal diets.

However, the presence of antinutritional factors (ANFs) in lupin (Alkaloids) and the rapid rumen degradation characteristics make them unsuitable to be included in the unprocessed form in ruminant diets (Goelema, personal communication). Within species of lupin there are sweet and bitter varieties. The bitter contain from 10 to 20 g/kg of toxic alkaloids and should not be offered to animals: even sweet varieties may contain low level of alkaloids. Goelema (personal communication) reported that *lupinus albus* had alkaloid of 4.3 g/kg. For safety the alkaloid content must be less than 0.6 g/kg (McDonald et al., 1988). Van Straalen et al. (1990) reported that Kd of lupin was 12.87%/h and it was highest among beans, horse beans, peas, soy beans (raw) and soy beans (toasted) with Kd of 8.24, 10.28, 8.95, 10.24 and 6.76 %/h. Both Valentine et al. (1988) and Cros et al. (1991) found the Kd of protein of WLS was even higher (48 and 22.1%/h). Also poor performances of lactating dairy cows fed lupin seeds have been reported (Guilaume et al., 1987), these could be partly due in a reduced supply of true protein to the small intestine.

Attempts (Cros et al., 1992; Kibelolaud et al., 1993) have been made to reduce rumen breakdown of protein, reduce ruminal production of NH_3 and the cost of urea synthesis (Cros et al., 1992) and increase the quantity of protein reaching the small intestine of ruminants by heat treatment. The intensity of effect is a function of time of exposure and temperature reached (Stern et al., 1985; Pena et al., 1986; Annexstad et al., 1987; Waltz and stern, 1989). Heat treatment also may destroy a number of ANFs in some legume seeds (Rackis et al., 1986; Van der poel, 1990)

Several articles (Annexstad et al., 1987, Arieli et al., 1989, Cros et al., 1992, Antoniewicz, 1993, Benchaar et al., 1994) have demontrated that heat treatment does increase the BCP but little information has been reported concerning the benefit of heating this proteaginous seed to reduce protein breakdown in the rumen and to increase small intestine availability of rumen undegraded dietary nitrogen. Also the optimal heating conditions have not been found for each legume seed. Heating above the optimal temperature may overprotect the protein so that the protien is neither fermented in the rumen nor digested in the samll intestine (Stern et al., 1985). Also Prolonged treatment with high temperature could lead to reactions (Maillard reaction) between carbonhydrates and amino acids, which severely reduce the availability of amino acids for the animal.

In vitro enzyme digesibility is a fast and not expensive method to screen wheather the heating damage

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Received June 2, 1998; Accepted September 18, 1998

the total digesibility.

The objectives of our study was to determine the effects of dry roasting on rumen protein degradation characteristics of WLS in dairy cows to evluate raw and dry roasted WLS as source of ruminally bypass crude protein (BCP) and intestinal available protein in dairy cows, to optimize rumen fermentation in shifting protein digestion from rumen to intestines and therefore reducing unnecessary nitrogen losses from rumen and to determine the optimal dry roasting conditions for dairy feed industry.

MATERIALS AND METHODS

Materials

WLS (*Lupinus albus*) were obtained from a commercial feed company (Peter Gibbs Stock Feeds in Australia). The chemical composition of the WLS are shown in table 4.

Treatments

RWLS were dry roasted at 3 different temperatures (110, 130, 150°C) for 15, 30 and 45 minutes in a complete block design with A and B series as shown in table 1. A, B series was used to test whether the dry roasting conditions were kept constantly and reliable.

RWLS were used as a control. For each treatment, about 1.5 kg was roasted in the lab oven (Qualtex Solidstat. universal series 2000 designed in Australia by Watson Victor LTD). The conditions of processing are shown in table 1. After roasting, the samples were allowed to cool down to ambient temperature and were ground through a 3 mm screen (Hammer mill AEG TYP AM80N*2).

In vitro enzyme digestibility

About 0.5 g of sample material weighed to an accuracy of ± 0.1 mg, was incubated in a 100 ml plastic tubes with 25 ml 50°C pepsin (1:10,000, AR, Cat No: 102598, ICN Biochemicals, USA) / HCl solution (HCl, 0.125 M; pepsin 4.8 g liter $^{-1}$) in the incubator at 50°C for 48 hours, then add 50 ml 50°C acetate/cellulase (AR, Maruzen Chemicals Co., LTD, Japan) buffer solution (acetate buffer, 2.9 ml glacial acetic, 6.8 g sodium acetate ($CH_3COONa: 3H_2O$) liter^{-1}, pH: 4.6; cellulase 6 g liter^{-1}), adjusted pH 4.5 for continue incubation at 50°C for further 48 hours and then filter residue using a vacuum filtering system. DM, Ash of residue was determined. (Mecleod et al., 1978)

Animal and animal diet

Six dry Holstein Friesian cows (table 2), of average weight 580 Kg were previously equipped with a rumen cannula with an internal diameter of 10 cm (Silicon rubbers, handmade, Ellinbank Dairy Center, Victoria, Australia) for measuring rumen degradability and were kept at Ellinbank Dairy Center in the grassland.

All cows received a diet consisting of crushed barley and oaten hay according to the requirements of dairy cows, chemical analysis of which are shown in table 3. The barley and oaten hay purchased locally from Northern District (Victoria, Australia). Water was always available. The cows were fed twice daily at 08:30 and 16:30. A one week period of adaptation was allowed.

Table 1. Treatments A (1 control+9 treatments) and B (1 control+9 treatments)) and the dry roasting condition of WLS

Treatments	Dry roasting Temp. (°C)	SD
Raw whole lupin seeds A series (RWLS A)		
110°C/15 min A	110.0	1.0
110°C/30 min A	109.5	0.5
110°C/45 min A	109.5	0.5
130°C/15 min A	129.5	0.5
130°C/30 min A	130.0	0.0
130°C/45 min A	131.0	1.0
150°C/15 min A	150.0	0.0
150°C/30 min A	149.5	0.5
150°C/45 min A	149.5	0.5
Raw whole lupin seeds B series (RWLS B)		
110°C/15 min B	111.0	1.0
110°C/30 min B	111.0	0.0
110°C/45 min B	111.0	1.0
130°C/15 min B	130.0	0.0
130°C/30 min B	130.5	0.5
130°C/45 min B	130.5	0.5
150°C/15 min B	150.0	0.0
150°C/30 min B	150.0	0.0
150°C/45 min B	150.0	0.0

Note: Each treatment was measured at least 5 times.

Table 2. Basic data of the animal used for the rumen incubation

	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5	Cow 6
Cow No.	307	330	348	344	338	304
Breed	HFa	HFa	HFa	HFa	HFa	HFa
Weight (kg)	629	549	577	591	550	602
Birth Year	93	93	93	93	93	93
No. Lactation	2	2	2	2	3	2
Fat Yield (kg)	239	242	220	242	193	282
Protein Yield (kg)	168	162	162	183	152	223
Milk Yield (kg)	4,160	5,131	4,832	5,729	4,801	6,200
Last Insemination day	14/11/96	30/12/96	30/11/96	2/11/96	6/1/97	1/2/97
Cow milk stageb	dry	dry	dry	dry	dry	dry

Notes: a: HF= Holstein and Fristian; b: Cow stage at incubation time; The data provided by Ellinbank Dairy Center (Victoria, Australia).

Table 3. The chemical analysis[a] of crushed barley and oaten hay

Composition	Barley	Oaten hay
DM (%)	89.5	85.0
NIRSb, CP (%)	14.6	5.2
Ash %	2.6	-
NDF %	20.0	63
Total P %	0.28	-
Total K %	0.58	-
Total S %	0.13	-
Total Na %	0.04	-
Total Ca %	0.04	-
Total Mg %	0.12	-
Total B (mg/kg)	3	-
Total Fe (mg/kg)	82	-
Total Mn (mg/kg)	16	-
Total Cu (mg/kg)	5	-
Total Zn (mg/kg)	15	-
NIRS, ME (% DM)	12.6	7.8
NIRS, Digestibility	85.0	60.0

Notes: a: The date provided by Ellinbank Dairy Center (Victoria, Australia); b: NIRS= Near Infra-Red Reflectance Spectroscopy.

In Sacco protein degradability

Protein rumen degradation characteristics (RDC) of WLS in the rumen of the 6 dairy cows were determined using the *in sacco* method. Incubation of all treatments in the rumen was with 5 g DM in nylon bags (size: 10*17cm) with the pore size of approximately 44μm (Switzerland 1807710014 I 044 Nytal ASTM 325-44) as described by Tamminga et al. (1990). The rumen incubations were performed according to the 'gradual addition/all out' schedule. Incubations were carried out for 24, 12, 8, 4 and 2 hours; bags were inserted at 20:00, (next day) 08:00, 12:00, 16:00 and 18:00 and removed at 20:00 hours respectively. The 48 hour rumen incubation were carried out from 20:00 till 20:00 two days later. All treatments were randomly allocated over all cows and the whole incubation period. After incubation, the bags containing the residues were rinsed under a cold stream of tap water to remove excess ruminal contents and microbes on the surface to stop microbial activity, washed with cool water without detergent in a commercial washing machine (Fisher & Paykel, Smart Drive 500) for 55 minutes without spinning and subsequently dried at 60°C for 24 hour in the oven. The 0 hour incubation samples were only put in the washing machine under the same conditions. Samples were stored in a cool room (4°C) until analysis. The residue was ground through a 1 mm screen and analyzed for chemical composition.

Chemical analysis and calculation

Feed and rumen residues of 0, 2, 4, 8, 12, 24 and 48 hours of all 20 treatments were analyzed for DM, Ash and Nitrogen. DM was determined by drying at 105°C to constant weight. Ash was determined by ashing at 550°C to constant weight. N was analyzed by NCS instruments (NA 1500 NCS FISONS), and CP content was obtained by N multiplication by 6.25.

Analysis of RDC: Important RDC in the rumen are: the soluble (fraction (S); the fraction which will not be degraded (U) irrespective of time of incubation in the rumen; the fraction which is not soluble, but potentially degradable (D); the fractional rate of degradation (Kd) of the fraction D (Van Straalen and Tamminga, 1990). Part of DM and CP could be washed out of the bags without incubation in the rumen. This proportion (S) was considered to be degraded very rapidly and completely. The rumen undegradable proportion (U) was estimated from the degradation curve. The remaining proportion (D), degradable but insoluble, can be calculated as 100-S-U. The fractional rate of degradation of this proportion was called Kd.

Results of *in sacco* incubations were calculated using the NLIN procedure of the statistical package SAS (SAS, 1991) using iterative least squares regression (Gauess-Newton method) by the following equation: $R(t) = U + D * \exp^{-Kd*(t-T0)}$, Where: R(t) stands for residue (in %) of the amount of incubated material after t hours of rumen incubation and T0 for the lag phase (h) in which no degradation takes place. Based on the residues after rumen incubation the effective degradability in the rumen and amount of %BCP were calculated using the method of Ørskov and McDonald (1979) and the new Dutch protein evaluation system (Tamminga, 1994). Percentage of bypass crude protein (%BCP) was calculated as: % BCP = U + D * Kp / (Kp+Kd); Bypass crude protein (BCP) was calculated as: BCP = 1.11 * CP * %BCP /100, where: Passage rates (kp) of 0.06 /h was adopted based on international data (Tamminga et al., 1994); BCP and CP in g/kg, DM; The factor 1.11 in the formula was taken from the French PDI-system (Verite and Peyraud, 1989), the regression coefficient of *in vivo* on *in sacco* degradation data.

Statistical analysis

Statistical analysis were carried out using the statistical package SAS. Analysis of variance was carried out using Proc GLM (SAS). $Y_{ijk} = m + Temp_i + Time_j + Series_k + Temp*Time_{ij} + e_{ijk}$, where: Y = degraded fraction; i = 1,2,3,4; j = 1,2,3,4; K=1, 2

Comparison of mean of temperature, time and interaction effects, on degradation characteristics as well as *in vitro* DM and OM digestibility (IVDMD and IVOMD) were carried out by Tukeys studentized range Test (HSD or Tukey Test)

RESULTS

Chemical composition

The chemical composition of WLS is presented in table 4. Dry roasting increased DM content but did not change CP content on a DM basis.

In vitro enzyme digestibility

The effect of dry roasting on in vitro enzyme digestibility of WLS is presented in table 4. Dry

Table 4. Dry matter and chemical composition of raw and dry roasted WLS

Temp. (°C)		110			130			150		
Time (min.)	Raw	15	30	45	15	30	45	15	30	45
Chemical composition of whole lupin seeds										
DM (g/kg)	921.26[b]	920.95[b]	930.40[ab]	933.15[ab]	928.77[ab]	927.78[ab]	931.92[ab]	932.98[ab]	935.06[a]	933.72[a]
	(3.01)	(1.81)	(2.24)	(2.45)	(2.78)	(3.49)	(1.84)	(6.89)	(1.69)	(0.42)
OM (g/kg DM)	894.01	892.64	904.19	905.62	899.37	901.00	905.05	904.68	908.92	932.88
	(0.88)	(0.94)	(2.44)	(0.20)	(2.23)	(1.99)	(2.55)	(5.60)	(1.42)	(36.79)
CP (g/kg, DM)	386.54	387.88	380.69	377.25	379.76	384.97	380.07	392.35	384.97	386.04
	(10.74)	(7.07)	(15.64)	(5.30)	(3.01)	(12.14)	(11.58)	(9.68)	(2.12)	(2.26)
Ash (g/kg, DM)	27.2	28.3	26.2	27.5	29.4	26.8	26. 9	28.3	26.1	25.8
	(0.21)	(0.09)	(0.02)	(0.23)	(0.05)	(0.15)	(0.07)	(0.13)	(0.03)	(0.19)
OMFPa (g/kg, DM)	507.48	504.76	523.52	528.35	519.63	516.06	524.98	512.35	526.86	521.83
	(11.58)	(8.01)	(13.22)	(5.50)	(0.76)	(14.15)	(9.04)	(15.31)	(0.66)	(3.68)

Notes: The figures in the bracket are SD; a: OMFP= organic matter free crude protein (OM-CP); Means with the same letter are not significantly different, Tukeys Studentized Range (HSD) Test, Alpha = 0.05.

Table 5. Effect of dry toasting on in vitro enzyme digestibility of WLS

Temp. (°C)		110			130			150		
Time (min.)	Raw	15	30	45	15	30	45	15	30	45
DM (g/kg)	921.26[a]	920.95[a]	930.40[ab]	933.15[ab]	928.77[ab]	927.78[ab]	931.92[ab]	932.98[ab]	935.06[a]	933.72[a]
OM (g/kg DM)	894.01[a]	892.64[a]	904.19[a]	905.62[a]	899.37[a]	901.00[a]	905.05[a]	904.68[a]	908.92[a]	932.88[a]
In vitro enzyme digestibility (%)										
IVDMD	88.64	89.11	88.65	89.77	88.70	90.96	90.35	89.83	90.49	90.47
sd	(1.91)	(1.81)	(1.59)	(1.40)	(1.98)	(1.80)	(1.58)	(0.74)	(2.26)	(1.25)
IVOMD	98.34	98.36	98.36	98.74	98.47	98.58	98.59	98.35	98.55	98.54
sd	(0.34)	(0.54)	(0.70)	(0.53)	(0.54)	(0.51)	(0.67)	(0.67)	(0.58)	(0.57)

Notes: IVDMD, IVOMD: In vitro DM, OM enzyme digestibility; Treatment temperatures had no significant effects on in vitro digestibility of DM (P=0.352) and OM (P=0.899); Treatment times had no significant effects on in vitro digestibility of DM (P=0.351) and OM (P=0.934) in whole lupin seeds. Tukeys studentized Range (HSD) Test, Alpha=0.05; means with the same letter in same row are not significantly different.

Table 6. Effect of dry roasting on rumen degradation characteristics (mean and asymptotic standard error) and bypass (BCP) of CP in treatment A and B group of whole lupin seeds

Temp. (°C)		110			130			150		
Time (min.)	Raw	15	30	45	15	30	45	15	30	45
CP (g/kg, DM)	386.54[a]	387.88[a]	380.69[a]	377.25[a]	379.76[a]	384.97[a]	380.07[a]	392.35[a]	384.97[a]	386.04[a]
CP Rumen Degradation Characteristics (RDCa) of treatment A group (control A +9 treatments)										
Sa (%)	30.97	32.00	34.65	29.85	27.60	29.83	29.77	28.49	31.37	23.86
Da (%)	69.03	67.92	65.35	70.15	72.40	67.37	59.96	62.86	42.05	40.74
Kda (%/h)	9.12	9.55	8.64	10.99	9.11	6.74	10.05	8.15	3.92	3.46
	(1.30)	(0.86)	(0.88)	(0.68)	(1.00)	(1.07)	(0.95)	(0.92)	(0.90)	(0.89)
Ua (%)	0.00	0.09	0.00	0.00	0.00	2.80	10.27	8.65	26.58	35.40
	(3.31)	(2.03)	(2.44)	(1.57)	(2.92)	(3.97)	(1.86)	(2.77)	(4.09)	(5.28)
T0a (/h)	0.00	0.00	0.87	1.13	0.96	0.00	0.00	1.40	0.00	0.00
	(0.42)	(0.26)	(0.27)	(0.14)	(0.28)	(0.52)	(0.27)	(0.29)	(0.67)	(0.81)
%BCPa (%)	27.39	26.30	26.79	24.77	28.75	34.52	32.69	35.30	52.02	61.24
BCPa (g/kg DM)	119.81	114.69	116.48	102.71	121.89	150.80	140.87	156.41	219.75	261.34
CP Rumen Degradation Characteristics (RDC[b]) of teatment B group (control B +9 treatments)										
Sb (%)	32.63	32.01	28.53	30.55	32.55	25.59	24.42	26.70	26.19	27.63
Db (%)	65.79	67.55	70.22	69.45	66.91	72.29	72.50	56.65	45.39	30.89
Kdb (%/h)	11.30	9.21	9.64	9.96	9.57	8.74	9.68	8.34	4.88	3.66
	(0.67)	(0.97)	(0.58)	(0.90)	(0.87)	(0.34)	(0.95)	(1.05)	(0.54)	(2.94)
Ub (%)	1.58	0.44	1.25	0.00	0.54	2.12	3.08	16.65	28.42	41.48
	(1.29)	(2.44)	(1.52)	(2.29)	(2.38)	(1.02)	(2.37)	(2.72)	(2.13)	(12.91)
T0b (/h)	0.40	0.29	0.91	1.08	1.68	0.57	0.15	1.09	0.00	1.60
	(0.15)	(0.30)	(0.15)	(0.22)	(0.21)	(0.11)	(0.28)	(0.33)	(0.37)	(2.15)
%BCPb (%)	24.40	27.09	28.19	26.11	26.32	31.54	30.83	40.35	53.46	60.67
BCPb (g/kg DM)	102.61	115.12	115.67	110.43	110.32	131.78	127.26	172.66	227.60	261.04

Notes: %BCP: percentage of bypass crude protein of whole lupin seeds; % BCP =D*Kp/(Kp+Kd)+U (Kp=6%/h); BCP (g/kg)=1.11*CP (g/kg, DM)*% BCP/100; Means with the same letter are not significantly different, Tukeys Studentized Range (HSD) Test, Alpha = 0.05.

The Series (A, B) effects: P=0.381 for S; P=0.993 for D; P=0.141 for Kd; P=0.076 for T0; P=0.381 for U; P=0.923 for %BCP; P=0.442 for BCP. Therefore there were no significantly effects on RDC (S, D, Kd, T0, U) and BCP(%BCP, BCP) of A and B series (p>0.05).

roasting did not alter the in vitro digestibility with increasing times and temperatures.

Rumen degradation characteristics (RDC) of crude protein

The effects of dry roasting on rumen protein degradation characteristics of treatments A and B groups of WLS are presented in table 6. There were no series effects on RDC of whole lupin seeds. P value of S, D, Kd. T0, U, %BCP and BCP for series effects were 0.381, 0.993, 0.141, 0.076, 0.381, 0.923 and 0.442, respectively. It indicated that dry roasting conditions were reliable and constant heating method.

RDC of intergration of A and B series: Based on the best fit of data to the model, though S of CP (figure 1) varied from 32.0% in 110°C/15min (31.8% in raw) to 27.1% in 150°C/45min, treatment temperatures and times had no significant effects on S ($p>0.05$).

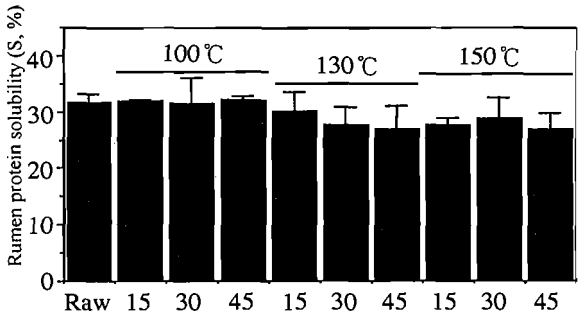

Figure 1. Effect of dry roasting on rumen solubility (S) of crude protein of whole lupin seeds in dairy cows

With increasing temperatures and times, U (figure 2) was dramaticly increased from 0% (110°C/45') and 0.8% (Raw) to 38.4% (150°C/45'). The interaction effects of dry roasting temperature and time were strongly significant ($p<0.01$).

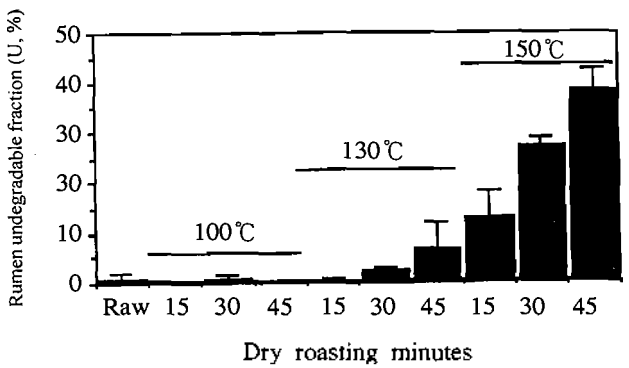

Figure 2. Effect of dry roasting on rumen undegradable fraction (U) of CP of whole lupin seeds in dairy cows

D of CP varied from 67.4% in raw to 35.8% in 150°C/40min (figure 3). D was increased by 1.6%, 1.7%, when temperature was 110°C and 130°C, respectively, then decreased by 30.8% at the temperature of 150°C relative to the control. The effect of temperatures and times interaction was significant ($p<0.05$).

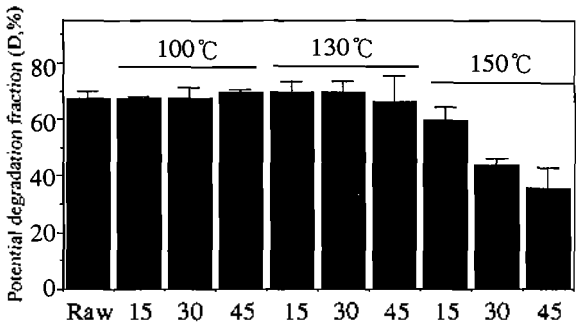

Figure 3. Effect of dry roasting on D fraction of crude protein in whole lupin seeds in dairy cows

Kd of CP varied from 10.2%/h in raw to 3.6%/h in 150°C/45min (figure 4). Average of Kd at 110, 130, 150°C group were 9.7, 8.9, 5.4%/h, respectively. The effect of temperature and time interaction was strongly significant ($p<0.01$). Compared with raw, Kd of 150°C/15 min, 150°C/30 min and 150°C/45 min was reduced by 19.2%, 56.9% and 65.1%, respectively.

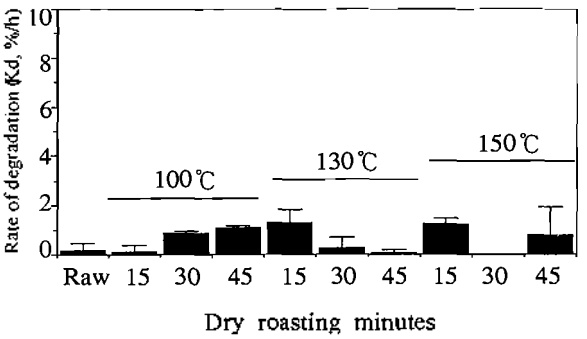

Figure 4. Effect of dry roasting on rumen degradation rate (Kd) of crude protein of whole lupin seeds in dairy cows

The lag time (T0) of rumen degradation is shown in figure 5. Neither treatment time nor temperature significantly affected T0.

BCP and %BCP, the most important components of whole lupin seed nutrition value in dairy cows, were a function of Kd, U and D fractions. %BCP varied from 25.9% in raw to 61.0% in 150°C/45' as shown in figure 6. It increased by 2.5%, 18.8% and 95.0% at 110, 130, 150°C. At the temperature of 150°C, it tremendously increased. Compared with the raw, %BCP of the treatment of 150°C/45 was highest and increased 2.4 times.

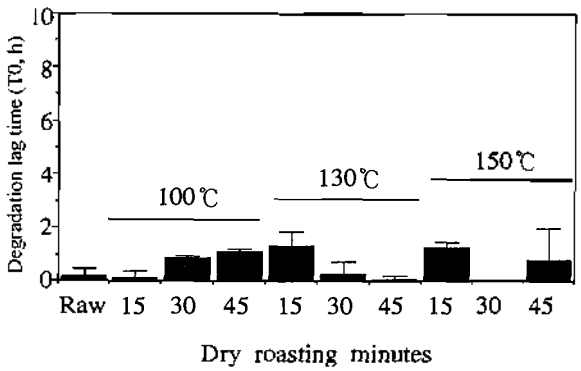

Figure 5. Effect of dry roasting on rumen degradation lag time of crude protein of whole lupin seeds in dairy cows

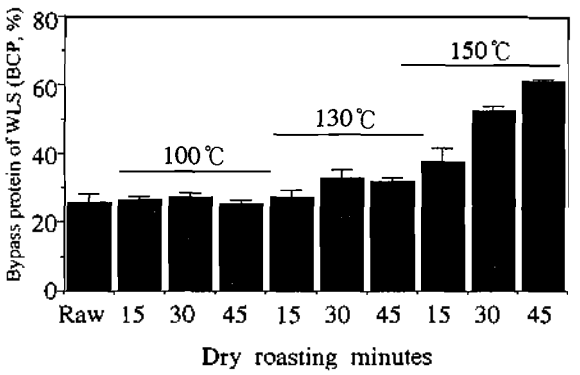

Figure 6. Effect of dry roasting on percentage of bypass crude protein of whole lupin seeds in dairy cows

The changes of BCP had the same pattern as %BCP. It varied from 111.2 in raw to 261.2 g/kg in DM in 150°C/45min as shown in figure 7. It did not significantly changed at 110°C (112.5 g/kg) but increased by 17.3% at 130°C (130.5 g/kg). When temperature reached 150°C, it dramaticlly increased by 96%. The interaction effect was very strongly significanlty ($p<0.001$). BCP of 150°C/45min was increased 2.3 times compared with the raw. The important results were that %BCP and BCP had little change until 150°C for 15, 30 or 45 min.

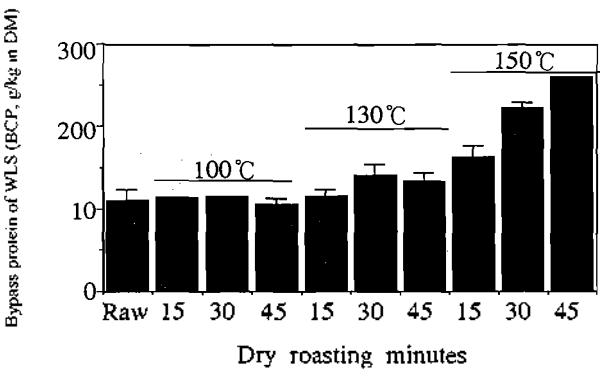

Figure 7. Effect of dry toasting on bypass crude protein of whole lupin seeds in dairy cows

DISCUSSION

In vitro enzyme digestbility

Dry roasting did not affect digestibility *in vitro* but significantly increased protein retention with increasing temperature and time. It may suggest that lack of overprotection of WLS as a result of this heat treatment. This is in agreedment with the previous studies by Kibelolaud et al. (1993) in that the whole tract digestibility of extruding lupin at 110, 130, 150 or 180 °C was generally unchanged; Cros et al. (1991) in that WLS proteins were affectively protected from ruminal breakdown by extrusion without adverse effect on protein total digestibility.

Nutritional value

RWLS have a high rate of degradation (10.2%/h) and very low U fraction 0.8%, which contribute to a high degradability (74.1%) after being incubated in the rumen, resulting in only 25.9% bypass protein into the intestines. It is very close to the result reported by Van Straalen et al. (1990) at IVVO, Netherlands that bypass crude protein of lupin was 24%, but quite inconsistent with the results obtained by Valentine et al.(1988), Cros et al. (1992), Aguilera et al. (1992) and Kibeloaud et al. (1993) with BCP of 8.1, 4.9, 9.7 and 6.6%, respectively. The table 7 is the summary of in sacco protein degradability of whole lupin seeds abtained by different authors. From the table 7, the reasons for these different figures may de due to the variety, growth periods or environment in the fields as well as in sacco technique procedures (i.e. assumed rate of passage (Kp), sample size, feed particle size, bag size, bag pore size, washing procedures). Its usefulness depends on the standardization of different variables. Despite its limitations (Yu, 1998), the results of in sacco degradation still better reflect true degradation than results from in vitro and enzymatic methods (Tamminga, 1990). For measurement of feed protein which escape microbial degradation in the rumen, the in sacco nylon bag technique is still internationally accepted.

In this experiment, dry roasting did not alter the in sacco protein solubility (S), potential protein degradation fraction (D), undegradable fraction (U) as well as the rate of degradaton (Kd) until the temperature reached 130°C for 30 or 45 min and 150°C for 15, 30 or 45 min. It did not alter the amount of bypass protein (BCP) of WLS at 110°C for15 min (26.7%), 30min (27.5%) or 45 min (25.4%) and 130°C for15 min (27.5%), relative to the RWLS (25.9%). Dry roasting increased the BCP only at higher temperature of 130°C for 30 (33.0%) or 45 min (31.8%) or at 150°C for 15 (37.8%), 30 (52.7%) or 45 min (61.0%).

It indicated that lower heating temperatures had no affect at all on rumen degradation characteristics. It did not reduce rumen protein degradability and increased protein escaped rumen to small intestine. It had effect on rumen protien degradation only when temperature and time reached certain point. In this study dry roasting

Table 7. Protein degradability characteristics of lupin

Authors	Lupin	var.	Particle size	Rumen protein degradation characteristics (RDC) CP (%)	S (%)	D (%)	U (%)	Kd (%/h)	T0 (h)	D[c] (%)	Kp[e] (/h)	BCP (%)
[a]Valentine et al. (1988)	*lupinus angustifolius*	*illyarrie*	1 mm	32.0	6.0 (2.7)	86.2 (2.9)	7.8	48 (4)	-	84.4[d]	0.06[d]	15.6[d]
Van Straalen et al. (1990)	lupin	-	3 mm	34.2	25.5	74.3	0.2	12.87	-	76	0.06	24
[b]Cros et al. (1991)	*lupinus albus*	*lublanc*	1 mm	38.8	80.7 (0.7)	18.3 (0.7)	1.0	22.1	-	95.1	0.06	4.9
Aguilera et al. (1992)	*lupinus albus*	*multolupa*	2 mm	40.7	36.27	62.51		9.8		90.3 87.2	0.0154 0.0222	9.7 12.8
Kibelolaud et al. (1993)	*lupinus albus*	*lublanc*	1 mm	38.8	75.3	23.7	1.0	19.2	-	93.4	0.06	6.6
[b]Yu et al. (our study)	*lupinus albus*	-	3 mm	38.7 (1.1)	31.8 (1.17)	67.4 (2.29)	0.8 (1.1)	10.21 (1.54)	0.2 (0.28)	74.1 (2.1)	0.06	25.9 (2.1)

Notes: a: the number in bracket is SE. b: the number in bracket is SD; c: D=degradability; d: Calulated or assumed by Author; e: assumped Kp; -: not mentioned.

only at 130 or 150°C reduced the rumen degradability with increasing time and temperature. These findings are similar to published observations carried out with a variety of protein supplements subject to different protective methods involving heating. Yu et al. (1995) found that pressure toasting did not increase bypass protein of horse bean at 100°C. McMeniman et al. (1979) found that heating faba beans at 105°C did not increase total flow of protien to the duodenum of cattle. Yu et al. (1998) reported that dry roasting had no effects at all on the faba bean when roasting temperature was 110°C. All these findings indicate that lower heating temperature did not have a pronounced effect on ruminal degradation and was inadequate for protecting these protein.

The susceptibility to heat treatment

The different legume seeds have their own characteristics, their own protein degradation characteristics and their own susceptibility to heat treatment of the protein. Yu et al. (1998) found dry roasting at lower temperature of 110°C for 15, 30 and 45 min did not result in decreasing S, Kd, increasing D and BCP, but resulted in a higher rumen protein degradability than the raw faba bean. It decreased BCP by 18% at 110°C. It caused more crude protein to be feremented in the rumen. It increased BCP by 21% at 130°C and 145% at 150°C. In this study dry roasting at 110°C had no effects at all on S, D, Kd, U, BCP. It neither incresed nor decreased RDC.It only increased BCP at higher temperature of 130 and 150 °C with increasing by 17% (moderately increased) and 95% (sharp increased) relative to the raw. But Yu et al. (1995) reported that pressure toasting even at lower temperatures of 100°C for 15 or 30 min had significantly effects on RDC. It reduced S, Kd, increased D and BCP. Aguilera et al. (1992) found that autoclaved lupin seeds at 120°C for 30 min decreased S from 36.27 to 9.90%, Kd (9.8 to 3.6%/h) of protein degradation and increased D from 62.51 to 90.10%. Effective degradability of protein was strongly reduced from 87.2 to 65.4% by heat treatment in lupin seeds. Kibelolaud et el. (1993) extruded lupin at 110, 130, 150 and 180°C reduced CP S from 75.3 to 79.2, 55.8, 49.5 and 46.4%, respectively, the rate of degradation from 19.2 to7.3, 7.6, 2.8 and 2.5 %and degradatibility from 93.4 to 89.8, 79.9, 65.1 and 61.8%.

Though dry roasting increased rumen bapass protein of both lupin seed and faba bean at higher temperature of 130 and 150°C in dairy cows, the reasons of increasing BCP were quite different. For faba beans (Yu et al., 1998), it was beause dry roasting reduced S and Kd, increasing potential degradation fraction without significantly altering undegradable fraction (U). All these effects resulted in increasing faba bean rumen BCP. This was not case for lupin seed. Dry roasting didnt alter S, decreased D, it increased U fraction in stead of decreasing S, increasing D and no changing U fraction in faba bean. Dry roasting reduce Kd fraction on both lupin and faba bean.

Therefore, the susceptibility to heat treatment of the protein in the legume seeds assayed varied widely, indicated that extrapolations from one legume species to another may be misleading.

CONCLUSIONS

Dry roasting of WLS at higher temperatures has the potential to increase supply of bypass protien without damageing the digestibility *in vitro* thus improving the nutritional quality of lupin seed by increasing the amount of protein supplied to the small intestine, which could be benefit to high production of cows. It might be effective in shifting crude protein degradation from rumen to intestine to reduce unnecessary nitrogen (N) loss in the rumen. The Intestinal availibility of individual amino acid, especially first limiting amino acicd, need to be further investigated.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. D. Dalley (Dairy Research Institute, Ellinbank and Gippsland Region, Australia) for providing experimental station and technical assistances; Mr. R.Toe (Agricultural Lab), Mrs. F. Nowniaz (Animal Production Lab, Institute of Land and Food Resources, The University of Melbourne, Australia) for helpful assistance in chemical analysis.

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