Determination of Optimal Conditions of Pressure Toasting on Legume Seeds for Dairy Deed Industry: I. Effects of Pressure Toasting on Nutritive Values of *Lupinus albus* in Lactating Dairy Cows

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ABSTRACT : Whole *lupinus albus* seeds were pressure toasted at temperatures of 100, 118 and 136℃ for 3, 7, 15 and 30 min to study rumen degradation and post-rumen digestion and to determine optimal heating conditions for the Dutch dairy feed industry. *In sacco* nylon bag and mobile bag techniques were employed for rumen and intestine incubations to determine ruminal degradation characteristics and intestinal digestion of crude protein (CP) in 4 lactating rumen cannulated and 4 lactating intestinal cannulated Dutch dairy cows fed 47% hay and 53% concentrate according to Dutch dairy requirements. Measured rumen degradation characteristics were soluble fraction (S), undegradable fraction (U), potentially degradable fraction (D), lag time (T0) and rate of degradation (Kd) of insoluble but degradable fraction. Percentage bypass feed protein (BCP), ruminal microbial protein synthesised based on available nitrogen (N_MP) and that based on available energy (E_MP), true protein supplied to the small intestine (TPSI), truly absorbed BCP (ABCP), absorbed microbial protein (AMP) in the small intestine, endogenous protein losses in the digestion (ENDP), true digested protein in the small intestine (TAP or DVE in Dutch) and degraded protein balance (PDB or OEB in Dutch) were totally evaluated using the new Dutch DVE/OEB System. Pressure toasting decreased ($p<0.001$) rumen degradability of CP. It reduced S ($p<0.05$) and Kd ($p=0.06$), increased D ($p<0.05$) and U ($p<0.01$) but did not alter T0 ($p>0.05$), thus resulting in dramatically increased BCP ($p<0.001$) with increasing time and temperature from 73.7 (raw) up to 182.5 g/kg DM (136℃/15 min). Although rumen microbial protein synthesised based on available energy (E_MP) was reduced, true protein (microbial and bypass feed protein) supplied to the small intestine (TPSI) was increased ($p<0.001$) from 153.1 (raw) to 247.6 g/kg DM (136℃/15 min). Due to digestibility of BCP in the intestine not changing ($p>0.05$) average 87.8%, the absorbed BCP increased ($p<0.001$) from 62.3 (raw) to 153.7 g/kg DM (136℃/15 min). Therefore DVE value of true digested protein in the small intestine was significantly increased ($p<0.001$) from 118.9 (raw) to 197.0 g/kg DM (136℃/15 min) and OEB value of degraded protein balance was significantly reduced ($p<0.001$) from 147.2 (raw) to 63.1 g/kg DM (136℃/15 min). It was concluded that pressure toasting was effective in shifting degradation of CP of *lupinus albus* from the rumen to small intestine without changing intestinal digestion. Further studies are required on the degradation and digestion of individual amino acids and on the damaging effects of processing on amino acids, especially the first limiting amino acids. (***Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 8 : 1205-1214***)

Key Words : Rumen Degradation Characteristics, Intestinal Digestion, Dve, Oeb, *In Sacco*, Mobile Bag Technique

INTRODUCTION

Lupinus albus seeds, also called sweet lupinus seeds (McDonald et al., 1988), are legume seeds with low levels of alkaloids of 0.02% -0.43% (Singh et al., 1995; Goelema, 1994), are free of antinutritional factors such as trypsin inhibitor, haemagglutonins and lectins (Robinson and McNiven, 1993), have relatively high protein (30-35%), and lipid contents (8-12%) (Hill, 1977) and a metabolisable energy content of 13.2 MJ/kg DM for ruminants (McDonald et al., 1988). They can be grown in temperate climates such as in the Netherlands (McDonald et al., 1988). Thus they represent a source of protein and energy in diets for lactating dairy cows in this region. Production of *lupinus albus* in Holland is 110, 000 tons which is sufficient for the Dutch feed industry (Goelema, 1994).

However the rapid rumen degradation characteristics and traditional processing methods cause reduces in their nutritive values for ruminants. Published studies indicate that *lupinus albus* has very rapid rumen degradation characteristics with a high degradation rate (Kd) of 12.9 to 48%/h and effective degradability of 76 to 95.1%, respectively (Valentine et al., 1988; Van Straalen and Tamminga, 1990; Cros et al., 1991; Aguilera et al., 1992; Kibelolaud et al., 1993). Such rapid rumen degradation of CP could cause an imbalance between feed breakdown and microbial protein synthesis, and result in N loss from rumen (Yu et al., 1998a). Studies with lactating dairy cows fed raw *lupinus albus* (Guillaume et al., 1987; May et al., 1993) reported poor performance which has generally been attributed to the high proportion of rapidly degradable crude protein in *lupinus albus*

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(Singh et al., 1995).

Dairy cows require large quantities of protein which are available and absorbed in the small intestine, not in the rumen, to maintain high milk production. This can be enhanced by decreasing the degradability of feed protein in the rumen (Yu et al., 1998a). Therefore the rapid degradability of *lupinus albus* must be reduced without damaging intestinal digestion.

Heat treatment has been widely used to decrease protein degradation in the rumen and increase the supply of dietary protein to the duodenum (Singh et al., 1995). Pressure toasting, a current interest in our department to reduce legume seeds degradation in the rumen, has given very promising results (Goelema, 1994), but the optimal processing conditions for such seeds have not yet been found. Heating under optimal conditions may not increase bypass protein content and heating above optimal conditions may overprotect the protein so that the protein is neither degraded in the rumen nor digested in the small intestine (Yu et al., 1996).

In dairy cows, protein entering the small intestine originates from three different sources: 1) rumen bypass feed protein (feed protein escaping from rumen fermentation); 2) microbial protein (microorganisms leaving the forestomachs with the digesta; 3) endogenous protein (enzymes and mucosal cells excreted in the lumen of the gastrointestinal tract) (Antoniewicz et al., 1992). To fully and accurately evaluate the above nutritive values of raw and pressure toasted *lupinus albus* in dairy cows, proven evaluation method must be used. The *in sacco* and mobile bag techniques which are internationally accepted methods (Tamminga and Jansman, 1993), together with the newly developed Dutch Protein Evaluation DVE/OEB system (Tamminga et al., 1994) were employed to measure protein degradation and digestion in the rumen and small intestine.

The objectives of this study were to determine rumen degradation and intestinal digestion characteristics of raw and pressure toasted *lupinus albus* using nylon bag techniques in cannulated lactating dairy cows and to fully evaluate ruminant nutritional values of protein using the new Dutch DVE/OEB system in order to define the optimal pressure toasting conditions of *lupinus albus* for the Dutch dairy feed industry.

MATERIALS AND METHODS

Feedstuffs

Whole *lupinus albus* seeds were obtained from the Dutch commercial company. The chemical composition of raw whole *lupinus albus* seeds are present in Results (table 2).

Pressure toasting design

Whole *lupinus albus* were pressure toasted at 3 different temperatures (100, 118 and 136℃) for 3, 7, 15 and 30 min in an incomplete block design. All treatments were carried out in duplicate resulting in 22 treatments in total divided into A and B series as shown in the table 1. The treatment of 100℃/3 min and 136℃/30 min were dropped due to no expected significant difference between the raw and 100℃/3 min and the risk of overheating, respectively.

Table 1. The design of pressure toasting treatments of A and B series of *lupinus albus* seeds

	Pressure toasting minutes							
Temperature	A series				B series			
100°C	-	7	15	30	-	7	15	30
118°C	3	7	15	30	3	7	15	30
136°C	3	7	15	-	3	7	15	-

Notes: -: not determined.

Table 2. Chemical compositions of the raw and pressure toasted whole *lupinus albus* seeds

Temp. (℃)		100			118				136		
Time (min.)	Raw	7	15	30	3	7	15	30	3	7	15
Chemical compositions											
DM (g/kg)	909.35^{b} ±0.49	916.55^{ab} ±6.01	919.75^{ab} ±2.62	925.95^{a} ±3.32	921.15^{ab} ±3.46	918.40^{ab} ±5.80	916.05^{ab} ±2.62	911.45^{ab} ±0.35	914.40^{ab} ±1.27	917.30^{ab} ±6.65	914.10^{ab} ±5.52
OM (g/kg, DM)	969.27 ±0.06	969.34 ±0.04	969.56 ±0.08	969.71 ±0.18	969.60 ±0.11	969.57 ±0.04	969.49 ±0.32	969.50 ±0.17	969.67 ±0.12	969.48 ±0.24	969.26 ±0.13
Ash (g/kg, DM)	30.74 ±0.06	30.66 ±0.04	30.44 ±0.08	30.29 ±0.18	30.40 ±0.11	30.44 ±0.04	30.52 ±0.32	30.50 ±0.17	30.35 ±0.12	30.52 ±0.24	30.74 ±0.13
N (g/kg, DM)	52.29 ±0.55	52.77 ±0.13	52.53 ±0.08	52.44 ±0.49	52.80 ±0.16	51.92 ±0.54	52.51 ±0.21	52.38 ±0.08	52.67 ±0.48	53.19 ±0.28	53.19 ±0.29

Notes: ±SD; Means with the same letter in the same raw are not significantly different ($p>0.05$).

Processing was carried out at *Wageningen Feed Processing Center (WFPC)* using a laboratory scale pressure toaster as described by Van der Poel (1990). After toasting, the *lupinus albus* seeds were dried at 35℃ for 18 h in the oven, allowed to cool down to ambient temperature and then coarsely ground through a 3 mm screen (Hemmer Mill AEG TYP AM80N*2)

Animals and diets

Four lactating Holstein Friesian cows were each fitted with a large rumen cannula with an internal diameter of 10 cm (Bar Diamond Inc., Parma, Idaho, USA) for measuring rumen degradability were housed at the tie stall at Wageningen Agricultural University. All cows received a diet consisting of a commercial pelleted concentrate (940 VEM (energy), 120 DVE (true protein digested in the small intestine)) and hay (666 VEM, 53 DVE) according to Dutch lactating dairy cow feed requirement.

Four intestinal cannulated lactating Dutch dairy cows, housed on a tie-stall and fed pelleted commercial concentrate and hay according to Dutch feeding standards, were used to determine intestinal digestion by the mobile bag technique at the research institute of IVVO (The Netherlands).

All the cows were individually fed twice daily at 08:00 h and 16:00 h. Water was always available. A 14 days period of adaptation was allowed.

The animal used in these experiments were cared for in accordance with the guidelines Dutch Animal Care and Use.

Rumen incubation

Ruminal degradation characteristics of *lupinus albus* in the rumen of 4 lactating dairy cows were determined using the *in sacco* method. Incubation of all treatments in the rumen were with 5.5 g dry matter in coded nylon bags (10*17 cm) with the pore size of approximately 40 m (Nylot, Switzerland). The rumen incubations were performed according to the gradual addition/all out' schedule. Incubations were carried out for 24, 12, 8, 4 and 2 h, bags were inserted at 17:00, (next day) 05:00, 09:00, 13:00, 15:00 and removed at 17:00 h, respectively. The 48 h rumen incubations were carried out from 20:00 untill 20:00 two days later. All treatments were randomly allocated over all cows and the whole incubation period.

After incubation, the bags were removed from rumen and rinsed under a cold stream of tap water to remove excess ruminal contents and microbes on the surface to stop microbial activity. The bags were washed with cool water without detergent in a commercial washing machine for 55 min without spinning and subsequently dried at 60℃ for 24 h. The 0 h incubation samples were only put in the washing machine under the same conditions. Dry samples were stored in a cool room (4℃) until analysis. The residues were pooled according to feed treatment and incubation time and then ground through a 1 mm screen and analysed for DM, ash and N.

Intestinal digestion

The 4 intestinal cannulated lactating Dutch dairy cows were used to determinate intestinal digestion by the mobile bag technique. Some extra bags were incubated in the rumen to provide sufficient material for the intestinal studies. The feeds was preincubated for 12 h (Goelema, 1994). After rumen incubation, the bags were removed and handled as described previously. However, the residues were freeze dried and pooled according to feed treatments. Approximately 0.5 g (DM) of rumen residues was then weighed into small coded nylon bags (5×3 cm, pore size 40 μm), 24 bags per treatment. Prior to incubation in the small intestine the bags were incubated in a solution of pepsin-HCl solution (1 g pepsin (2000 FIP u/g, Merck 7190 in 1 ltr 0.1 N HCl) at 39℃ for 1 h. Every 20 min, 4 mobile bags were taken at random and inserted into the intestine through the duodenal cannula. The bags were retrieved from faeces. Checks were made every 2 h after bag introduction, and the collection time was recorded for each bag. The retrieved bags were stored at -20℃ until all the bags had been recovered. These bags were washed in a washing machine for 2 h at 40℃ without spinning. The residues were freeze dried, weighed and pooled according to feed treatments and analysed for N, DM and ash after pooled residues were ground using a 1 mm screen.

Laboratory analysis

Chemical analysis were performed according to the Animal Nutrition Department (WAU)'s standard procedures (Boer, 1995). Laboratory samples of the feeds, rumen residues and mobile bags residues of all treatments of *lupinus albus* were prepared by grinding to pass a 1 mm mesh after freeze drying and analysed for DM, Ash and N. DM was determined by drying at 105℃ to constant weight. Ash was determined by ashing at 550℃ to constant weight. N was analysed by Kjeldahl digestion and distillation (Gerhardt Vadopest 6, Germany) and crude protein (CP) content was obtained as N×6.25.

Calculation model

1) Rumen degradation

Important degradation characteristics of CP were: the soluble fraction (S) which is assumed to be degraded rapidly and completely; the rumen undegradable fraction (U); the fraction which is not

soluble, but potentially degradable (D=100-S-U).

The degradation rate (kd, %/h) of the fraction D (Van Straalen and Tamminga, 1990) was calculated using the NLIN procedure of the statistical package SAS (SAS, 1991) using iterative least squares regression (Gauss-Newton method) according to the first order degradation model (Ørskov and McDonald, 1979), including a test for lag phase (T0) preceding the onset of rumen degradation by the following equation $R(t)=U+D\times exp\text{-}kd(t\text{-}T0)$, where R(t) stands for residue (in %) of the amount of incubated material after t h of rumen incubation.

Percentage of bypass feed CP (%BCP) was calculated according the following formula (Tamminga et al., 1994): % BCP = $U+D\times Kp/(Kp+Kd)$. Bypass feed CP in g/kg DM was calculated as: BCP = 1.11 ×CP (g/kg DM))×%BCP/100, where passage rates (kp) of 6%/h was adopted based on international data (Tamminga and Jansman, 1993).

Fermented organic matter (FOM) was calculated as: FOM=DOM-CFAT-BCP-BST (g/kg DM), where, DOM: digested organic matter; CFAT: crude fat; BST: bypass feed starch (DVE/OEB, 1994); Subsequently microbial protein synthesised in the rumen was estimated from available energy as: E_Mp=0.15×FOM (g/kg, DM), where the factor 0.15 stands for that 150 g of microbial protein was assumed to be synthesised per kg FOM (DVE/OEB, 1994).

2) Intestinal digestion

Organic matter and crude protein digested in the small intestine were calculated on the basis of rumen residues (Hvelplund and Weisbjerg, 1991). Digestibility of bypass feed protein in the small intestine (dBCP) was calculated as: $dBCP(\%)=(BCP-U_n)/BCP$, where, U_n: undigested fraction in the rumen and intestine. So the true absorbed bypass protein in the small intestine was calculated as: ABCP (g/kg DM) = dBCP× BCP/100. The true protein supplied to the small intestine (TPSI) was calculated as: TPSI=BCP+0.75 MP (g/kg DM), where, the factor 0.75 means that 75% of microbial N was present in amino acids, the remaining part of N in nucleic acids (DVE/OEB, 1994). Truly absorbed microbial protein (AMP) in the small intestine was estimated as: AMP=0.85×0.75MP (g/kg, DM), where, true digestibility of microbial protein was assumed to be 85%. Endogenous protein losses (ENDP) was calculated as: ENDP=75×UDM (g/kg DM), where, the factor 75 means that 75 g absorbed protein per kg dry matter in faecal excretion was required to compensate for endogenous losses (DVE/OEB, 1994).

Therefore truly digested protein absorbed from the small intestine (TAP, called DVE in Dutch) was estimated from the total of absorbed rumen bypass feed protein plus absorbed microbial protein minus endogenous losses: TAP (or DVE)=ABCP+AMP-ENDP (g/kg DM) (DVE/OEB, 1994).

The degraded protein balance (DPB or called OEB in Dutch) value, calculated as DPB (or OEB)= N_MP-EMP (g/kg DM), showed the balance or imbalance between microbial protein synthesis potential possible from available rumen degradable feed CP (N_MP) and that potential possible from the energy extracted during anaerobic fermentation in the rumen (E_MP).

Statistical analysis

Statistical analysis were carried out using the SAS (1991). Analysis of variance was by using Proc GLM of SAS (1991). The following linear model was used for data analysis: $Y_{hij}=m+series_h+Temp_i+Time_j+Temp\times Time_{ij}+e_{hij}$, where: Y=degradation or digestion fractions; h =1, 2; i = 1, 2, 3, 4; j = 1, 2, 3, 4, 5.

Differences between means were tested by Tukey's Studentized Range Test (HSD) (SAS, 1991).

RESULTS

Chemical compositions of raw and toasted *lupinus albus*

The chemical composition of raw and pressure toasted *lupinus albus* is given in table 2. Raw *Lupinus albus* seeds contained DM of 909.4 g/kg, OM of 969.3 g/kg DM, ash of 30.7 g/kg DM and N of 52.3 g/kg DM. Pressure toasting did not change the OM, ash and N contents on dry matter basis of raw and dry roasted treatments ($p>0.05$).

Rumen degradation characteristics

Effects of pressure toasting on rumen degradation characteristics of CP of raw and treated *lupinus albus* are presented in table 3. Pressure toasting decreased ($p<0.001$) rumen degradability of CP. The temperature of pressure toasting had more effect than the time on rumen degradation characteristics. It significantly reduced S ($p<0.001$) from 47.6 (raw), to 38.2, 31.4 and 30.8%, altered D ($p<0.001$) from 52.0 (raw), to 61.6, 63.1 and 44.9%, reduced Kd ($p<0.05$) from 9.7 (raw), to 5.2, 4.7 and 6.7%/h and dramatically increased U ($p<0.001$) from 0.3 (raw), to 0.1, 5.5 and 24.3% at 100, 118 and 136℃ of pressure toasting temperature, respectively. Pressure toasting time, however, had no significant effects on S, D, Kd and U ($p>0.05$). Both temperature and time had strong significant effects on BCP ($p<0.001$), which increased with increasing temperatures and times from 19.4% or 73.7 g/kg DM (raw) to 49.5% or 182.5 g/kg DM (136℃/15 min) (figure 1a). Lag time (T0) ($p>0.05$) was not affected either by toasting temperature or time.

Pressure toasting significantly affected on the rumen microbial protein synthesis based on available

N, also called rumen degraded protein ($p<0.001$). It was reduced from 253.1 (raw) to 149.9 g/kg DM (136 ℃/15 min) (figure 1b). Although the rumen microbial protein synthesised based on available energy (E_MP) was reduced ($p<0.001$) from 105.9 (raw) to 86.9 g/kg DM (136℃/15 min) (figure 1c), total true protein supplied to the small intestine (TPSI), consisting of BCP and true microbial protein (MP) (figure 1d), was

Table 3. Effect of pressure toasting on rumen degradation characteristics of whole *lupinus albus* seeds in lactating dairy cows

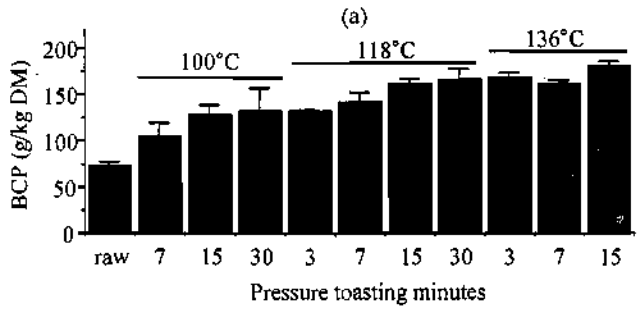
Temp.(℃)		100			118				136		
Time (min)	Raw	7	15	30	3	7	15	30	3	7	15
CP(g/kg, DM)	326.82 ±3.44	329.79 ±0.84	328.32 ±0.53	327.72 ±3.05	330.00 ±0.98	324.51 ±3.36	328.16 ±1.28	327.38 ±0.53	329.19 ±3.01	332.44 ±1.77	332.41 ±1.81
Rumen degradation characteristics of crude protein (CP)											
S (%)	47.63^{a} ±1.45	39.66^{ab} ±4.60	36.96^{ab} ±6.45	38.08^{ab} ±7.12	34.35^{ab} ±1.11	32.88^{ab} ±7.46	29.95^{b} ±1.06	29.95^{b} ±1.06	28.91^{b} ±4.07	31.88^{ab} ±0.59	31.51^{ab} ±2.26
D (%)	52.04 ±0.97	58.90 ±5.23	63.04 ±6.45	61.92 ±7.12	56.73 ±0.28	66.08 ±8.94	66.15 ±4.45	66.15 ±4.45	54.99 ±14.28	42.27 ±6.10	37.42 ±1.53
U_r (%)	0.34^{c} ±0.48	0.45^{c} ±0.63	0.00^{c} ±0.00	0.00^{c} ±0.00	8.94^{bc} ±1.39	1.05^{c} ±1.48	3.90^{c} ±5.52	3.90^{c} ±5.52	16.10^{abc} ±10.21	25.85^{ab} ±6.69	31.07^{a} ±0.74
T0 (h)	0.26 ±0.37	1.46 ±0.13	2.00 ±1.00	2.21 ±2.45	1.04 ±0.59	0.86 ± 0.64	1.46 ±0.50	1.46 ±0.50	1.09 ±0.44	0.39 ±0.54	0.04 ±0.05
Kd (%/h)	9.68 ±1.33	6.73 ±0.92	4.62 ±0.33	4.18 ±0.79	6.37 ±0.64	4.28 ±0.38	3.86 ±1.07	3.86 ±1.07	5.13 ±1.55	8.72 ±4.02	6.21 ±0.04
% BCP	19.41^{d} ±2.11	28.84^{cd} ±3.88	35.58^{bc} ±2.55	36.77^{bc} ±7.06	36.51^{bc} ±0.18	39.55^{abc} ±2.31	44.56^{ab} ±1.60	44.56^{ab} ±1.60	46.59^{ab} ±1.74	44.10^{ab} ±0.78	49.46^{a} ±1.56
BCP (g/kg, DM)	73.69^{d} ±3.83	105.55^{cd} ±13.95	129.66^{bc} ±9.52	133.63^{bc} ±24.43	133.72^{bc} ±0.26	142.49^{abc} ±9.78	162.30^{ab} ±5.20	162.30^{ab} ±5.20	170.21^{ab} ±4.79	162.73^{ab} ±3.72	182.48^{a} ±4.72
RDP (g/kg, DM)	253.13^{a} ±0.38	224.24^{ab} ±14.79	198.66^{bc} ±8.99	194.09^{bcd} ±27.48	196.29^{bc} ±1.23	182.02^{bcd} ±6.42	165.86^{cd} ±6.48	165.86^{cd} ±6.48	158.98^{cd} ±7.79	169.71^{cd} ±1.96	149.93^{d} ±6.53
E_MP (g/kg, DM)	105.94^{a} ±0.62	98.46^{ab} ±3.34	95.41^{bc} ±2.17	94.17^{bc} ±3.18	95.92^{bc} ±0.98	94.19^{bc} ±5.02	89.82^{bc} ±0.54	89.82^{bc} ±0.54	89.56^{bc} ±1.29	89.52^{bc} ±0.54	86.88^{c} ±0.81
TPSI (g/kg, DM)	153.14^{e} ±3.36	179.39^{de} ±11.44	201.21^{cd} ±7.89	204.26^{bcd} ±22.04	205.65^{bcd} ±0.47	213.12^{abcd} ±6.01	229.66^{abc} ±4.79	229.66^{abc} ±4.79	237.38^{ab} ±3.83	229.87^{abc} ±3.32	247.64^{a} ±4.12

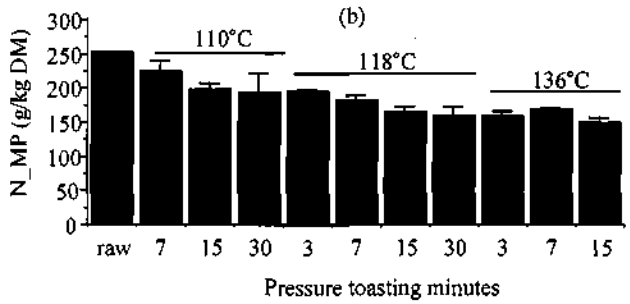
Notes: ±SD; BCP: rumen bypass crude protein; RDP=N_MP: rumen degradable crude protein (RDP), also called rumen microbial protein synthesized based on available nitrogen (N_MP); E_MP: rumen microbial protein synthesized based on available energy; TPSI; true protein supplied to the small intestine. Means with the same letter in the same raw are not significantly different ($p>0.05$).

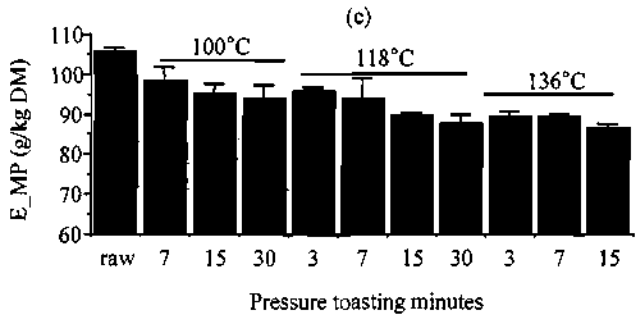
Table 4. Effect of pressure toasting on intestinal digestion of whole *lupinus albus* seeds in lactating dairy cows

Temp. (°C)		100			118				136		
Time (min.)	Raw	7	15	30	3	7	15	30	3	7	15
Intestinal digestion											
dBCP (%)	84.50 ±1.90	90.11 ±0.12	88.90 ±1.32	88.75 ±0.88	89.39 ±1.12	88.44 ±0.25	88.83 ±0.57	86.69 ±1.90	88.26 ±2.43	87.66 ±3.25	84.24 ±0.21
ABCP (g/kg, DM)	62.30^{c} ±4.63	95.11^{bc} ±12.70	115.33^{ab} ±10.18	118.70^{ab} ±22.85	119.52^{ab} ±1.27	126.03^{ab} ±9.02	144.18^{a} ±5.55	144.90^{a} ±6.76	150.16^{a} ±0.10	142.54^{a} ±8.78	153.70^{a} ±3.61
AMP (g/kg, DM)	67.54^{a} ±0.40	62.77^{ab} ±2.13	60.82^{bc} ±1.39	60.04^{bc} ±2.03	61.15^{bc} ±0.63	60.05^{bc} ±3.20	57.26^{bc} ±0.35	56.01^{c} ±1.25	57.10^{bc} ±0.83	57.07^{bc} ±0.35	55.39^{c} ±0.52
ENDP (g/kg, DM)	10.89 ±0.02	12.24 ±0.62	11.97 ±0.36	12.29 ±0.25	11.41 ±0.47	11.61 ±1.78	12.31 ±0.13	12.92 ±0.11	11.85 ±0.28	12.43 ±0.01	12.25 ±0.04
TAP (DVE) (g/kg, DM)	118.95^{c} ±4.21	145.64^{bc} ±9.94	164.18^{ab} ±8.43	166.45^{ab} ±21.06	169.26^{ab} ±2.35	174.46^{ab} ±4.04	189.13^{a} ±5.34	187.99^{a} ±5.41	195.40^{a} ±1.00	189.35^{a} ±5.37	196.85^{a} ±3.05
PDB (OEB) (g/kg, DM)	147.19^{a} ±0.24	125.78^{ab} ±11.44	103.25^{bc} ±6.81	99.92^{bcd} ±24.30	100.37^{bcd} ±0.25	88.83^{bcd} ±0.01	76.04^{cd} ±5.94	72.25^{cd} ±10.05	69.42^{cd} ±6.50	80.19^{cd} ±1.41	63.05^{d} ±5.73

Notes: SD; Means with the same letter in the same raw are not significantly different ($p>0.05$). dBCP: Digestibility of rumen bypass protein in the small intestine; ABCP: truly absorbed bypass protein in the small intestine; AMP: truly absorbed rumen synthesized microbial protein in the small intestine; ENDP: endogenous protein losses in digestion; TAP (DVE): true digested protein in the small intestine; PDB (OEB): degradaed protein balance.







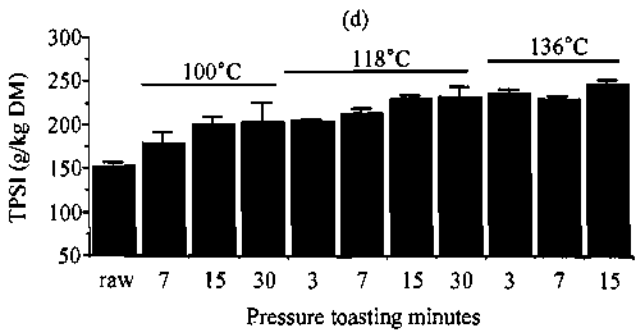
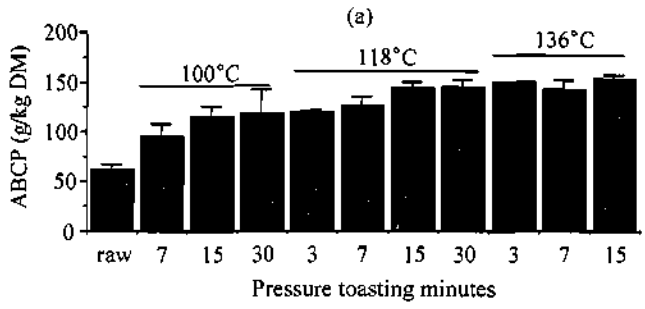
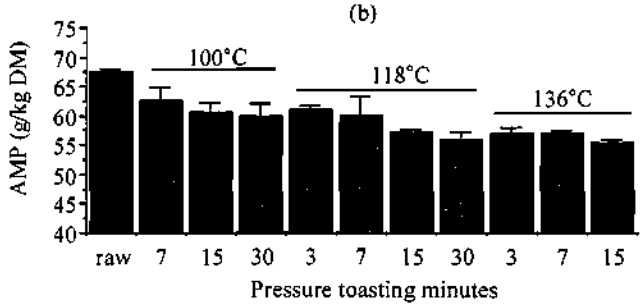
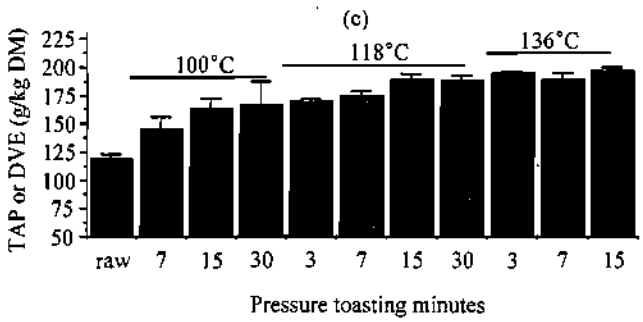


Figure 1. Effects of pressure toasting on rumen bypass feed protein (BCP) (a), microbial protein synthesis based on available nitrogen (N_MP) (b) and that based on available energy (E_MP) (c), and true protein supplied to the small intestines (TPSI) (d)







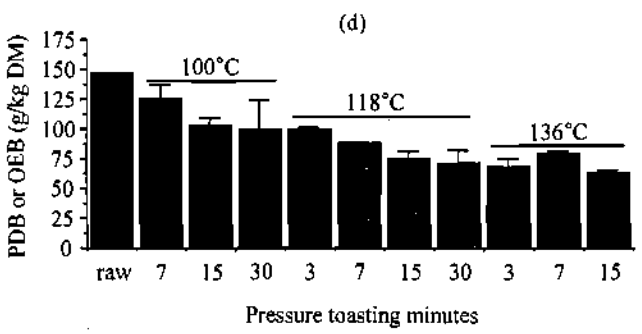


Figure 2. Effects of pressure toasting on the absorbed bypass feed protein (ABCP) (a), truly absorbed rumen synthesized microbial protein (AMP) (b) and true digested protein (TAP or DVE) in the small intestine (c), and protein degraded balance (PDB or OEB)

significantly increased ($p<0.001$) from 153.1 (raw) to 247.6 g/kg DM (136℃/15 min) by pressure toasting due to the large amount of increased bypass feed protein (BCP) supplied to the small intestine which overcompensated the reduction of microbial protein (MP) synthesised from available energy.

Intestinal digestion

The results of intestinal digestion are present in table 4. Pressure toasting did not affect digestibility of BCP ($p>0.05$) in the intestine which averaged 87.8%. So the increasing pattern of the absorbed BCP (ABCP) ($p<0.001$) (figure 2a) was the same as the BCP. It was increased from 62.3 (raw) to 153.7 g/kg DM (136℃/15 min). Also pressure toasting did not change the endogenous protein losses for all treatments ($p>0.05$) which averaged 12.0 g/kg DM. Although the estimated absorbed microbial protein (AMP) in the small intestine was reduced ($p<0.001$) from 67.5 in raw to 55.4 g/kg DM in 136℃/15 min (figure 2b), the total absorbed protein (TAP) or DVE was significantly increased ($p<0.001$) by pressure toasting (figure 2c). Compared with the raw sample, TAP (DVE) was increased by 33.4, 51.4 and 62.9% at roasting temperatures of 100, 118 and 136℃, respectively.

The protein degradable balance (PDB) values or OEB is the difference between microbial protein synthesised based on available N and microbial protein synthesised based on available energy. It was significantly decreased ($p<0.001$) as shown in figure 2d from 147.2 (raw), to 109.7, 84.4 and 70.9 g/kg DM as the roasting temperatures rose to 100, 118 and 136℃.

DISCUSSION

DVE/OEB system

The new Dutch protein evaluation system, the DVE/OEB system, has been recently developed by several research institutes in the Netherlands, as reported by Tamminga et al. (1994). In this system each feed has a DVE value composed of the digestible true protein contributed by feed protein escaping rumen degradation; microbial protein synthesised in the rumen and a correction for endogenous protein losses in the digestive tract. Each feed also has a degraded protein balance (OEB) reflecting the difference between the potential microbial protein synthesised based on degraded feed crude protein and that based on energy available for microbial fermentation in the rumen. In this system, the strong elements of other recently developed protein evaluation systems are also considered and additional new elements are introduced, including undegraded starch (BST), fermentation products (FP) in ensiled feeds, the role of energy balance in protein supply and the way in which requirements change in the course of lactation. This system make it possible to feed protein more accurately according to the real demands of the dairy cow and prevent unnecessary losses of nitrogen (Tamminga et al., 1994).

Pressure toasting had significant effects on rumen degradation characteristics and intestinal digestion of protein of *lupinus albus* in lactating dairy cows as present in the Results section. Use of this new DVE/OEB system made the evaluation of the nutritive values of raw and toasted *lupinus albus* more accurate and detailed. But there were several points in present study that need to be pointed out.

Bypass feed protein (BCP) was calculated from a fixed value for passage rate (Kp) of 6%/h for all treatments and raw samples. The fermented OM (FOM) was derived from digested OM (DOM) by substructing crude fat (CFAT), bypass feed protein (BCP), bypass feed starch (BST). Since *lupinus albus* has only 1.5% of starch in the kernel (Hove, 1974), the rumen bypass feed starch of *lupinus albus* was assumed to be zero.

Per kg of FOM, 150 g microbial crude protein was assumed to be synthesised for each of the treatments; 75% of microbial N was present in amino acids and 85% of digestibility of microbial protein was assumed. Therefore absorbed microbial protein for each treatment of pressure toasting was estimated as: $AMP=0.85\times0.75\times0.15\times FOM$.

The digestibilities of bypass feed protein and organic matter in the small intestine were $87.8\%\pm1.9$ and $84.5\%\pm1.0$, respectively, for each treatment, which were not significantly different between treatments, were measured using the mobile bag technique. It should be remembered that using the mobile bag technique to measure digestibility in the intestines usually overestimates digestibility because the bags are recovered from faeces and some protein might disappear in the large intestine (Hvelplund, 1985; Voigt et al., 1985). Also the previous study verified that digestibility by mobile bag technique was higher than that by the optimal three-step in vitro enzyme methods (87 vs 91; 90 vs 98%, for faba bean and lupin, respectively).

In calculating endogenous losses (ENDP) in the digestive tract, it was assumed that 75 g of absorbed protein per kg dry matter in faecal excretion was required to compensate for endogenous losses for each pressure toasting treatment. ENDP was calculated as $0.075\times UDM$. UDM intake was separated into indigestible organic matter (UOM) and indigestible inorganic matter (UASH). In the DVE/OEB system, the digestible inorganic matter (UASH) was calculated from the crude inorganic matter (CASH) as $UASH=\%UASH/100\times CASH$. The 65% of maximum of

%UASH for each treatment was adapted (CVB, 1991a and 1991b).

The OEB value indicate whether feed protein breakdown and microbial protein balance or imbalance. If the OEB value is high, it means excessive N loss from the rumen. When OEB is negative, microbial protein synthesis may be impaired, because of a shortage of N in the rumen. The optimal OEB value in a ration is therefore zero or slightly above (Tamminga and Jansman, 1993). In present study, raw *Lupinus albus* had a high value of OEB (147.2 g/kg DM) which indicated an imbalance between feed N degradation and utilisation. Pressure toasting significantly reduced OEB ($p<0.001$) from 147.2 to 63.1 g/kg DM in 136℃/15 min but not to negative values.

Rumen degradation characteristics

Raw *lupinus albus* meal (3 mm) had a high degradation rate (9.7%/h), a highly soluble fraction (47.6%) and very small undegradable fraction (0.3%), which all contributed to a very high effective degradability (80.6%) after being incubated in the rumen, resulting in only 19.4% or 73.9 g/kg DM of bypass protein into the small intestine. Van Straalen and Tamminga (1990) reported the degradation rate of 12.8%/h, bypass protein of 24% of 3 mm raw *lupinus albus* meal at assumed passage rate of 6%/h, Yu et al. (1998b) reported the degradation rate of 10.2%/h, 25.9% of bypass protein at assumed passage of 6%/h of 3 mm raw *lupinus albus* meal and Goelema (1994) reported that the raw *lupinus albus* degradation characteristics of 20.0% or 77.7 g/kd of bypass protein, 80.0% of effective degradability, were all quite close to the present result as shown in table 5. But Cros et al. (1991) reported the degradation rate of 22.1%/h, bypass protein of 4.9% at the assumed passage of 6%/h of 1 mm *lupinus albus* meal, Aguilera et al. (1992) reported the degradation rate of 9.8%/h, effective degradability of 90.3% at measured passage of 1.54%/h of 2 mm raw *lupinus albus* meal and Kibelolaud et al. (1993) reported degradation rate of 19.2%/h, bypass protein of 6.6% at assumed of 6%/h of 1 mm *lupinus albus* (table 5). The above three published results indicated the raw *lupinus albus* had even more degradation characteristics than ours. Published and present results clearly indicate that raw *lupinus albus* do has a rapid degradation rate with little bypass protein going to the small intestine, and that the rumen degradation characteristics of *lupinus albus* largely depend on rumen incubation procedures such as feed preparation (particle size 1 mm, 2 mm or 3 mm) (finely ground feed is usually rapidly degraded), nylon bag pore size and assumed or measured passage rate value. Therefore international standard rumen incubation procedures for dairy cows need to be developed for comparing different feed, different lab, different processing methods.

Pressure toasting increased the flow of bypass protein to the small intestine with increasing times and temperatures by 73.7, 115 and 141% at temperatures of 100, 118 and 136℃. This was mainly caused by a reduction in the soluble fraction by 19.7, 34.0 and 35%, reducing degradation rate by 46.5, 51.1 and 31.0% and dramatically increasing rumen undegradable fraction by -58, 1506 and 7047% at toasting temperatures of 100, 118 and 136℃. These were similar to the results obtained by Goelema (1994) who showed that pressure toasting at 100℃ and 130℃ increased the amount of bypass crude protein by decreasing the soluble nitrogen fraction and decreasing the rate of degradation of the rumen undegradable fraction.

In present study, pressure toasting only increased potential degradation fraction at 100 and 118℃ but failed to increase it at 136℃. The reason for this is not clear but might be due to a large amount of the undegradable fraction increased at 136℃. This findings is close to the result by Yu et al. (1998b) that dry roasting only increased the potential degradation fraction at 110 and 130℃ but failed to increase it at 150℃. Increasing bypass feed protein were mainly a reduction in the soluble fraction and its degradation rate and dramatically increasing the undegradable fraction. However, Yu et al. (1996) showed that increasing rumen bypass protein of whole horse beans seeds was mainly caused by pressure toasting reducing the soluble fraction and degradation rate, and largely increasing potential degradation fraction (D) without changing undegradable fraction. All these results indicate that different feedstuffs (such as horse bean, lupin) have different responses (such as rumen

Table 5. Protein degradation of *lupinus albus*

Authors	Particle size	Kd (%/h)	Kp (%/h)	EDCP (%)	BCP (%)
Yu et al. (1998b)	3 mm	10.2	6	74.1	25.9
Goelema (1994)	3 mm	-	6	80.0	20.0
Kibelolaud et al. (1993)	1 mm	19.2	6	93.4	6.6
Aguilera et al. (1992)	2 mm	9.8	1.54	-	90.3
Cros et al. (1991)	1 mm	22.1	6	95.1	4.9
Van Straalen et al. (1990)	3 mm	12.8	6	76.0	24.0

degradation characteristics) to the same heating treatment (such as pressure toasting).

In the study of Goelema (1994), the rumen undegradable fraction of pressure toasted *lupinus albus* was assumed the same and fixed in all treatments to obtain the bypass feed protein. This was not case in present study that the rumen undegradable fraction was dramatically increased by pressure toasting.

Pressure toasting decreased microbial protein synthesised from available energy by 9%, 13% and 16% at toasting temperatures of 100, 118 and 136℃, respectively. This was mainly due to reduction in fermented organic matter of 11, 15 and 17%. However the true protein supplied to the small intestines was increased by 27, 44 and 56%. This occurred because pressure toasting increased the amount of bypass protein which largely overcompensated reduced microbial protein synthesis, resulting in increasing net total protein supplied to the small intestine. Similar results were found by Yu et al. (1998c) with dry roasted lupin seeds.

Intestinal digestion

Intestinal digestibility (87.7%) of bypass feed protein and endogenous protein losses in the digestive tract were not affected by pressure toasting. The absorbed bypass protein in the small intestine increased by 76, 114 and 139% at 110, 118 and 136℃, respectively, by pressure toasting. These results indicated that pressure toasting up to 136℃ increased protein digestion in the small intestine. It suggests that higher pressure toasting temperatures should be examined.

In this study, there were not significant differences between treatments on the digestibility of bypass feed protein. In contrast, Cros et al. (1991) reported that the intestinal digestibility of the rumen undegraded protein, using the mobile bag technique, were increased by extrusion from 63.2 (raw), to 91.2, 94.9 and 95.1% as the temperature rose to 120, 150 and 195℃, respectively. The digestibility of bypass feed protein in raw samples was much lower than in the present study. Yu et al. (1998c) had earlier shown that the estimated intestinal digestibility of rumen undegraded feed protein, using optimal three-step optimal procedures, was also increased from 87.0 (raw) to 88.9, 91.5 and 93.0% as dry roasting temperatures rose to 110, 130 and 150℃, respectively. The reason for such a contrasting pattern of intestinal digestibility in response to heat treatment is still not clear. It may be due to different processing and heating methods which will change the physical and chemical structure of *lupinus albus* to the different degrees.

In this study, the preincubation of 12 h was based on the findings by IVVO (Van Straalen and Tamminga, 1990) that rumen retention time of 8 or 16 h (for using the mobile bag technique to measure intestinal digestibility) and by Yu et al. (1998c) that rumen retention times of 8, 12 and 24 h (for using optimal three-step in vitro procedure to estimate intestinal digestibility) had no significant effect on the intestinal digestibility of rumen undegradable protein of lupin seeds. But it should be remembered that the effect of rumen retention time on intestinal digestion of rumen undegraded feed protein is variable and depends on the feedstuffs (Hvelplund, 1985; Rooke, 1985; Voigt et al., 1985; De Boer et al., 1987; Liu et al., 1994).

The effect of pressure toasting on the amino acid profile of *lupinus albus*, and the influence of possible changes on digestion in the rumen and small intestine need to be further investigated.

CONCLUSION

Pressure toasting was effective in shifting the degradation of the crude protein of *lupinus albus* from rumen to small intestine without changing intestinal digestion. Although no optimal pressure toasting condition could be determined in this study, it seems that 136℃/15 min was the best treatment due to its largest TAP (DVE) and lowest PDB (OEB) values.

Additional study information is required concerning the effects of pressure toasting on the degradation and digestion of individual amino acids, especially the first limiting amino acids, and possible effects on animal performance.

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