Influence of Dry Roasting of Whole Faba Beans *(Vicia faba)* and Whole Lupin Seeds *(Lupinus albus)* on Rumen Disappearance and Estimated Intestinal Digestion of CP Using the Optimal Three-Step *In Vitro* Technique in Dairy Cows

P. Yu*, A. R. Egan and B. J. Leury
Dept. of Animal Science, Institute of Land and Food Resources, University of Melbourne
Parkville, Victoria 3052, Australia

ABSTRACT : The effects of dry roasting whole faba beans (WFB) and whole lupin seeds (WLS) at 110, 130 or 150℃ for 15, 30 or 45 min on rumen (RDCP%), estimated intestinal (IDCP%) and total tract disappearance of CP (TDCP%) and intestinal availability (IARUCP%) of rumen undegraded CP (RUCP%) were determined. The RDCP values were estimated by *in sacco* technique by incubating nylon bags for 8, 12 and 24 h in the rumen of dairy cows. The IDCP and IARUCP values were estimated using a sequence of ruminal incubation, *in vitro* incubation in acid-pepsin for 1 h and then in pancreatin for 24 h of three-step *in vitro* procedure technique. Dry roasting at 130 and 150℃ decreased RDCP with correspondingly increasing IDCP. The IDCP value generally increased from 12.3 (raw) to 8.6, 14.8 and 39.6% (WFB) and from 28.3 (raw) to 33.7, 36.2 and 56.2% (WLS) at 8 h rumen incubation; from 2.9 (raw) to 2.9, 4.6 and 23.3% (WFB) and from 19.6 (raw) to 19.0, 24.0 and 46.6% (WLS) at 12 h rumen incubation; from 1.3 (raw) to 1.9, 1.7 and 11.0% (WFB) and from 4.4 (raw) to 4.2, 10.7 and 36.7% (WLS) at 24 h rumen incubation as the temperatures rose to 110, 130 and 150℃, respectively. The TDCP values were always high and increased by time in the rumen, the average values of which were 97.9, 96.6; 99.2, 96.9 and 99.6, 98.7% for WFB and WLS, respectively, at 8, 12 and 24 h rumen incubation. But within the same retention time, TDCP was generally unchanged. The average IARUCP increased from 87.3 (raw) to 87.4, 88.7 and 92.0% (WFB); from 87.6 (raw) to 88.9, 91.5 and 93.0% (WLS) at roasting temperatures of 110, 130 and 150℃, respectively. It was concluded that dry roasting can shift the digestion of CP from rumen to the lower gastrointestinal tract without depressing the digestion of RUCP. The best processing condition in this study was dry roasting at 150℃ for 45 min in terms of effects on the disappearances and availability of CP. Research data on intestinal availability of individual amino acids need to be further investigated. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 7 : 1054-1062*)

Key Words : Lupin Seed, Faba Bean, Intestinal Digestion, Rumen Degradation, Three-Step *In Vitro* Technique

INTRODUCTION

Yu et al. (1998) have shown that dry roasting of WFB and WLS at temperature of 130 or 150℃ for 15 to 45 min, is an effective method of reducing RDCP thus increasing RUCP, but no studies have been published concerning the effects of dry roasting on IDCP, as well as the IARUCP of WFB and WLS. Therefore no conclusion of optimal processing conditions are available for feed processing industry. Considering the improvement in RUCP that results from duration of (15 to 45 min) dry roasting at temperature (130 to 150℃), it is of value to determine the availability of the protein fraction of roasted WFB and WLS throughout the gastro-intestinal tract. Such information is critical if a commercial WFB and WLS supplement high in RUCP are to be developed by feed industry using a dry roasting heating process for dairy cows.

There are several ways to measure or estimate protein postruminal digestion such as *in vivo*, mobile bag technique and *in vitro* techniques, of which the majority are only applicable to the mixture of undegraded feed protein, microbial protein and endogenous protein. None of them meet all the technique criteria as stated by Calsamiglia and Stern (1995), which should 1) closely simulate physiological conditions of ruminants, including potential effects of ruminal fermentation; 2) be rapid, reliable, and inexpensible; 3) be applicable to a wide variety of protein supplements and 4) accurately reflect differences in protein digestion.

However, the three-step *in vitro* procedure, using optimal conditions of pepsin-pancreatin, provide an alternative to the use of intestinally cannulated animals for estimating intestinal digestion of individual protein supplements. The results (Calsamiglia and Stern, 1995) showed that this new three-step procedures closely simulated physiological conditions in the animal. It was sensitive to heat damage of feed protein. Compared with *in vivo* determination of intestinal digestion of CP, the three-step *in vitro* procedure provided evidence of reliability, resulted in a substantial reduction in cost and labor and could be routinely used for screening intestinal digestion of

* Corresponding Author: P. Yu.
Received September 24, 1998; Accepted February 18, 1999

Table 1. Treatments and the dry roasting conditions of whole faba beans (WFB) and whole lupin seeds (WLS) (where, each treatment was measured at least 5 times)

Treatments	Dry roasting					
	WFB		WLS, A series		WLS, B series	
	Temp. (℃)	SD	Temp. (°C)	SD	Temp. (°C)	SD
Raw	RWFB (control)		RWLS, A (control)		RWLS, B (control)	
110℃/15 min	110.0	0.0	110.0	1.0	111.0	1.0
110℃/30 min	111.3	1.2	109.5	0.5	111.0	0.0
110℃/45 min	110.9	1.6	109.5	0.5	111.0	1.0
130℃/15 min	130.0	0.0	129.5	0.5	130.0	0.0
130℃/30 min	129.8	0.5	130.0	0.0	130.5	0.5
130℃/45 min	130.0	0.0	131.0	1.0	130.5	0.5
150℃/15 min	149.5	0.7	150.0	0.0	150.0	0.0
150℃/30 min	150.0	0.0	149.5	0.5	150.0	0.0
150℃/45 min	150.0	0.0	149.5	0.5	150.0	0.0

Table 2. The chemical compositions of raw and dry roasted whole faba beans (WFB) and whole lupin seeds (WLS)

Temp. (℃)		110			130			150		
Time (min)	Raw	15	30	45	15	30	45	15	30	45
Whole faba beans										
DM (g/kg)	885.90	895.60	900.70	910.20	919.00	920.60	923.40	924.10	935.30	941.00
CP (g/kg DM)	317.33	317.45	319.89	318.82	323.04	324.25	318.19	322.00	320.35	310.37
Ash (g/kg DM)	34.77	34.17	33.75	33.40	33.73	34.22	34.33	35.28	34.00	34.22
OM (g/kg DM)	965.23	965.83	966.25	966.60	966.27	965.78	965.67	964.72	966.00	965.78
CF (g/kg DM)	20.42	19.87	18.67	17.12	18.20	16.34	15.98	16.24	14.57	13.98
Starch (g/kg DM)	410.99	415.14	412.12	420.06	404.46	402.13	407.73	401.15	395.06	400.74
Whole lupin seeds										
DM (g/kg)	921.26^{ab}	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}
CP (g/kg DM)	386.53^{a}	387.88^{a}	380.64^{a}	377.29^{a}	379.75^{a}	384.94^{a}	380.07^{a}	392.34^{a}	382.06^{a}	386.05^{a}
Ash (g/kg DM)	27.25^{a}	28.32^{a}	26.21^{a}	27.53^{a}	29.40^{a}	26.78^{a}	26.88^{a}	28.30^{a}	26.15^{a}	25.84^{a}
OM (g/kg DM)	972.75^{a}	971.69^{a}	973.79^{a}	972.48^{a}	970.61^{a}	973.22^{a}	973.13^{a}	971.70^{a}	973.86^{a}	974.16^{a}
CF (g/kg DM)	53.88^{a}	53.21^{ab}	53.13^{ab}	50.68^{abc}	49.68^{bc}	48.43^{cd}	45.33^{de}	44.06^{e}	43.90^{e}	41.68^{e}

Means with different letters in the same row are significantly different ($p<0.05$) (Tukey Studentized Range (HSD) Test).

proteins in ruminants. The use of estimates of intestinal digestion in combination with estimates of protein degradation in the rumen may provide estimated values of intestinally absorbable dietary protein derived from individual ingredients. These data would be very useful for quality control of processed proteins and for determining an overall value of protein supplements for ruminants.

The objectives of this study were to a) estimate intestinal digestion of RUCP using optimal three-step *in vitro* procedure; b) determine the effects of dry roasting on IARUCP; c) determine the effects of dry roasting on the heat damage protein; d) determine the effects of rumen preincubation on pepsin-pancreastin digestion of feed protein and e) determine the optimal processing conditions.

MATERIALS AND METHODS

Feedstuffs

Whole faba beans (*Vicia faba*) and WLS (*Lupinus albus*) were obtained from commercial feed company (Peter Gibbs Stock Feeds, Australia). The chemical compositions are shown in table 2.

Technological treatments

Raw WFB and WLS were dry roasted at 3 different temperatures (110, 130 and 150℃) for 15, 30

and 45 min in a complete block design (with A and B series for WLS) as shown in table 1. The A and B series were used to test whether the conditions of dry roasting were kept constant and reliable.

Raw WFB and WLS were used as controls. 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 though a 3 mm screen (Hammer mill, AEG TYP AM80N*2).

Animals and diets

The rumen preincubation of WFB and WLS were separately carried out in the different dairy research centers.

For WFB rumen incubation, six dry Holstein Friesian cows, of average weight 620 kg previously equipped with a rumen cannula with an internal diameter of 10 cm (silicon rubbers, handmade) were used at Kyabram Dairy Center (Victoria, Australia) in the feedlot. The detail of the animal and diets was described by Yu et al. (1998).

For WLS rumen incubation, six dry Holstein Friesian cows, of average weight 580 kg and previously equipped with a rumen cannula were used at Ellinbank Dairy Center in the grass land with group feeding. The cows received a diet consisting of crushed barley and oaten hay. Water was always available. The cows were fed twice daily at 08:30 and 16:30. A 12 d period of adaptation was allowed.

The animal used in these experiments were cared for in accordance with the guidelines established by Australian Council of Animal Care.

Rumen preincubation

Approximately 5 g DM of test feeds were weighted into nylon bags (1017 cm) with the pore size of approximately 44 μm (Switzerland 1807710014 I 044 Nytal ASTM 325-44) and suspended for 8, 12 and 24 h in the rumen of cannulated cows.

Depending on CP content and degradability of the feed tested, 8 to 15 bags were required to provide at least 6 g of residual per feed treatment.

The detail of rumen preincubation procedures was described by Yu et al. (1998).

Intestinal digestion of the residual CP was determined using optimal conditions of three-step *in vitro* procedure. Results were compared with those obtained when identical samples were processed without ruminal exposure.

Determining intestinal digestion

Whole faba beans and WLS were evaluated to determine the sensitivity of the technique to heat-damaged proteins and the effect of dry roasting on intestinal digestion of RUCP using the three-step *in vitro* procedure described as the following.

1) Reagents

Pepsin: Sigma P-7012, 1:60,000, activity: 2,500-3,500 units per mg protein; Pancreatin: Sigma P-7545, activity equivalent to 8U.S.Pharmacopeia (USP) specifications; Thymol: Sigma T-0501; 0.1 N HCl containing 1 g/L of pepsin, pH 1.9; 1 N NaOH; 0.5 M phosphate solution, pH 7.8, containing 3 g/L of pancreatin and 50 ppm of thymol; 100% (wt/vol) trichloroacetic acid (TCA) solution.

2) Procedure

The first step : Nylon bags containing tested feed samples (3 mm) were suspended in the rumen (see the section of Rumen preincubation in detail).

The second step (pepsin digestion) : Weighed the rumen residue and nonruminal preincubation samples to contain 15 mg of N into a 50 ml centrifugation tube; Added in 10 ml of a 0.1N HCl solution containing 1 g/l of pepsin, at pH 1.9, vortexed and incubated for 1 h in a 38℃ shaking water bath.

The third step (pancreatin digestion) : After incubation, added 0.5 ml of a 1N NaOH solution and 13.5 ml of a pancreatin solution (0.5M KH2PO4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/L of pancreatin; Samples were vortexed and incubated in a shaking water bath (38℃) for 24 h. Samples were vortexed approximately every 8 h; After a 24 h incubation, 3 ml of a 100% (wt/vol) trichloroacetic acid solution was immediately added to the tubes to stop enzyme action and precipitate undigested proteins; All tubes were vortexed and allowed them to stand for 15 min; Samples were centrifuged at 10,000*g* for 15 min and analyzed insoluble N.

Chemical analysis and calculation

1) Chemical analysis

Feed treatments and rumen residues of 8, 12 or 24 h were analyzed for ash, DM and N content. DM was determined by drying at 105℃ to constant weight. Ash was determined by ashing at 550℃ to constant weight. N of feed and rumen residues were analyzed by NCS instruments (NA 1500 NCS FISONS) and N of three-step *in vitro* digestion residuals were analyzed by the Kjeldahl method and CP content were obtained by N multiplication by 6.25. Previous study showed that using NCS instruments and using Kjeldahl method to analyze N had the same accuracy. Starch in WFB was analyzed according to Yu (1995). Crude fat (CF)

in WFB and WLS was determined by AOAC (1984).

2) Calculation

Rumen disappearance of CP: RDCP for each incubation (8, 12 or 24 h) was percent of initial, calculated as RDCP (%) = (CP-RUCP)*100/CP, where RUCP after 8, 12 or 24 h incubation.

Estimated intestinal availability of RUCP: IARUCP was calculated as IARUCP (%) = (RUCP-RRUCP)/ RUCP*100, where RRUCP is the residual CP after rumen incubation plus estimated intestinal digestion by three-step *in vitro* technique.

Estimated total tract disappearance: sum of rumen plus estimated intestinal disappearance of CP were percent of initial, calculated as TDCP (%) = (CP-RRUCP)/CP *100.

Estimated intestinal disappearance: IDCP by three-step *in vitro* technique was calculated as IDCP (%) = TDCP-RDCP.

Statistical analysis

Results are analyzed as a completely randomized design using the GLM procedures of SAS (1991). Statistical differences were declared at $p<0.05$ using Tukeys Test (SAS, 1991).

RESULTS

The chemical compositions of raw and dry roasted WFB and WLS are presented in table 2. Dry roasting significantly increased DM contents and decreased CF ($p<0.05$). This could be attributed to the water evaporating. Dry roasting did not significantly affected CP, starch and ash ($p>0.05$). The lower CF content of dry roasted WLS and WFB agreed with the results observed by others (Cros et al., 1991; Kibelolaud et al., 1993). Due to very lower starch content in WLS, no starch chemical analysis results were given.

The rumen and intestinal disappearance of CP of

Table 3. Disappearance of CP of raw and dry roasted whole faba beans (WFB) and estimated intestinal availability of rumen undegraded CP (RUCP) after 8, 12 and 24 h of *in sacco* rumen incubation, using optimal three-step *in vitro* procedure

Temp. (℃)		110			130			150		
Time (min)	Raw	15	30	45	15	30	45	15	30	45
8 h incubation										
RDCP%	85.30^{c}	92.28^{a}	89.78^{ab}	87.85^{bc}	86.13^{bc}	84.82^{c}	78.87^{d}	75.10^{d}	62.08^{e}	35.92^{f}
	(0.15)*	(0.47)	(1.66)	(0.39)	(0.40)	(1.60)	(0.64)	(1.79)	(1.78)	(3.26)
IDCP%	12.26	6.62	8.13	11.07	12.38	12.61	19.39	22.00	35.68	61.09
TDCP%	97.56	98.90	97.91	98.92	98.51	97.43	98.26	97.01	97.76	97.01
	(0.16)	(0.22)	(0.22)	(0.21)	(0.15)	(0.32)	(0.29)	(0.20)	(0.74)	(0.75)
IARUCP%	90.25^{bc}	85.82^{c}	89.32^{bc}	91.15^{ab}	89.24^{bc}	89.66^{bc}	91.76^{ab}	92.00^{ab}	94.08^{ab}	95.32^{a}
	(0.99)	(2.84)	(2.14)	(1.77)	(1.10)	(2.11)	(1.38)	(0.81)	(1.94)	(1.17)
12 h incubation										
RDCP%	96.67^{ab}	97.15^{a}	97.48^{a}	95.55^{abc}	95.81^{abc}	95.47^{abc}	93.45^{bc}	92.87^{c}	81.91^{d}	50.05^{e}
	(0.25)	(0.20)	(0.24)	(2.42)	(0.32)	(0.18)	(0.10)	(0.59)	(1.57)	(2.72)
IDCP%	2.90	2.53	2.17	4.03	3.75	4.09	5.86	6.28	16.33	47.14
TDCP%	99.57^{a}	99.68^{a}	99.65^{a}	99.58^{a}	99.56^{a}	99.56^{a}	99.31^{a}	99.15^{a}	98.24^{b}	97.19^{c}
	(0.11)	(0.08)	(0.03)	(0.05)	(0.07)	(0.10)	(0.17)	(0.21)	(0.30)	(0.46)
IARUCP%	87.09^{b}	88.56^{ab}	86.23^{b}	87.56^{b}	89.38^{ab}	90.42^{ab}	89.43^{ab}	88.05^{ab}	90.30^{ab}	94.29^{a}
	(3.38)	(3.00)	(0.97)	(1.46)	(1.81)	(2.14)	(2.65)	(2.96)	(1.64)	(0.94)
24 h incubation										
RDCP%	98.52^{a}	98.37^{a}	98.35^{a}	96.98^{a}	98.25^{a}	97.90^{a}	98.05^{a}	98.82^{a}	94.60^{b}	71.59^{c}
	(0.04)	(0.12)	(0.09)	(2.29)	(0.14)	(0.03)	(0.16)	(0.12)	(0.16)	(0.76)
IDCP%	1.25	1.36	1.41	2.82	1.51	1.81	1.65	0.89	4.91	27.14
TDCP%	99.77^{a}	99.73^{a}	99.76^{a}	99.80^{a}	99.76^{a}	99.71^{a}	99.70^{a}	99.71^{a}	99.51^{a}	98.73^{b}
	(0.02)	(0.04)	(0.02)	(0.04)	(0.08)	(0.05)	(0.04)	(0.04)	(0.10)	(0.29)
IARUCP%	84.76^{b}	83.78^{b}	85.93^{b}	88.17^{b}	86.32^{b}	86.65^{b}	85.31^{b}	87.48^{b}	90.86^{ab}	95.82^{a}
	(1.39)	(2.81)	(1.28)	(2.93)	(4.57)	(2.32)	(2.55)	(2.17)	(1.83)	(0.84)

Notes: * : standard deviation; Means with different letters in the same row are significantly different ($p<0.05$) (Tukey Studentized Range (HSD) Test); RDCP: rumen disappearance of CP; IDCP: estimated intestine disappearance of CP; TDCP: estimated total tract disappearance of CP; IARUCP: estimated intestinal availability of rumen undegraded CP.

Table 4. Disappearance of CP of raw and dry roasted white lupin seeds (WLS) and estimated intestinal availability of rumen undegraded CP (RUCP) after 8, 12 and 24 h of *in sacco* ruminal incubation, using optimal three-step *in vitro* procedure

Temp. (℃)		110			130			150		
Time (min)	Raw	15	30	45	15	30	45	15	30	45
8 h incubation										
RDCP	68.21^{a}	62.71^{ab}	63.31^{ab}	62.02^{ab}	62.10^{ab}	60.58^{ab}	59.60^{ab}	50.08^{bc}	39.66^{cd}	31.83^{d}
	(3.21)*	(2.83)	(3.59)	(2.43)	(4.76)	(0.79)	(3.02)	(7.37)	(0.66)	(7.42)
IDCP	28.32	33.92	33.00	34.17	35.21	35.97	37.34	46.63	56.90	64.92
TDCP	96.53	96.63	96.31	96.19	97.31	96.55	96.94	96.71a	96.56^{a}	96.75^{a}
	(0.61)	(0.62)	(0.94)	(0.48)	(0.40)	(0.75)	(0.47)	(0.95)	(1.35)	(1.40)
IARUCP	89.81^{ab}	90.44^{ab}	91.30^{ab}	89.49^{b}	92.55^{ab}	91.71^{ab}	92.33^{ab}	93.31^{ab}	94.67^{ab}	95.16^{a}
	(1.79)	(1.75)	(1.09)	(1.32)	(0.76)	(2.37)	(1.76)	(2.43)	(2.34)	(2.56)
12 h incubation										
RDCP	76.80^{a}	79.88^{a}	74.30^{a}	80.27^{a}	73.10^{a}	71.64^{a}	74.37^{a}	66.36^{ab}	49.11^{bc}	34.78^{c}
	(8.76)	(3.08)	(4.80)	(1.10)	(7.25)	(0.59)	(7.28)	(4.33)	(1.24)	(2.07)
IDCP	19.59	16.93	22.36	17.77	24.05	25.40	22.41	30.74	47.45	61.56
TDCP	96.39	96.81	96.66	98.04	97.15	96.86	96.78	97.10^{a}	96.56^{a}	96.34^{a}
	(0.27)	(0.43)	(0.42)	(0.20)	(0.24)	(0.73)	(0.55)	(0.74)	(1.14)	(1.22)
IARUCP	87.39^{cd}	85.71^{d}	88.20^{bcd}	89.64^{abcd}	91.10^{abc}	88.76^{bcd}	89.88^{abcd}	90.50^{abcd}	93.17^{ab}	94.26^{a}
	(1.07)	(1.93)	(1.97)	(1.04)	(0.77)	(2.59)	(1.42)	(2.42)	(2.29)	(1.92)
24 h incubation										
RDCP	95.22^{a}	94.06^{ab}	94.10^{ab}	97.98^{a}	95.35^{a}	82.70^{ab}	86.06^{ab}	77.47^{b}	55.81^{c}	49.07^{c}
	(3.22)	(3.54)	(3.56)	(1.22)	(1.80)	(8.85)	(4.60)	(1.00)	(0.70)	(6.71)
IDCP	4.42	5.49	5.51	1.72	3.47	15.51	13.24	19.89	41.98	48.29
TDCP	99.64^{a}	99.55^{a}	99.61^{a}	99.70^{a}	98.82^{ab}	98.21^{ab}	99.30^{ab}	97.36^{b}	97.79^{ab}	97.36^{b}
	(0.07)	(0.10)	(0.16)	(0.21)	(1.28)	(0.51)	(0.69)	(0.28)	(1.26)	(0.89)
IARUCP	85.54^{a}	86.84^{a}	88.30^{a}	89.77^{a}	90.50^{a}	94.28^{a}	92.58^{a}	89.04^{a}	94.93^{a}	95.25^{a}
	(2.88)	(2.74)	(4.65)	(7.37)	(6.58)	(2.02)	(4.45)	(0.65)	(2.87)	(1.59)

Notes: * : standard deviation; Means with different letters in the same row are significantly different ($p<0.05$) (Tukey Studentized Range (HSD) Test); RDCP: rumen disappearance of CP; IDCP: estimated intestine disappearance of CP; TDCP: estimated total tract disappearance of CP; IARUCP: estimated intestinal availability of rumen undegraded CP.

raw and dry roasted WFB and WLS after 8, 12, and 24 h of *in sacco* ruminal incubation are shown in table 3 and 4. The RDCP value decreased by dry roasting at 130 and 150℃. Rumen disappearance generally decreased from 85.3 (raw) to 89.9, 83.3 and 57.7% (WFB) and from 68.2 (raw) to 62.7, 60.8 and 40.5% (WLS) at 8 h rumen incubation; from 96.7 (raw) to 96.7, 94.9 and 74.9% (WFB) and from 76.8 (raw) to 78.2, 73.0 and 50.1% (WLS) at 12 h rumen incubation; from 98.5 (raw) to 97.9, 98.1 and 88.3% (WFB) and from 95.2 (raw) to 95.4, 88.0 and 60.8% (WLS) at 24 h rumen incubation as the temperature rose to 110, 130 and 150℃, respectively.

The IDCP value generally increased by dry roasting. Estimated intestinal disappearance generally increased from 12.3 (raw) to 8.6, 14.8 and 39.6% (WFB) and from 28.3 (raw) to 33.7, 36.2 and 56.2% (WLS) at 8 h rumen incubation; from 2.9 (raw) to 2.9, 4.6 and 23.3% (WFB) and from 19.6 (raw) to 19.0, 24.0 and 46.6% (WLS) at 12 h rumen incubation; from 1.3 (raw) to 1.9, 1.7 and 11.0% (WFB) and from 4.4 (raw) to 4.2, 10.7 and 36.7% (WLS) at 24 h rumen incubation as the temperature rose to 110, 130 and 150℃, respectively.

The TDCP value were always high (97.0 to 99.7%; 96.1 to 99.6% for WFB and WLS, respectively). The TDCP data increased by time in the rumen, average of which were 97.9, 96.6; 99.2, 96.9 and 99.6, 98.7% for WFB and WLS, respectively, after 8, 12 and 24 h rumen incubation.

In general, dry roasting decreased RDCP and increased IDCP which resulted in TDCP being not depressed by dry roasting.

The IARUCP value of dry roasted WFB and WLS after 8, 12 and 24 h *in sacco* rumen incubations were presented in Table 3 and 4. The average IARUCP increased from 87.3 (raw) to 87.4, 88.7 and 92.0 (WFB); from 87.6 (raw) to 88.9, 91.5 and 93.0 (WLS) as temperature rose to 110, 130 and 150℃, respectively.

Table 5. Digestion (%) of CP of raw and dry roasted whole faba beans (WFB) and whole lupin seeds (WLS), using three-step *in vitro* procedure but without rumen preincubation

Temp. (℃)		110			130			150		
Time (min)	Raw	15	30	45	15	30	45	15	30	45
Whole faba beans										
Digestion, *in vitro*	80.98	82.01	79.89	81.83	82.01	81.89	79.87	82.25	79.29	81.95
	(1.12)*	(2.39)	(3.35)	(1.67)	(1.83)	(2.56)	(2.96)	(1.81)	(1.60)	(1.80)
Whole lupin seeds										
Digestion, *in vitro*	86.34	87.65	85.51	85.03	86.38	87.30	86.43	86.15	85.98	87.30
	(1.45)	(2.33)	(2.97)	(1.98)	(2.34)	(1.45)	(2.57)	(2.76)	(2.92)	(1.82)

Notes: * : standard deviation. Means with different letters in the same row are significantly different ($p<0.05$) (Tukey Studentized Range (HSD) Test).

The digestion of CP of raw and dry roasted WFB and WLS, using three-step *in vitro* procedure but without rumen preincubation is in table 5. There were no significant difference between treatments for both WFB and WLS. The average of CP digestion were 81.2% for WFB and 86.4% for WLS. But compared with the digestion of CP after rumen incubation (tables 3 and 4), the digestion of CP in all treatments for WFB and WLS, no matter how long the rumen retention time was, significantly increased (88.3 and 91.3% for WFB and WLS, respectively). It indicated the preincubation is an important step in the three-step *in vitro* procedure to measure CP disappearances.

DISCUSSION

Protein postruminal digestion measurements

Duodenally cannulated animals, preferably with cannulae in the beginning and at the end of the small intestine, provide information on the apparent absorption of intestinal protein. Alternatives are to infuse protein sources in the abomasum or at the beginning segment of the small intestine and to measure the increased faecal protein output (Schwarting and Kaufmann, 1978) or increased ileal protein flow (Hvelplund, 1985). A limitation is that infusion is not restricted to protein, but that the feedstuffs also contain DM other than protein, which causes the release of extra endogenous protein. This is likely to result in an underestimation of the true absorption, particularly if the increased ileal flow is measured (Van Straalen and Tamminga, 1990). Also large variation in *in vivo* intestinal digestion among protein supplements also has been reported (Stern et al., 1985; Waltz et al., 1989). Obtaining estimates of protein digestion in the small intestine is expensive and labor-intensive and it requires the use of surgically prepared animals.

A recently developed method is the mobile nylon bag technique (Sauer et al., 1983). With this method, small quantities of feedstuffs are included in small nylon bags, incubated in the rumen and subsequently introduced into the beginning segment of the small intestine and after passage though the intestinal tract, recovered from the faeces or ileum. This technique has a high potential value, because the capacity to evaluate individual feedstuffs is quite high. However, although this technique uses a more physiological approach (Hvelplund, 1985) and results from fecal collection of bags showed reasonable correlation with *in vivo* intestinal CP digestion (r=0.81, Hvelplund, 1985), an interaction between type of feed and site of collection (ileal vs. fecal) has been reported (Hvelplund, 1985) and may invalidate these results. Hvelplund (1985) calculated that amount of protein within the nylon bags that was digested in the large intestine was 50 and 27% of that leaving the ileum for soybean meal and rapeseed meal, respectively. In addition, bag pore size, animal diet, large intestinal fermentation and bacterial contamination may also contribute to variation (Hvelplund, 1985; Rooke, 1985; Voigt et al., 1985).

Various *in vitro* methods that have been developed, including ADIN (Goering et al., 1972), enzymatic procedures (Britton et al., 1987). None of them meet all the technique criteria as stated before.

The newly developed optimal three-step *in vitro* procedure (Calsamiglia and Stern, 1995), closely simulated physiological conditions in the animal, provided evidence of reliability, resulted in a substantial reduction in cost and labor and could be routinely used for screening intestinal digestion of proteins in ruminants. The use of estimates of intestinal digestion in combination with estimates of protein degradation in the rumen may provide estimated values of intestinally absorbable dietary protein derived from individual ingredients as stated before.

Rumen disappearance

Dry roasting reduced RDCP as the temperature

rose to 130 and 150℃ at 8, 12 and 24 h rumen incubation for both WFB and WLS. This confirms that heating reduced RDCP in the reports (Yu, 1995; Yu et al., 1996). The results indicated that each seed has their own degradation characteristics. The degradation of WFB was higher than that of WLS. Dry roasting had more effects on WLS than on WFB and reduced rumen degradability of WLS higher than of WFB. The RDCP value was significantly higher for raw feeds than for dry roasted feeds as a temperature to 130 and 150℃. However, with longer incubations, the effect of dry roasting gradually decreased. This is consistent with other reports (Cros et al., 1991) dealing with extruded WLS.

Intestinal disappearance

Treatment of feedstuffs with heat can reduce RDCP and also IDCP when feedstuffs are overprotected. In this study, dry roasting of WFB and WLS significantly depressed RDCP, the TDCP value was not significantly affected. Consequently, IDCP increased with increasing temperature and roasting time. No consistant effects of dry roasting on TDCP of dry roasted WFB and WLS were observed within each incubation time. Therefore a compensation occurs between rumen and intestinal CP disappearance resulting in a very high CP digestibility in the whole tract for both WFB (98.8%) and WLS (97.4%). These findings observed were similar to those obtained by others using the mobile nylon bag technique with various seeds: canola meal (Moshtaghi and Ingalls, 1992), lupin (Cros et al., 1991; Kibelolaud et al., 1993) and horse beans (Cros et al., 1992).

Cros et al. (1991) reported that extruding WLS increased IDCP from 8.1 (control) to 10.6, 15.6 and 26.8% as the temperature of the extrusion rose to 120, 150 and 195℃, respectively without depressing TDCP (97.5 to 98.7%) of N. Arieli et al. (1989) also showed that extrusion tended to elevate the intestinal digestibility of N from 64 to 76%. In contrast, the study by Arieli et al. (1989) showed that processing cottonseeds at 180℃ diminished the intestinal digestibility (22 vs. 64%). The study by Calsamiglia and Stern (1995) show that heating soybean meal at 165℃ for more than 2.5 h resulted in a decrease in estimated intestinal CP digestion, using three-step *in vitro* procedure.

Robinson and Tamminga (1984), separated feedstuffs into protein sources in which TDCP is unaffected or increased by time in the rumen. The data of protein sources of WFB and WLS obtained in this study fell into their second category that TDCP increased by time in the rumen. In contrast, Kibelolaud et al. (1993) reported that TDCP of extruded WLS was unaffected by incubation time. These indicate that processing method had different effects on TDCP even in the same feedstuff.

The average IARUCP using optimal three-step *in vitro* procedure was high and increased from 87.3 (raw) to 87.4, 88.7 and 92.0 (WFB); from 87.6 (raw) to 88.9, 91.5 and 93.0 (WLS) as dry roasting temperature rose to 110, 130 and 150℃, respectively. These results are in agreement with Kibelolaud et al. (1993) who observed that IARUCP of extruding WLS were very high for raw (85.6%) and extruded (98.2%) WLS and Cros et al. (1991) who reported that IARUCP, using mobile bag technique, increased by extrusion from: 63.2, 91.2, 94.9 and 95.1% as the temperature rose to 120, 150 and 195℃, respectively. But the IARUCP of raw WLS (63%) in Cross study (1991) was much lower than ours (88%). The reason for that are not clear. According to Cross explains, the raw RUCP in WLS may be least available in the intestine much due to the readily digested proteins are degraded by microorganisms, leaving only more refractory portions. But it did not happen in our raw samples.

Rumen retention time on intestinal availability

The effect of rumen retention time on IARUCP is variable and depends on the feedstuff (Hvelplund, 1985; Rooke, 1985; Voigt et al. 1985; De Boer et al., 1987). In this study, rumen retention time for 8, 12 and 24 h had no significant effect on IARUCP, using three-step *in vitro* procedure, for both WFB and WLS. This is in agreement with the results from mobile nylon bag experiments from IVVO (Van Straalen and Tamminga, 1990) that incubation in the rumen for 6 or 18 h had no significant effect on IARUCP.

Rumen preincubation on pepsin-pancreatin digestion

Because the feed reaching the abomasum is modified by ruminal fermentation (Calsamiglia and Stern, 1995), it is reasonable to test the effect of ruminal preincubation on pepsin-pancreatin digestion of feed protein. In this study, rumen preincubation increased ($p<0.05$) IARUCP in all treatments for both WFB (81.2 vs. 88.6%) and WLS (86.4 vs. 91.3%) for nonruminal vs. ruminal preincubation, respectively, when samples were preincubated in the rumen, suggesting that rumen play an important role in protein digestion. But Calsamiglia and Stern (1995) reported that there were no differences in pepsin-pancreatin digestion of proteins when sample were preincubated in preliminary tests. However, in their recent study (Calsamiglia and Stern, 1995), the effect of ruminal preincubation on pepsin-pancreatin digestion was mixture. The digestion of CP remaining after 16 h of ruminal incubation was less compared with digestion of samples not incubated in the rumen for meat and bone meal, hydrolyzed feather meal and fish meal (82.1 vs. 55.6%, 80.1 vs. 70.2%; and 87.5 vs.

81.1% for non ruminal vs. ruminal preincubation, respectively). This indicated that digestible protein in these sources was mostly degraded in the rumen, and only a small fraction of RUCP is available for digestion in the intestine. The digestion of CP remaining after 16 h of ruminal incubation had no effect ($p>0.05$) on soybean meal, corn gluten meal and blood meal (91.5 vs. 95.7%; 89.7 vs. 92.8% and 91.7 vs. 89.9% for nonruminal vs. ruminal preincubation, respectively). This indicated RUCP in these feeds was readily digestible in the small intestine. The techniques assume that microbial contamination of protein supplements is negligible. Erasmus et al. (1994) indicated that microbial contamination of residues after ruminal incubation ranged from 0.9 to 8.6% of total N in protein supplements. Results for the current study and Calsamiglia and Stern (1995) suggest that digestion of the protein leaving the rumen may be different from that of the original feed and justifies the need to use the three-step procedure to estimate intestinal digestion of RUCP of feedstuffs.

Three-step *in vitro* procedure

In this study, the results of IARUCP of WFB and WLS, using three-step *in vitro* procedure, were lower than the values reported by Van Straalen and Tamminga (1990), using mobile bag technique (87 vs. 91%; 90 vs. 98%, for WFB and WLS, respectively). The reason for that is probably due to overestimation with the mobile nylon bag technique when bags are recovered from the faeces because of disappearance of CP in the large intestine (Hvelplund, 1985; Voigt et al., 1985).

Animal feed intake and frequency may affect IARUCP. At a high level of feed intake, Hvelplund (1984) found a decreased apparent N digestion in the small intestine. Tamminga and Ketelaar (1988) reported decreased intestinal digestion measured with the mobile nylon bag technique was due to a much shorter transit time at a high level of intake. Calsamiglia and Stern (1995) reported that values obtained using the mobile bag technique agree with results from the three-step *in vitro* procedure for soybean meal, corn gluten meal, fish meal and meat and bone meal (De Boer et al., 1987) but were greater than true digestion estimated *in vivo* (Titgemeyer et al., 1989). It should be noted that, in contrast with data from experiments using animals fitted with duodenal and ileal cannulas, the three-step *in vitro* procedure estimates abomasal (pepsin) and intestinal (pancreatin) digestion. Because protein may be digested and absorbed from abomasal (Webb et al., 1992), less digestible dietary protein reaches the small intestine. Therefore, values obtained with the three-step *in vitro* procedure are expected to be somewhat higher than those estimated using cows fitted with duodenal and ileal cannulas (Calsamiglia and Stern, 1995).

CONCLUSIONS

Dry roasting of WFB and WLS at 130 and 150℃ was effective in increasing RUCP without adverse effect on TDCP. The increased RUCP and IARUCP could benefit rapidly growing calves and high producing dairy cows.

In term of RDCP, IDCP, IARUCP and TDCP, dry roasting at 150℃ for 45 min was the best processing conditions in this study.

However, although based on the CP results of WFB and WLS are very promising, intestinal availability of individual amino acid, especially first limiting amino acids which is necessary information for proper evaluation of the processing of these seeds need to be further investigated. Also whether dry roasting temperature or time could go further high need to be further investigated for dairy feed processing industry.

REFERENCES

AOAC. 1984. Association of official analytical chemists, Official Methods of Analysis. Washington DC.

Arieli, A., A. Ben, Moshe, S. Zamwel and H. Tagari. 1989. *In situ* evaluation of the ruminal and intestinal digestibility of heat-treated whole cottonseeds. J. Dairy Sci. 72:1228-1223.

Britton, R. A., T. J. Klopfenstein, R. Cleale, F. Goedeken and V. Wilkerson. 1987. Methods of estimating heat damage in protein sources. MP. Univ. Nebr. Agric. Exp. Stn. 52:19-21.

Calsamiglia, S. and M. D. Stern. 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73:1459-1465.

Cros, P., C. Benchaar, C. Bayourthe, M. Vernay and R. Moncoulon. 1991. *In situ* evaluation of the ruminal and intestinal degradability of extruded whole lupin seed nitrogen. Reprod. Nutr. Dev. 31:575-583.

Cros, P., M. Vernay, C. Bayourthe and R. Moncoulon. 1992. Influence of extrusion on ruminal and intestinal disappearance of amino acids in whole horsebean. Can. J. Anim. Sci. 72:359-366

De Boer, G., J. J. Murphy and J. J. Kennelly. 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977-982.

Erasmus, L. J., P. M. Botha, C. W. Cruywagen and H. H. Meissner. 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen undegradable protein form various sources. J. Dairy Sci. 77:541-551.

Goering, H. K., C. H. Gordon, R. W. Hemken, D. R. Waldo, P. J. Van Soest and L. W. Smith. 1972. Analytical estimates of nitrogen digestibility in heat damaged forages. J. Dairy Sci. 55:1275-1280.

Hvelplund, T. 1984. Intestinal digestion of protein in dairy cows. Can. J. Anim. Sci. 64 (Suppl.):193-194.

Hvelplund, T. 1985. Digestibility of rumen microbial protein and undegraded dietary protein estimated in the small intestine of sheep and by *in sacco* procedure. Acta

Agric. Scand. 25 (Suppl.):132-144.

Kibelolaud, A. R., M. Vernay, C. Bayourthe and R. Moncoulon. 1993. Effect of extruding on ruminal disappearance and lower gastrointestinal tract digestion of white lupin seeds. Can. J. Anim. Sci. 73:571-579.

Moshtaghi Nia, S. A. and J. R. Ingalls. 1992. Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. Can. J. Anim. Sci. 72:83-88.

Robinson, P. H. and S. Tamminga. 1984. Present knowledge of protein digestion and absorption in ruminants. Ubers. Tierernah. 12:119-130.

Rooke, J. A. 1985. The nutritive value of feed proteins and feed protein residues resistant to degradation by rumen microorganisms. J. Sci. Food Agric. 36:629-637.

SAS. 1991. User's Guide: Statistics, Version 6 Edition. SAS Inst., Inc., Cary, NC.

Sauer, W. C., H. Jorgensen and R. Berzins. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci.. 63:233-237.

Schwarting, G. and W. Kaufmann. 1978. Die verdaulichkert des proteins beim wiederkauer. Zeitschift fur Tierphysiologie Tierernahung und Futtermittelkunde, 40: 6-18.

Stern, M. D., K. A. Santos and L. D. Satter. 1985. Protein degradation in rumen and amino acid absorption in small intestine of lactating dairy cattle fed heat treated whole soybean. J. Dairy Sci. 68:45-56.

Tamminga, S. and R. Ketelaar. 1988. Resistance against ruminal degradation of feeds for ruminants. Report IVVO No. 192.

Titgemeyer, E. C., N. R. Merchen and L. L. Berger. 1989. Evaluation of soybean meal, corn gluten meal, blood meal and fish meal as sources of nitrogen and amino acids disappearing form the small intestine in steers. J. Anim. Sci. 67:262-275.

Van Straalen, W. M. and S. Tamminga. 1990. Protein degradation of ruminant diets, In: Feed Evaluation (J. Wiseman and D. J. A. Cole, Eds), Butterworth, London. pp. 55-73.

Voigt, R., B. Piatkoeski, H. Engelmann and E. Rudolph. 1985. Measurement of protein solubility in common feedstuffs. J. Dairy Sci. 56:1052-1057.

Waltz, D. M., M. D. Stern and D. J. Illg. 1989. Effect of ruminal protein degradation of blood meal and feather meal on the intestinal amino acid supply to lactating cows. J. Dairy Sci. 72:1509-1518.

Webb, K. E., J. C. Matthews and D. B. Dirienzo. 1992. Peptide absorption: A review of current concepts and future perspectives. J. Anim. Sci. 70:3248-3257.

Yu, P., 1995. Influence of pressure toasting on rumen degradation characteristics and *in vitro* digestibility of horse beans. MSc. Thesis. Wageningen Agricultural University, The Netherlands.

Yu, P., J. H. G. Holmes, B. J. Leury and A. R. Egan. 1998. Influence of dry roasting on rumen protein degradation characteristics of whole faba bean *(Vicia faba)* in dairy cows. Asian-Aus. J. Anim Sci. 11(1):35-42.

Yu, P., J. O. Goelema, J. H. G. Holmes and S. Tamminga. 1996. Influence of pressure toasting on rumen degradation characteristics of lactating dairy cows. Proceeding of the 8th AAAP Animal Science Congress (Japan). pp. 694-695.