

## Influence of Dry Roasting on Rumen Protein Degradation Characteristics of Whole Faba Bean (*Vicia faba*) in Dairy Cows

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**ABSTRACT** : Whole faba beans (WFB) were dry roasted at different temperatures (110, 130, 150°C) for 15, 30, 45 minutes to determine the optimal heating conditions of time and temperature to increase nutritional value. Ruminant degradation characteristics of crude protein (CP) of WFB were determined by the nylon bag incubation technique in dairy cows fed 60% hay and 40% concentrate. Measured characteristics of crude protein (CP) were soluble (washable) fraction (S), undegradable fraction (U), lag time (T<sub>0</sub>), potentially degradable fraction (D) and the rate of degradation (K<sub>d</sub>) of insoluble but degradable fraction. Based on measured characteristics, percentage bypass crude protein (%BCP) and bypass crude protein (BCP in g/kg) were calculated. Degradability of CP was reduced by dry roasting ( $p < 0.01$ ). S was reduced rapidly with increasing time and temperature, from 49.0% in the raw WFB (RWFB) to 26.3% in 150°C/45 min. D varied from 50.7% in RWFB to 73.7 % in 150°C/45'. U varied from 0% in 130°C/45', 150°/30' and 150°/45' to 0.66% in 110°/45' (0.24% for the RWFB). Lag time (T<sub>0</sub>) varied from 1.58 h in 130°C/

30' to 2.40h in 150°C/45' (1.87 h for RWFB). K<sub>d</sub> varied from 24.2% in the 110°C/30' to 4.3% in 150°C/45' (21.4% for the RWFB). K<sub>d</sub> was significantly reduced with time and temperature. All these effects resulted in increasing %BCP from 8.9% in the 110°C/15', 11.3% in the RWFB to 43.1% in the 150°C/45'. Therefore BCP increased from 31.3 and 39.9 to 148.4 g/kg respectively. Both %BCP and BCP at 150°C/45 increased nearly 4 times over the raw faba beans. The effects of dry roasting temperature and time on %BCP and BCP seemed to be linear up to the highest values tested. Therefore no optimal dry roasting conditions of time and temperature could be determined at this stage. It was concluded that dry roasting was effective in shifting crude protein degradation from rumen to intestine to reduce unnecessary nitrogen (N) loss in the rumen. To determine the optimal treatment, the digestibility of each treatment should be measured in the next trial using mobile bags technique.

**(Key words** : Faba Beans, Dry Roasting, Rumen Protein Degradation, Bypass Protein, Cows)

### INTRODUCTION

A major difference between ruminant and non-ruminant species is that for the ruminant protein quality is dependent upon the availability of amino acids leaving the rumen, rather than in the ingested diet. The protein becomes available to the animal only after digestion in and absorption from the small intestine (Hvelplund et al., 1992). Dairy cows require large quantities of protein to maintain high milk production. This can only be assured if sufficient true protein is available to be absorbed from the small intestine. Therefore dairy cows with high milk production need not only nitrogen available for rumen microbial protein synthesis, but also digestible dietary crude protein bypassing the rumen. This can be enhanced

by decreasing the degradability of crude protein in the rumen to reduce the extensive metabolism of protein taking place there Faba bean (FB); legume seeds, which are particularly high in non-structural carbohydrate and CP (Cerning-Beroard, 1977), a good source of lysine but deficient in sulfur containing amino acids (S-AA) and tryptophan (Bailey et al., 1970, 1972), have attracted attention in recent years and appear to be the protein source and starch source best suitable to the ecological and climatic condition of many countries. Despite the fact WFB have an attractive protein content, their use in dairy cow feeding is limited because the crude protein (CP) is degraded very rapidly in the rumen causing an imbalance between feed breakdown and microbial protein synthesis. This results in unnecessary N-loss from the rumen (Tamminga, 1993) and therefore these seeds are not

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suitable to be used in an unprocessed form in ruminant diets.

One of the possibilities to reduce rumen degradation is heat treatment (Tamminga, 1993). The intensity of the 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); particle size and moisture during processing also influence the possible effects of processing. The decrease in rumen protein degradation should lead to an increasing intestinal availability of protein. A number of publications (Annexstad et al., 1987; Arieli et al., 1989; Cros et al., 1992; Antoniewicz, 1993; Benchaar et al., 1994) indicated that heat treatment does increase the bypass protein but optimal heating conditions have not been found for each legume seed. Heating above the optimal temperature may overprotect the protein so that the protein is neither fermented in the rumen nor digested in the small intestine (Stern et al., 1985). Also prolonged treatment with high temperature could lead to reactions (Maillard reaction) between carbohydrate and amino acid which severely reduce the availability of amino acids for the animal. Dry roasting is a technology of current interest to improve the nutritive value of legume seeds. Literature on the nutritive value of dry roasting WFB for ruminant has not been found.

In this trial we studied the influence of dry roasting of faba beans at different time and temperature on degradation characteristics of crude protein in the rumen of dairy cows to increase the amount of bypass protein, obtain maximum bypass protein and to determine the optimal dry roasting conditions for the dairy feed industry.

## MATERIALS AND METHODS

### Feedstuffs:

Whole faba beans (*vicia faba*) were obtained from a commercial feed company (Peter Gibbs Stock Feeds in Australia). Debris in faba beans were peas, of which contents was 2.5 g in 1570.7 g. The chemical composition of the whole faba bean seeds are shown in table 3.

### Technological treatments

RWFB were dry roasted at 3 different temperatures (110, 130, 150°C) for 15, 30 and 45 minutes in a complete block design as shown in table 1.

Raw whole faba beans 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).

**Table 1.** Treatments (1 control + 9 treatments) and the dry roasting condition of WFB (Where, each treatment was measured at least 4 times)

Treatments	Dry roasting	
	Temp. (°C)	SD
RWFB		
110°C/15 min	110.0	0.00
110°C/30 min	111.3	1.21
110°C/45 min	110.9	1.55
130°C/15 min	130.0	0.00
130°C/30 min	129.8	0.45
130°C/45 min	130.0	0.00
150°C/15 min	149.5	0.71
150°C/30 min	150.0	0.00
150°C/45 min	150.0	0.00

### Animal and Animal diet

Six dry Holstein Friesian cows, of average weight 620 Kg were previously equipped with a rumen cannula with an internal diameter of 10 cm (Silicon rubbers, handmade, Kyabram Dairy center, Victoria, Australia) for measuring rumen degradability and were kept at Kyabram Dairy Center (Victoria, Australia) in the feedlot.

**Table 2.** The chemical analysis\* of commercial pelleted concentrate of dairy cows

Composition	Content
Dry matter	87.6 %
Crude Protein	12.0 %
Non protein N	1.3 %
Urea	0.5 %
Crude fat	2.0 %
Crude fibre	15.0 %
Added salt	1.0 %
Fluorine	0.02%
Vitamin A	6,000 IU/kg
Vitamin D <sub>3</sub>	500 IU/kg

\* The data provided by manufacturer, Ridley Agriproducts PTY, LTD, except DM content.

All cows received a diet consisting of 3.5 kg/day commercial pelleted concentrate, Barastoc Hi-Lac-Hi-E Dairy Pellets (Ridley Agriproducts PTY. LTD), chemical

analysis of which are shown in in table 2 and 5.4 kg/day (83.7% DM) sub-clover hay purchased locally from Goulburn Valley irrigated hay farm (Victoria, Australia).

Water was always available. The cows were individually fed twice daily at 08:00 and 16:00, 2.7 kg sub-clover and 1.75 kg pellets. The feeding level was according to the dairy cow requirements calculated by Rumnut 3.3 (Dept. of Agriculture, Reading University, UK). A 12 day period of adaptation was allowed.

### Protein degradability using *in sacco* method

Protein degradation characteristics of whole faba beans 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 (10×17cm) with the pore size of approximately 44 μm (Switzerland 1807710014 I 044 Nylal 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 21:00, (next day) 09:00, 13:00, 17:00 and 19:00 and removed at 21:00 hours respectively. The 48 hour rumen incubation were carried out from 21:00 till 21:00 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 10 treatments were analysed 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 ruminal degradation characteristics:

Important degradation characteristics in the rumen are:

1. The soluble (washable) fraction (S);
2. The fraction which will not be degraded (U)

irrespective of time it is incubated in the rumen;

3. The fraction which is not soluble, but potentially degradable (D);

4. 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 (Gauss-Newton method) by the following equation:

$$R(t) = U + D \times \exp -Kd \times (t-t_0)$$

Where: R(t) stands for residue (in %) of the amount of incubated material after t hours of rumen incubation and t<sub>0</sub> 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 bypass crude protein (%BCP) were calculated using the method of Ørskov and McDonald (1979) and the new protein evaluation system (Tamminga, 1994).

Percentage of bypass crude protein (%BCP) was calculated as:

$$\% \text{BCP} = U + D \times Kp / (Kp + Kd)$$

Bypass crude protein (BCP) was calculated as:

$$\text{BCP} = 1.11 \times \text{CP} \times \% \text{BCP} / 100$$

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; %BCP in %; The factor 1.11 in the formula was taken from the French PDI-system, the regression coefficient of *in vivo* on *in sacco* degradation data.

Statistical analysis of treatment differences of temperature and time were carried out using the statistical package SAS. Analysis of variance was carried out using Proc GLM (SAS).

$$Y_{ij} = m + \text{Temp}_i + \text{Time}_j + \text{Temp} \times \text{Time}_{ij} + e_{ij}$$

where: Y = degraded fraction; i = 1,2,3,4; j = 1,2,3,4

Comparison of temperature effect or time effect on degradation characteristics were carried out by Tukey's studentized range Test (HSD or Tukey Test).

Since the determination of degradation characteristics yielded one result per treatment, no statistical test was carried out for the results of each combination of time and temperature of dry roasting.

## RESULTS

### Chemical composition

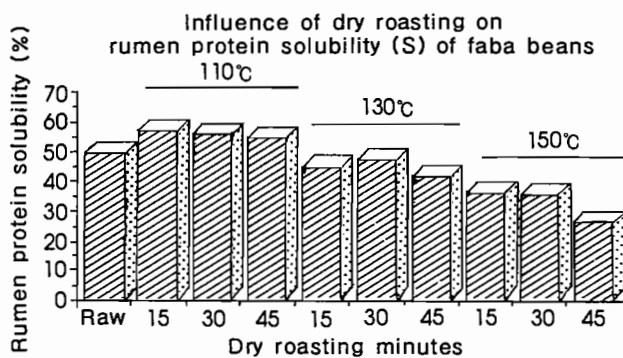
The chemical composition of whole faba beans seeds is presented in table 3.

**Table 3.** Dry matter and chemical composition of raw whole faba beans (RWFB)

Composition	Content (g/kg, DM)
Dry matter	885.9 ± 0.60
Organic matter	855.1 ± 0.73
Starch	364.1 ± 0.85
Crude protein	281.1 ± 1.67
Ash	30.8 ± 0.13

### Degradation characteristics of crude protein

Based on the best fit of data to the model, S of CP (figure 1) varied from 57.6% in 110°C/15 (49.0% in raw) to 26.3% in 150°C/45', being reduced by about 50%. When treatment temperature was lower (110°C), S increased by about 15% at all treatment times. S was reduced by 10% and 34% respectively when treatment temperatures were 130°C and 150°C, relative to the control. The effect of treatment time was not significant but temperature was strongly significant ( $p < 0.01$ )



**Figure 1.** Effect of dry roasting on rumen solubility of crude protein of whole faba beans in dairy cows.

Although U of CP varied from 0% (130°C/45', 150°C/15', 150°C/30', 150°C/45') to 0.66% (110°C/45') (0.24% for control) (table 4), the effects of both dry roasting time and temperature on U fraction were not significant ( $p > 0.05$ ).

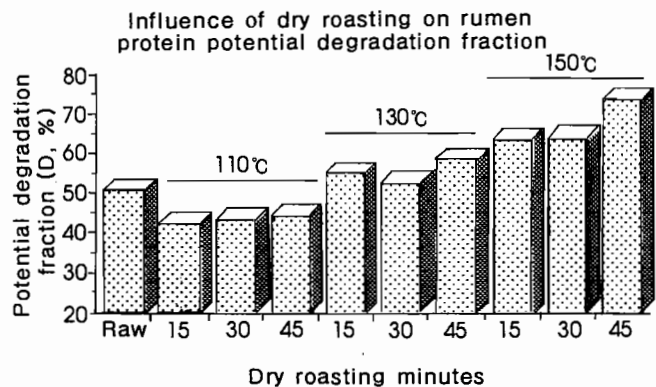
D of CP varied from 42.1% in 110°C/15' to 73.7% in 150°C/40' (figure 2). D was reduced by 14% when temperature was 110°C then increased by about 15% and

32% at the temperature of 130 and 150°C, respectively, relative to the control. The effect of temperature was strongly significantly ( $p < 0.01$ ).

**Table 4.** Effect of dry roasting on rumen protein degradation characteristic of U\* in WFB

Treatments	CP (k/kg DM)	U (%)
RWFB (control)	317.3	0.24
110°C/15 min	317.4	0.30
110°C/30 min	319.8	0.55
110°C/45 min	318.8	0.66
130°C/15 min	323.0	0.25
130°C/30 min	324.2	0.31
130°C/45 min	318.2	0.00
150°C/15 min	322.0	0.11
150°C/30 min	320.4	0.00
150°C/45 min	310.4	0.00

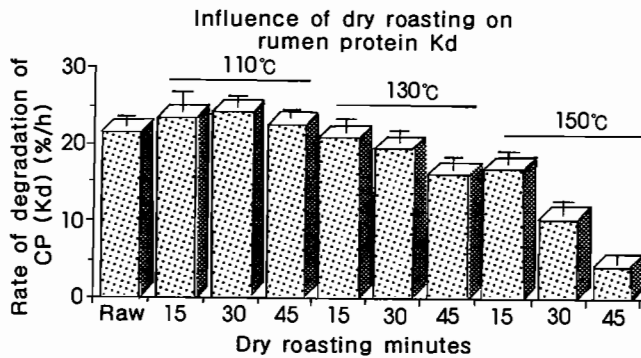
\*U: rumen undegradable proportion (U).



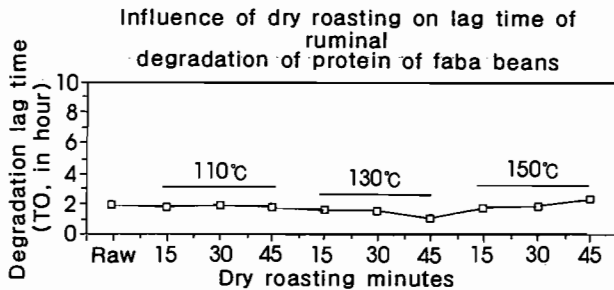
**Figure 2.** Effect of dry roasting on D fraction of crude protein in whole faba beans.

Kd of CP varied from 21.4 in raw and 24.2% in 110°C/30' to 4.3% in 150°C/45' (figure 3). The effect of temperature was strongly significantly ( $P < 0.01$ ). When temperature was 110°C, average of Kd was 23.5% and higher than the raw (21.4%). When temperature was 130°C and 150°C means of Kd were 18.7% and 10.5%, respectively. Compared with raw, Kd of 150°C/15', 150°C/30' and 150°C/45' was reduced by 22%, 51% and 80%, respectively, but the effect of time was not significant ( $p > 0.05$ ).

The lag time ( $T_0$ ) of rumen degradation is shown in figure 4. Neither treatment time nor temperature affected  $T_0$ .

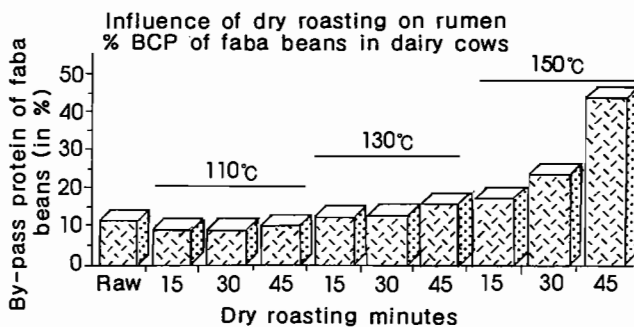


**Figure 3.** Effect of dry roasting on rumen degradation rate (Kd) of crude protein of whole faba beans in dairy cows.



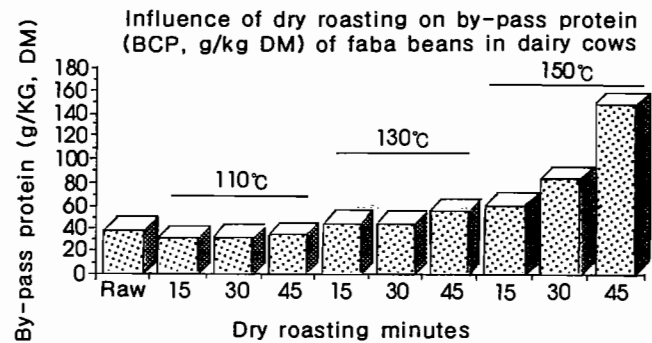
**Figure 4.** Effect of dry roasting on rumen degradation lag time of crude protein of whole faba beans in dairy cows.

BCP and %BCP, the most important components of faba beans' nutrition value in dairy cows, were a function of Kd and D fractions. %BCP varied from 11.3% in raw and 8.9% in 110°C/15' to 43.1% in 150°C/45' as shown in figure 5. It decreased by 18% at the temperature of 110°C and then it increased by 21% at 130°C. At the temperature of 150°C, it tremendously increased by 145%. Compared with the control, %BCP of the treatment of 150°C/45' was highest and increased 3.8 times.



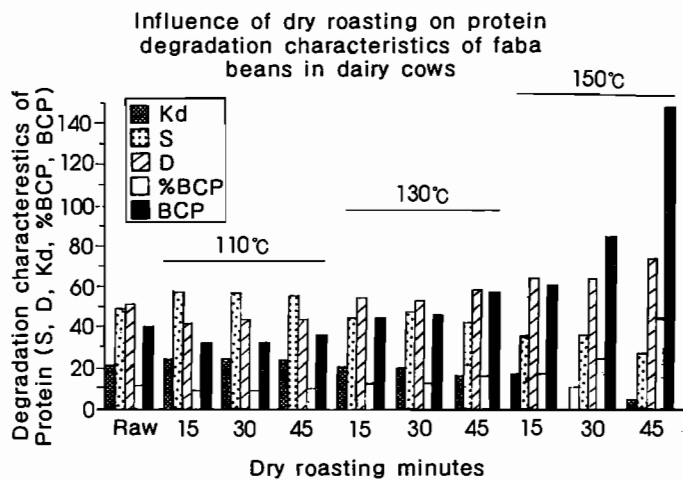
**Figure 5.** Effect of dry roasting on rumen degradation of percentage of crude protein of whole faba beans in dairy cows.

The changes of BCP had the same pattern as %BCP. It was varied from 39.9 in raw and 31.3 in 110°C/15 to 148.42 g/kg in 150°C/45' as shown in Figure 6. It decreased by 17% at 110°C and then increased by 23% at 130°C. When temperature was 150°C, it increased by 144%. BCP of 150°C/45' was increased 3.7 times compared with the raw. The important results were that %BCP and BCP had an initial decline and then little change until 150°C for 30 or 45 min.



**Figure 6.** Effect of dry roasting on bypass crude protein of whole faba beans in dairy cows.

To make more clear the influence of dry roasting on rumen degradation of whole faba beans, Kd, S, D, %BCP and BCP were put in one graph as shown in figure 7.



**Figure 7.** Effect of dry roasting on rumen protein degradation of whole horse beans (S, D, and %BCP in; Kd in %/h; %BCP in g/kg DM).

## DISCUSSION

### In sacco degradation

*In vitro* methods are commonly used to screen the

effects on protein digestibility (Babinzky et al., 1990; Aufrere and Cartailier, 1988). The circumstances under which the degradation takes place using these methods are standardized and do not follow the dynamics in rumen fermentation especially comminution and mixing. *In vitro* analysis is usually done over one time period but multiple times permit some idea of dynamics. *In sacco* method for measuring degradation of feedstuffs in the rumen allow incubation of nylon bags in the rumen for various lengths of time. Between the different methods to estimate ruminal protein and carbohydrate digestion, the *in sacco* technique offers a better way to simulate the rumen environment. The results of *in sacco* degradation probably better reflect true degradation than results from *in vitro* and enzymatic methods.

However this technique also has its limitations. The first is that the residue present in the bags after incubation may be contaminated with microbial N, which would overestimate rumen escape N. But there is no effect on protein rich feedstuffs, such as legume seeds. Compared with N residues in such protein rich feedsuffs, microbial N contamination may be ignored. The second limitation is that rate of degradation by this technique has to be combined with an (often assumed) rate of passage (Kp) in order to estimate the effective protein escape from the rumen. Its usefulness depends on the standardization of different variables (i.e. bag porosity, sample size, feed particle size, washing procedures). Despite its limitation it is a technique which yields useful information on rumen degradation characteristics (Tamminga, 1990).

### Nutritional value

Raw whole faba beans have a very high rate of degradation (21.4%/h), high soluble fraction (49.0%) and low potentially degradable fraction (50.7%), which all contribute to a very high degradability (88.7%) after being incubated in the rumen, resulting in only 11.3% of bypass protein into the intestines. Ørskov (1982) reported values of percentage disappearance of nitrogen at  $k=0.02 \text{ h}^{-1}$  for faba beans close to those obtained in the present experiment (82.9%). Yu (1996) reported that faba beans degradability of 82.7% in dairy cows was also close to the present result.

Very high Kd (21.4%/h) of raw faba beans in this experiment was unexpected compared with 7.4%/h of faba beans (*vicia faba cv Alfred*) reported in previous work (Yu, et al., 1996). The reasons for this large figure may be due to the difference of variety of faba beans used in the experiments or difference of growth periods or environment in the fields. Faba beans obtained in early age usually have a high rate of degradation in the

rumen.

The decrease observed in the degradability as a result of the high-temperature dry roasting from 88.7% (raw) to 56.9% (150°C/45) is similar to published observations carried out with a variety of protein supplements subject to different protective methods involving heating. Pressure toasting decreased rumen degradability from 0.83 (raw) to 0.47 (136°C/15') (Yu, et al., 1996). Extrusion reduced rumen degradability from 0.90 for untreated (faba beans) to 0.66 for extruded faba beans and also reduced enzymatic degradation (*Streptomyces griseus*) from 0.75 to 0.35 and 0.84 to 0.74 respectively after 1 and 24 hours (Aufrere, et al., 1991). Autoclaving at 121°C decreased ruminal degradability of horse beans from 0.82 (untreated horse beans) to 0.52 (10 min.), 0.46 (20 min.), 0.39 (30 min.) (Antoniewicz, 1993).

In this study, dry roasting decreased the soluble fraction by 46%, dramatically decreased Kd by 5 times and increased D 45% in 150°C/45' compared with raw faba beans. Tamminga (1994) found that heat treatments reduced the size of the soluble fraction and slowed down the rate of degradation. Steaming of beans at high temperature (136°C) can improve the protein value dramatically. The intensity of this effect is a function of both the temperature reached and the time of exposure (Stern et al., 1985; Annexstad et al., 1987; Waltz and Stern, 1989). The effect is caused by both crosslinking within and between proteins and irreversible binding between aldehyde groups of carbohydrates and amino groups, largely  $\epsilon$ -amino groups of lysine; the former are plentiful in legume seeds.

The dramatic increases of %BCP and BCP were mainly because dry roasting at higher temperature reduced rate of degradation (Kd), soluble fraction (S) and increased the degradation part (D) thus increasing the amount of crude protein reaching the intestines. Similar effects of heat-treatment of seeds (cotton; lupins; canola) on CP degradation in the rumen have been reported (Mir et al., 1984; Arieli et al., 1989; Cros et al., 1992).

McMeniman and Armstrong (1979) found that heating faba beans did not increase total flow of protein to the duodenum of cattle, but faba beans were prepared at 105°C, which probably was inadequate for protecting their protein. For rapeseed (Lindberg, 1984) and soy bean meals (Mir et al., 1984) as well as canola and soybeans (Deacon et al., 1988), moderate heating of the meals and seeds did not have a pronounced effect on ruminal degradation. It was surprising to find in this experiment that dry roasting at lower temperature (110°C) did not result in decreasing S, Kd, increasing the D and BCP, but resulted in a higher rumen protein degradability than raw

faba beans. The dry roasting at 110°C caused more crude protein to be fermented in the rumen. The reasons for this are still not clear. The use of lower temperature in faba beans roasting may change physical and chemical structure which could result in increasing rumen degradation.

Whether the total tract disappearance and intestinal digestibility of undegraded rumen protein of faba beans were different from raw faba beans, whether there was an overprotection effect, whether individual AA from residue and raw beans were different and whether residual AA composition is different from the original AA will be investigated in another trial using mobile bags technique. Ganey et al. (1979) found no systematic changes in AA composition in the residue of heated whole faba beans left in bags after rumen incubation. Benchaar et al. (1994) indicated that extruded whole faba beans (195°C, 25s) increased AA flow to the duodenum and disappearance in the small intestine and diets containing extruded whole horse beans (faba beans) increased availability of total essential AA in the small intestine compared with diets containing raw whole faba beans. Cros et al. (1992) demonstrated that heat treatment (extrusion) did not alter the AA profile, but AA composition of faba beans' protein fractions that bypass the rumen may markedly differ quantitatively and qualitatively from their original composition. Extruding the beans increased intestinal disappearance of most of the AA, but variation in disappearance among AA was substantial. These results indicate that rumen undegraded proteins differ in their potential as a source for absorbable AA in the intestine; processing altered the protein quality of rumen undegraded fraction. For accurate prediction of this intestinal protein supply, information is also needed on the relative contributions of feed protein and microbial protein. Rumen degradation characteristics of non-structural carbohydrate of faba bean should be measured, because in optimizing rumen fermentation too large amounts of soluble or rapidly degradable carbohydrates may give rise to an excessive VFA production resulting in a low pH, which will slow down the degradation of structural carbohydrates and also result in excessive NH<sub>3</sub> production followed by urea excretion (Tamminga, 1990). Escape from rumen degradation of non-structural carbohydrates may be beneficial, because they can be digested in the small intestine. This usually results in a reduced milk fat content and somewhat enhanced milk protein content (Tamminga, 1990).

In our experiment, bypass crude protein was increased only at 130°C and 150°C; crude protein rumen degradability at 110°C was increased which made bypass

protein less than the raw whole faba beans.

## CONCLUSIONS

Dry roasting was effective in shifting crude protein degradation from rumen to intestine to reduce unnecessary nitrogen (N) loss in the rumen. Dry roasting of whole faba beans at higher temperatures has the potential to increase supply of escape protein and improves the nutritional quality of faba beans by increasing the amount of protein supplied to the small intestine, which could be a benefit to high production cows.

But no optimal dry roasting condition could be determined at this stage. It appears that 150°C/45' was the best combination of time and temperature due to its largest amount of BCP. To determine the optimal treatment, digestibility in the intestine of all treatments should be measured using mobile bags technique.

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