Effect of pressure toasting on the rumen degradability and intestinal digestibility of whole and broken peas, lupins and faba beans and a mixture of these feedstuffs


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Abstract

The effects of pressure toasting of whole and broken peas, lupins and faba beans on in situ degradability of protein and starch and intestinal digestibility of protein were studied. To test for associative effects on rumen degradability and intestinal digestibility after toasting, a mixture of peas, lupins and faba beans was examined and results were compared with weighted averages of separately processed feedstuffs. Pressure toasting for 3 min at 132°C decreased in situ protein degradability of peas, lupins and faba beans and in situ starch degradability of peas and faba beans, especially when broken versus whole seeds were processed. Undegraded intake protein (%UIP) increased after toasting whole or broken seeds from 25% to 44% and 52% for peas, from 22% to 47% and 51% for lupins and from 20% to 48% and 57% for faba beans, respectively. Undegraded intake starch (%UISTA) increased from 39% to 50% and 53% after toasting whole and broken peas and from 33% to 53% and 60% for toasted whole and broken faba beans, respectively. Total tract protein digestibility, measured after 12 h rumen and subsequent intestinal incubation, remained unchanged for peas and faba beans, but decreased from 99% to 98% for toasted whole lupins and to 97% for toasted broken lupins. For toasted whole and broken faba beans, pressure toasting increased %UISTA from 33% to 53% and 60%, respectively. After pressure toasting, washable fractions (W) of all legume seeds decreased for both constituents, the fractional rate of degradation (kd) of protein decreased, while the kd of starch increased. It was concluded that protein degradability decreased after pressure toasting, without seriously affecting its total tract protein digestibility. Toasting a mixture of peas, faba beans and lupins resulted in higher starch degradabilities than expected, based on the separately treated feedstuffs. The kd’s of the mixtures were higher than expected: 5.49 versus 4.29% h⁻¹ for whole seeds and 5.01 versus 4.18% h⁻¹ for

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broken seeds, respectively. Consequently, %UISTA was lower than expected (47% vs. 51% for whole seeds and 50% vs. 57% for broken seeds). © 1998 Elsevier Science B.V.

Keywords: Peas; Lupins; Faba beans; Pressure toasting; Rumen degradability; Intestinal digestibility; Protein; Starch

1. Introduction

Because of their high protein content, peas (*Pisum sativum*), lupins (*Lupinus spp.*) and faba beans (*Vicia faba*) may be of use in ruminant diets to balance other dietary ingredients low in protein (Dixon and Hosking, 1992). Recently, there has been renewed interest in these seeds because they can be grown in the European community (Aguilera et al., 1992). This makes them possible substitutes for imported protein sources such as soybean meal, although the protein content of these seeds is generally lower than in soybean meal.

The protein fraction in these leguminous seeds consists of 85–100% albumins and globulins (Van Straalen and Tamminga, 1990), which are highly soluble and easily degradable (up to 75%) in the rumen. Starch is the major storage carbohydrate in peas and faba beans, while for lupins it is β-(1,4) galactan (Hill, 1977). Literature on the rumen degradability of starch from legume seeds is rare. Recent data shows that starch from peas (Walhain et al., 1992) and faba beans is highly soluble and easily degradable (Tamminga et al., 1990; Nocek and Tamminga, 1991).

Although legume seeds provide rumen degradable protein and starch, supplies of rumen undegradable protein and starch are of special importance for high producing dairy cows (Chalupa, 1974; Satter, 1986; Nocek and Tamminga, 1991; Klopfenstein, 1996). Therefore, several treatments have been proposed to increase rumen escape protein through denaturation and Maillard reactions (Satter, 1986). Extrusion has been used to decrease protein degradability of horse beans (Cros et al., 1991), lupins (Cros et al., 1992; Kibelolaud et al., 1993) and peas (Benchaar et al., 1994) while protein degradability of lupins was reduced after roasting (Murphy and McNiven, 1994; Singh et al., 1995). Aguilera et al. (1992) showed that autoclaving was an effective method to reduce rumen protein degradability of peas, lupins, faba beans, vetch and bitter vetch. Differences among legumes were observed in response to the treatment.

Most treatments, where heat is involved, will increase starch degradability. This can be due to gelatinisation and to changes in the physical structure of the feedstuff, caused by the physical processes that are associated with the heat treatments. For example, Walhain et al. (1992) reported a decreased rumen protein degradability from 88% to 66%, after extruding peas at 140°C while starch degradability, calculated from their data, increased from 87% to 96%.

Structural changes of protein and starch after heat treatment affects rumen degradation depending on the temperature reached, the processing time, and the moisture content during processing (Lund, 1984; Stern et al., 1985; Cleale IV et al., 1987). In addition, seed particle size and the presence of hulls will influence the transfer of heat and moisture during processing (Goelema et al., 1997). Excessive heat treatment generally reduces
intestinal digestibility of dietary amino acids through the formation of indigestible Maillard products (Van Soest, 1994). Optimal conditions of treatments are generally defined as those which decrease rumen degradability without negatively altering postruminal digestion (Stern et al., 1985; Satter, 1986).

Additivity of nutritive characteristics is often assumed in calculating the nutritional value of diets or concentrates from the values of the individual ingredients. This may be correct after mixing individual (treated) feedstuffs only, but treatments which are applied to the mixture of feedstuffs, like for instance extrusion, expander treatment or pelleting, may involve interactions between ingredients (Vik-Mo and Lindberg, 1985; Murphy and Kenelly, 1986; Chapoutot et al., 1990; De Boever et al., 1995; Van Straalen et al., 1997). The digestive properties after processing a complete feed may therefore differ from the weighted average of its constituents. This experiment was conducted with the following objectives:

1. to measure the effect of pressure toasting on the rumen protein and starch degradability and the intestinal protein digestibility of peas, lupins, and faba beans.
2. to determine if the effects of pressure toasting on degradability and digestibility of these legume seeds are different for broken versus whole seeds.
3. to compare the rumen degradation and intestinal digestion characteristics of the toasted mixture of peas, lupins and faba beans with the weighted average of the individual seeds.

2. Material and methods

2.1. Samples and treatments

Lupins, peas and faba beans were obtained from a commercial supplier (ACM, Meppel, The Netherlands). Each batch of seeds was randomly divided into two equal parts; one of which was coarsely broken with a roller mill (Roskamp TP 900–36). The roller mill had three roller pairs and a capacity of 20 t h⁻¹. The gap width between the rollers was adjusted for each feedstuff to achieve maximum dehulling and minimal particle size reduction. The gap widths of the roller pairs 1, 2 and 3 were 4.15, 3.20, and 3.90 mm for peas, 4.20, 4.20 and 3.90 mm for lupins and 4.25, 4.50 and 5.20 mm for faba beans, respectively.

Two mixtures (1:1:1, weight basis) were made, consisting of either whole or broken seeds. The whole and broken seeds as well as the mixtures were processed for 3 min at 132°C in a laboratory scale pressurised toaster, as described by Van der Poel et al. (1990). Toasting treatments were repeated on two consecutive days. After toasting, the samples were oven dried at 35°C for 15 h. Untreated samples of the single feedstuffs were used as controls.

2.2. Rumen incubations

Rumen incubations were carried out according to Dutch standard methods (Centraal Veevoeder Bureau, 1996) in which four rumen-cannulated, lactating Holstein cross Friesian cows were used to measure ruminal crude protein and starch degradation. The
cows received about 17 kg of dry matter (DM) daily of a ration consisting of grass silage (48% of DM intake) and a commercial concentrate (90 g intestinal absorbable protein and 6.5 MJ NEL per kg).

Nylon with a pore size of 40 μm (PA 40/30, Nybolt, Switzerland) was used to prepare bags with an inner size of 10×19 cm. Feed samples were ground through a 3 mm sieve (Retsch ZM1 centrifugal mill). The bags were filled with about 5 g DM of the ground sample and incubated in the rumen for 0 (blank), 2, 4, 8, 12, 24 and 48 h. Treatments were randomly divided over cows.

After incubation, bags were immediately placed in cold water and rinsed with tap water to stop fermentation. Then the bags were washed in a domestic washing machine for 50 min with 70 l of cold water, without centrifugating. After washing, the bags were dried in an forced air oven at 60°C for 24 h, air equilibrated and weighed. Residues from the bags were pooled within time and treatment and ground through a 0.5 mm sieve (Retch ZM1 centrifugal mill).

2.3. Intestinal incubations

Two non-lactating Holstein cross Friesian cows, fitted with a cannula in the proximal duodenum, were used to measure intestinal protein digestibility. The cows received a daily ration of approximately 11 kg DM, consisting of maize silage (74% of DM intake) and grass silage.

Nylon with a pore size of 40 μm (PA 40/30, Nybolt, Switzerland) was used to prepare bags with an inner size of 3×7 cm. The bags were filled with approximately 0.5 g DM of the 12 h rumen incubation residue. Prior to incubation, the rumen incubation residue was prepared and handled as described above, but with freeze-drying instead of oven-drying.

Prior to incubation in the proximal duodenum the bags were incubated in a solution containing 1 g (2000 FIP U g⁻¹) pepsin in 1 l of 0.1 M HCL at 37°C for 1 h. Three bags were inserted into the proximal duodenum through the cannula of each cow after every 20 min. After insertion of 12 bags, a 20 min break was taken after which the procedure continued. Bags were retrieved from the faeces every 2 h and stored at −20°C until all the bags had been recovered. After thawing, the bags were rinsed, washed and freeze dried as described above. Residues were pooled within treatment and ground through a 0.5 mm sieve (Retch ZM1 centrifugal mill).

2.4. Chemical analysis

Feeds were analysed for DM, inorganic matter (ASH), crude protein (CP, 6.25×N), crude fat (CFAT), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and starch. Acid detergent insoluble nitrogen (ADIN) in the feed samples was determined by analysing the N content of ADF residues. Pooled rumen incubation residues were analysed for DM, ASH, CP and starch. Intestinal incubation samples were analysed for DM, ASH and CP.

DM was determined by drying to a constant weight at 103°C following ISO method 6496, ASH by combustion at 550°C according to ISO method 5984. N was determined with the Kjeldahl method with CuSO₄ as the catalyst, following ISO method 5983. CF
was analysed according to NEN method 5417 (1988). ADF and ADL were analysed according to Van Soest (1973). NDF was analysed according to the VVR/protocol NSP analyses (Anonymous, 1992). This method is similar to the method of Van Soest et al. (1991), but includes an incubation step with 1 ml heat stable amylase (Sigma 6814, 1350 U ml$^{-1}$) and 0.25 ml protease (Alcalase, 2.4 L, NOVO, 2.4 AU g$^{-1}$) in 60 ml of a phosphate buffer (pH 7). This incubation is carried out for 15 min at 40°C, after boiling and removal of the ND.

Starch was analysed according to the NIKO method (Brunt, 1992). Total starch was analysed by extracting soluble sugars with a 40% ethanol solution, followed by autoclaving for 3 h at 130°C and enzymatic breakdown (1 h at 60°C, pH 5) to glucose, using an enzyme cocktail containing amyloglucosidase, $\alpha$-amylase and pullulanase (A). Glucose was subsequently determined using hexokinase and G6P-dehydrogenase. The degree of gelatinisation (SGD) of starch was determined with two additional analyses where starch was analysed as above, but without the ethanol extraction (B) to quantify the amount of starch and lower sugars. Finally, the sample was hydrolysed with amyloglucosidase (60 U g$^{-1}$ sample) for 75 min at 50°C (pH 4.8) to determine gelatinised starch and soluble sugars (C).

The SGD was calculated as a percentage of total starch after correcting for lower sugars according to Eq. (1).

$$\text{SGD} = 100 \times \left\{ C - (B - A) \right\}/A$$  (1)

Protein dispersibility index (PDI) was determined according to a modified AACC 46–24 procedure, as described by Thomas et al. (1997). N-solubility index ($\text{NSI}_{\text{H}_2\text{O}}$) was determined according to a modified AOCS (1968) procedure, as described by Thomas et al. (1997).

### 2.5. Calculation of degradability and digestibility

Both CP and starch were classified into three fractions: a readily available fraction ($W$), measured as the fraction disappearing after washing (0 h incubation); an undegradable fraction ($U$), measured as the asymptote of the degradation curve at infinite incubation time; and a potentially degradable fraction ($D$) = 1 – $W$ – $U$. The fractional rate of degradation of the $D$ fraction ($k_d$, in % h$^{-1}$) was calculated using a first order degradation model, without a lag time, as described by Robinson et al. (1986). Undegraded intake crude protein (%UIP) and undegraded intake starch (%UISTA) were calculated for the Dutch standard outflow rate ($k_p$) of 6% h$^{-1}$, Eqs. (2) and (3), according to Tamminga et al. (1994). For starch, it was assumed that 10% of $W$ escapes rumen fermentation and $U$ is 0 (Tamminga et al., 1994).

$$\%\text{UIP} = U + D \times (k_p/(k_p + k_d))$$  (2)

$$\%\text{UISTA} = 0.1 \times W + D \times (k_p/(k_p + k_d))$$  (3)

The CP residue remaining after intestinal incubations (IUP) was used to calculate protein digestibility as a fraction of intake crude protein (%DP) and as a fraction of the undegraded intake protein (%DUP).
2.6. Statistical analysis

Analysis of variance was conducted using the General Linear Models (GLM) procedure of SAS (SAS, 1989) with the following model:

\[ Y_{ijk} = \mu + D_i + F_j + T_k + (F \times T)_{jk} + \epsilon_{ijk}, \]

where \( Y_{ijk} \) is the dependent variable under examination (residues, \( W, k_d, \%\text{UPI}, \%\text{UISTA}, \%\text{DP}, \%\text{DUP}) \), \( \mu \) the overall mean, \( D_i \) the treatment day effect (\( i=1, 2 \)), \( F_j \) the feed effect (\( j=1-4 \)), \( T_k \) the treatment effect (\( k=1-3 \)), \( (F \times T)_{jk} \) the interaction of feed and treatment, and \( \epsilon_{ijk} \) the residual error term.

The incubation residues and degradability characteristics of the treated mixtures were compared with the calculated weighted average of the single treated feedstuffs to test for additivity. When calculated means were outside the 95% confidence limits of the measured values, it was concluded that there was interaction. Treatment effects were compared by contrast statements, using the GLM procedure of SAS (SAS, 1989).

3. Results

3.1. Chemical composition

The chemical composition of the untreated seeds, as well as the measured and calculated OM, CP and CFAT contents of the mixture are in Table 1. The values agree with tabular values (Centraal Veevoeder Bureau, 1994) and those reported in other studies (Dixon and Hosking, 1992). The agreement between the measured and calculated values indicated that mixing was correct. A significant decrease of CP content was found for peas and lupins comparing BT and T (Table 2). Total starch content in peas and faba beans decreased after toasting (Table 3).

Table 1
Chemical composition of untreated peas, lupins, faba beans and their mixture

<table>
<thead>
<tr>
<th>Feedstuff</th>
<th>Peas</th>
<th>Lupins</th>
<th>Faba beans</th>
<th>Mixturea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured</td>
<td>Calculated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter (g kg(^{-1}))</td>
<td>939.5</td>
<td>950.3</td>
<td>948.0</td>
<td>948.6</td>
</tr>
<tr>
<td>In dry matter (g kg(^{-1}) DM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic matter</td>
<td>966.3</td>
<td>970.9</td>
<td>958.9</td>
<td>962.4</td>
</tr>
<tr>
<td>Crude protein</td>
<td>261.4</td>
<td>349.5</td>
<td>306.2</td>
<td>308.9</td>
</tr>
<tr>
<td>Crude fat</td>
<td>8.7</td>
<td>56.0</td>
<td>11.6</td>
<td>24.6</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>63.8</td>
<td>152.9</td>
<td>89.0</td>
<td>–b</td>
</tr>
<tr>
<td>Starch</td>
<td>404.3</td>
<td>–</td>
<td>413.1</td>
<td>–</td>
</tr>
<tr>
<td>NDF</td>
<td>118.5</td>
<td>248.7</td>
<td>151.9</td>
<td>–</td>
</tr>
<tr>
<td>ADF</td>
<td>78.0</td>
<td>196.6</td>
<td>111.6</td>
<td>–</td>
</tr>
<tr>
<td>ADL</td>
<td>2.2</td>
<td>8.5</td>
<td>3.2</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^{a}\)The mixture consisted of peas, lupins and faba beans on a 1:1:1 weight basis.

\(^{b}\)–: Not determined.
3.2. Protein degradability and digestibility

Untreated peas, lupins and faba beans were highly degradable in the rumen. UIP was 25% for peas, 22% for lupins and 20% for faba beans (Table 2).

Pressure toasting significantly increased %UIP by decreasing W and $k_d$ (Table 2). Total tract protein digestibility (%DP) was high and only slightly reduced after pressure toasting, although this reduction was significant for lupins.

Compared to toasted whole seeds, a decreased W was observed and this caused higher values of UIP for toasted broken lupins and faba beans, while for broken peas this was caused by a decreased $k_d$. Toasting broken seeds increased UIP without altering %DP or %DUP.

### Table 2
Effects of breaking and/or pressure toasting on the rumen degradation characteristics and intestinal digestion of crude protein

| Treatment | CP (g kg$^{-1}$ DM) | W (%) | $D$ (%) | $k_d$ (% h$^{-1}$) | %UIP | UIP (g kg$^{-1}$ DM) | %DUP | %DP | P-values of contrasts$^b$
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Peas</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>268.0</td>
<td>55.9</td>
<td>44.1</td>
<td>4.52</td>
<td>25.2</td>
<td>74.9</td>
<td>96.1</td>
<td>98.9</td>
<td>NS 0.0274</td>
</tr>
<tr>
<td>T</td>
<td>268.8</td>
<td>23.2</td>
<td>76.9</td>
<td>4.39</td>
<td>44.4</td>
<td>132.6</td>
<td>97.6</td>
<td>98.8</td>
<td>NS 0.001</td>
</tr>
<tr>
<td>BT</td>
<td>256.7</td>
<td>19.4</td>
<td>80.6</td>
<td>3.27</td>
<td>52.3</td>
<td>149.2</td>
<td>97.1</td>
<td>98.3</td>
<td>NS 0.001</td>
</tr>
<tr>
<td>Sem</td>
<td>3.15</td>
<td>7.37</td>
<td>7.37</td>
<td>0.30</td>
<td>5.19</td>
<td>14.71</td>
<td>0.37</td>
<td>0.17</td>
<td>NS 0.001</td>
</tr>
<tr>
<td><strong>Lupins</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>349.6</td>
<td>44.3</td>
<td>55.7</td>
<td>9.34</td>
<td>21.9</td>
<td>84.9</td>
<td>94.0</td>
<td>98.5</td>
<td>NS 0.0009</td>
</tr>
<tr>
<td>T</td>
<td>354.4</td>
<td>27.6</td>
<td>72.4</td>
<td>3.20</td>
<td>47.2</td>
<td>185.7</td>
<td>96.2</td>
<td>98.0</td>
<td>NS 0.0894</td>
</tr>
<tr>
<td>BT</td>
<td>332.5</td>
<td>22.5</td>
<td>77.5</td>
<td>3.10</td>
<td>51.2</td>
<td>188.9</td>
<td>95.5</td>
<td>97.4</td>
<td>0.0001 NS 0.0894</td>
</tr>
<tr>
<td>Sem</td>
<td>4.25</td>
<td>4.34</td>
<td>4.34</td>
<td>1.33</td>
<td>5.91</td>
<td>21.96</td>
<td>0.43</td>
<td>0.21</td>
<td>NS 0.0070 NS</td>
</tr>
<tr>
<td><strong>Faba beans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>314.0</td>
<td>67.1</td>
<td>33.0</td>
<td>3.90</td>
<td>20.0</td>
<td>69.6</td>
<td>96.0</td>
<td>99.1</td>
<td>NS 0.0259</td>
</tr>
<tr>
<td>T</td>
<td>315.9</td>
<td>25.7</td>
<td>74.3</td>
<td>3.26</td>
<td>48.2</td>
<td>169.1</td>
<td>97.9</td>
<td>98.9</td>
<td>NS 0.0259</td>
</tr>
<tr>
<td>BT</td>
<td>321.1</td>
<td>18.6</td>
<td>81.4</td>
<td>2.63</td>
<td>56.6</td>
<td>201.7</td>
<td>98.2</td>
<td>98.9</td>
<td>0.0001 0.0091</td>
</tr>
<tr>
<td>Sem</td>
<td>2.40</td>
<td>9.59</td>
<td>9.59</td>
<td>0.24</td>
<td>7.06</td>
<td>25.31</td>
<td>0.45</td>
<td>0.08</td>
<td>NS 0.0032</td>
</tr>
</tbody>
</table>

$^a$Treatments C: untreated; T: toasted; BT: broken seeds and subsequently toasted.
$^b$Contrasts treatment: T and BT vs. C and breaking: BT vs. T; NS: $P>0.10$.

`CP`: crude protein in g kg$^{-1}$ DM, W: washable fraction, $D$: potentially degradable fraction, $k_d$: fractional rate of degradation of $D$, %UIP: undegraded intake protein, as percentage of CP, UIP: undegraded intake protein %DP: total tract protein digestibility as percentage of CP, %DUP: intestinal digestibility of UIP.

### 3.2. Protein degradability and digestibility

Untreated peas, lupins and faba beans were highly degradable in the rumen. UIP was 25% for peas, 22% for lupins and 20% for faba beans (Table 2).

Pressure toasting significantly increased %UIP by decreasing W and $k_d$ (Table 2). Total tract protein digestibility (%DP) was high and only slightly reduced after pressure toasting, although this reduction was significant for lupins.

Compared to toasted whole seeds, a decreased W was observed and this caused higher values of UIP for toasted broken lupins and faba beans, while for broken peas this was caused by a decreased $k_d$. Toasting broken seeds increased UIP without altering %DP or %DUP.
Relative to peas and faba beans, lupins had the highest ADIN content (Table 4). Toasting increased ADIN for lupins and faba beans ($P \leq 0.05$) to 1.4% of total N for toasted broken lupins and 0.64% for broken toasted faba beans. ADIN correlated positively with UIP ($r^2 = 0.47$, $P = 0.0264$), but negatively with %DP and %DUP ($r^2 = -0.74$, $P = 0.0001$ and $r^2 = -0.77$, $P = 0.0281$, respectively).

PDI and NSI H$_2$O values (Table 4) were higher than W (Table 2), but all three parameters responded similarly to the toasting treatment. PDI and NSI H$_2$O correlated positively with W of protein (correlation coefficients ($r$) were 0.92 and 0.91, respectively; $P=0.0001$) and negatively with %UIP ($r=-0.79$ and $-0.77$; $P=0.0001$, respectively).

### 3.3. Starch degradability

Undegraded intake starch (%UISTA) of untreated peas and faba beans was 39% and 33% (Table 3), versus %UIP of 25% and 20%, respectively (Table 2). This indicated that starch was less degradable in the rumen than protein.

Pressure toasting decreased $W$, and increased the $k_d$ of starch. As a result, %UISTA increased by 29% for whole peas and 58% for whole faba beans (Table 3). Toasting broken faba beans increased %UISTA compared to whole beans due to a further reduction of $W$.

The decreased in situ starch degradability observed after pressure toasting was not consistent with the increased SGD after toasting (Table 4). SGD of untreated peas and faba beans was 11% and 5%, respectively, but exceeded 70% and 50% after toasting broken peas and faba beans, respectively.

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### Table 3

Effects of breaking and/or pressure toasting on the rumen degradation characteristics of starch$^a$

<table>
<thead>
<tr>
<th>Treatment$^a$</th>
<th>C</th>
<th>T</th>
<th>BT</th>
<th>Sem</th>
<th>P-values of contrasts$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td>Breaking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Peas</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch (g kg$^{-1}$ DM)</td>
<td>472.9</td>
<td>458.7</td>
<td>438.1</td>
<td>6.64</td>
<td>0.0111</td>
</tr>
<tr>
<td>W (%)</td>
<td>45.9</td>
<td>15.7</td>
<td>4.8</td>
<td>7.83</td>
<td>0.0001</td>
</tr>
<tr>
<td>$k_d$ (% h$^{-1}$)</td>
<td>3.47</td>
<td>4.44</td>
<td>4.87</td>
<td>0.29</td>
<td>0.0091</td>
</tr>
<tr>
<td>%UISTA</td>
<td>38.9</td>
<td>50.1</td>
<td>53.2</td>
<td>2.94</td>
<td>0.0011</td>
</tr>
<tr>
<td>UISTA (g kg$^{-1}$ DM)</td>
<td>183.9</td>
<td>229.8</td>
<td>233.4</td>
<td>11.49</td>
<td>0.0009</td>
</tr>
<tr>
<td><strong>Faba beans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch (g kg$^{-1}$ DM)</td>
<td>404.3</td>
<td>397.2</td>
<td>366.8</td>
<td>8.34</td>
<td>0.0168</td>
</tr>
<tr>
<td>W (%)</td>
<td>58.9</td>
<td>12.8</td>
<td>4.9</td>
<td>10.69</td>
<td>0.0001</td>
</tr>
<tr>
<td>$k_d$ (% h$^{-1}$)</td>
<td>2.96</td>
<td>4.15</td>
<td>3.55</td>
<td>0.24</td>
<td>0.0313</td>
</tr>
<tr>
<td>%UISTA</td>
<td>33.4</td>
<td>52.8</td>
<td>60.3</td>
<td>5.12</td>
<td>0.0001</td>
</tr>
<tr>
<td>UISTA (g kg$^{-1}$ DM)</td>
<td>135.1</td>
<td>209.9</td>
<td>220.8</td>
<td>17.06</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

$^a$Treatments C: untreated; T: toasted; BT: broken seeds and subsequently toasted.
$^b$Contrasts treatment: T and BT vs. C and breaking: BT vs. T; NS: $P > 0.10$.
$^c$W: washable fraction, $k_d$: fractional rate of degradation of the potentially degradable fraction, %UISTA: undegraded intake starch as percentage of starch, UISTA: undegraded intake starch
No differences were found between the measured and calculated CP in situ incubation residues, expressed as a percentage of the non-washable CP fraction, except for the 8 h residue of the toasted mixture. Hence, no differences were found between measured and calculated \(k_d\) of CP and %UIP.

The calculated starch residues, expressed as a percentage of the non-washable starch fraction, were mostly higher than measured values, indicating a higher actual starch degradation. The differences increased with time, especially after 8 and 24 h incubation. Due to the large variation, the difference was only significant for the 2 h incubation of toasted broken seeds.

As a resultant, measured \(k_d\) and %UISTA were different from the calculated values. The \(k_d\)'s were higher and, consequently, %UISTA was lower than calculated.

### 4. Discussion

#### 4.1. Chemical composition

Toasting decreased the starch content of peas and faba beans, which could be attributed to an increase in the fraction of soluble sugars. This, however, accounted for only about 30% of the decrease. Formation of atypical glycosidic bonds (Siljeström et al., 1989) was
proposed by Tovar and Melito (1996) as the reason for the decreased starch contents of black beans and lima beans after autoclaving for 15 min at 120°C, and might have occurred also after toasting. Finally, the effect of steam treatment on the protein matrix embedding the starch (Holm et al., 1985) may have rendered the starch inaccessible for hydrolyzing enzymes during analysis.

### 4.2. Protein degradability and digestibility

Several treatments have been used to decrease protein degradability of legume seeds. Decreased protein degradability after heat treatment has been attributed to formation of Maillard products from reducing sugars and amino acids and to cross-linking between and within proteins (Hurrel et al., 1976).

Literature on effects of pressurised steam treatments without additional shear forces on protein degradability of peas, lupins and faba beans is limited to results of Aguilera et al. (1992) for autoclaving. Although the treatment is similar to pressure toasting, temperature and processing time were 120°C and 30 min in the study of Aguilera et al. (1992) versus 132°C and 1.5 min in our study.

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>C</th>
<th>T</th>
<th>BT</th>
<th>Sem</th>
<th>P-valuesb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Interaction</td>
</tr>
<tr>
<td><strong>Mixture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP (g kg⁻¹ DM)</td>
<td>(310.5)</td>
<td>313.1</td>
<td>(313.1)</td>
<td>312.9</td>
<td>(303.4)</td>
</tr>
<tr>
<td>W (%)</td>
<td>(55.2)</td>
<td>24.7</td>
<td>(25.7)</td>
<td>20.1</td>
<td>(20.3)</td>
</tr>
<tr>
<td>D (%)</td>
<td>(44.8)</td>
<td>75.4</td>
<td>(74.3)</td>
<td>79.9</td>
<td>(79.7)</td>
</tr>
<tr>
<td>kₜ (h⁻¹)</td>
<td>(5.80)</td>
<td>3.99</td>
<td>(3.54)</td>
<td>3.13</td>
<td>(2.95)</td>
</tr>
<tr>
<td>%UIP</td>
<td>(22.8)</td>
<td>47.4</td>
<td>(46.8)</td>
<td>52.5</td>
<td>(53.5)</td>
</tr>
<tr>
<td>UIP (g kg⁻¹ DM)</td>
<td>(78.6)</td>
<td>164.7</td>
<td>(162.6)</td>
<td>182.4</td>
<td>(180.1)</td>
</tr>
<tr>
<td>%DUP</td>
<td>(95.4)</td>
<td>96.8</td>
<td>(97.2)</td>
<td>96.3</td>
<td>(97.0)</td>
</tr>
<tr>
<td>%DP</td>
<td>(98.8)</td>
<td>98.3</td>
<td>(98.5)</td>
<td>97.8</td>
<td>(98.2)</td>
</tr>
<tr>
<td>Starch (g kg⁻¹ DM)</td>
<td>(292.4)</td>
<td>280.5</td>
<td>(285.3)</td>
<td>277.0</td>
<td>(268.3)</td>
</tr>
<tr>
<td>W (%)</td>
<td>(51.9)</td>
<td>11.8</td>
<td>(14.3)</td>
<td>9.8</td>
<td>(4.9)</td>
</tr>
<tr>
<td>kₜ (h⁻¹)</td>
<td>(3.59)</td>
<td>5.49</td>
<td>(4.29)</td>
<td>5.01</td>
<td>(4.18)</td>
</tr>
<tr>
<td>%UISTA</td>
<td>(35.3)</td>
<td>47.2</td>
<td>(51.4)</td>
<td>50.2</td>
<td>(56.5)</td>
</tr>
<tr>
<td>UISTA (g kg⁻¹ DM)</td>
<td>(103.3)</td>
<td>132.5</td>
<td>(146.6)</td>
<td>139.0</td>
<td>(151.7)</td>
</tr>
<tr>
<td>SGD</td>
<td>(8.2)</td>
<td>19.8</td>
<td>(23.7)</td>
<td>55.6</td>
<td>(63.3)</td>
</tr>
<tr>
<td>PDI</td>
<td>(68.6)</td>
<td>24.6</td>
<td>(23.1)</td>
<td>18.4</td>
<td>(16.6)</td>
</tr>
<tr>
<td>NSH₂O</td>
<td>(61.4)</td>
<td>21.5</td>
<td>(20.1)</td>
<td>18.1</td>
<td>(16.1)</td>
</tr>
<tr>
<td>ADIN</td>
<td>(0.63)</td>
<td>0.79</td>
<td>(0.78)</td>
<td>0.82</td>
<td>(0.89)</td>
</tr>
</tbody>
</table>

*Note: Treatment C: untreated; T: toasted; BT: broken seeds and subsequently toasted; calculated values in parentheses.

*Interaction: measured values vs. calculated values; Contrast: Breaking: BT vs. T; NS: P>0.10.

*For abbreviations see Tables 2 and 3.*
Protein degradabilities of raw peas and lupins, reported by Aguilera et al. (1992) were consistent with our results, but %UIP of faba beans was higher than in our study. Their reported sensitivity to autoclaving varied between seeds, which was also observed in our study. For peas, lupins and faba beans, Aguilera et al. (1992) found increases of %UIP of 128%, 124% and 79%, while in our study the increases were 83%, 116% and 141%, respectively. Pressure toasting for 3 min at 132°C increased %UIP of faba beans more than autoclaving for 30 min at 120°C but due to the higher degradability of untreated beans in our study, %UIP of toasted beans (48%) was similar for the autoclaved beans (50%). The discrepancy between our results and those of Aguilera et al. (1992) could be due to the different processing conditions (temperature, but in particular processing time), differences in sample preparation (screen size) and in the procedure of in situ measurements (soluble fraction, diet, animals), apart from differences between batches and species of legume seeds.

Compared to toasting whole seeds, the toasting of broken seeds resulted in a higher %UIP, but this was only significant for peas and faba beans. During processing, transfer of heat and moisture to the seed kernel is delayed by the presence of the covering hull. Breaking seeds removes the hull, breaks the seeds into halves and thus facilitates the diffusion of heat to the kernel. This increases the effective processing time (the time in which the temperature of the whole seed equals the processing temperature) which was confirmed by the decreased PDI. Since the rate of the Maillard reaction decreases with lower moisture contents (Lea and Hannan, 1949; Cleale IV et al., 1987), diffusion of water may have played an additional role. Since ADIN is an indicator of Maillard polymerisation (Van Soest, 1994), the increased ADIN after the toasting of broken seeds compared to whole seeds is consistent with a more intensive heat treatment of broken seeds.

ADIN values of untreated lupins in the present study were lower than reported by Kung et al. (1991) and Murphy and McNiven (1994), but are similar to values of 1.6%, reported by Singh et al. (1995). Grinding fineness affects ADIN (Murphy and McNiven, 1994; Hussein et al., 1995) and may explain the discrepancy between our results and those reported by Kung et al. (1991), but not those of Murphy and McNiven (1994) and Singh et al. (1995). In the latter two papers, however, the reported ADF values were approximately 50% lower than found in our study and by Kung et al. (1991), which may have influenced ADIN. Based on the low ADIN values after toasting, it can also be concluded that denaturation rather than Maillard polymerisation is largely responsible for decreased protein degradability after toasting.

4.3. Starch degradability

To our knowledge, there are no studies reported of pressurised steam treatments on in situ starch degradability. In reported studies, application of heat is combined with shear forces, such as in expander treatment (Arieli et al., 1995), extrusion (Focant et al., 1990; Walhain et al., 1992; Arieli et al., 1995) or steam-flaking (Theurer, 1986). Therefore, the effects of heat and shear are confounded and effects on starch degradability cannot be solely attributed to either.

In our experiment W of starch decreased and $k_d$ slightly increased, which resulted in a decreased in situ starch degradability after pressure toasting. The application of moisture,
heat and shear during processing may induce several processes in starchy feedstuffs, such as swelling and gelatinisation (Theurer, 1986; Nocek and Tamminga, 1991). Swelling is not a likely cause for the decreased W, since heat-moisture treatments like pressure toasting decrease swelling power and solubility of starch (Hoover et al., 1993; Eliasson and Gudmundsson, 1996).

Sieve analysis of the material used for the rumen incubations, on the other hand, revealed that the ground toasted seeds contained a smaller fraction of particles <71 μm compared to the untoasted seeds. This may explain the decrease of W.

An increase in particle size decreased the k_d (Michalet-Doreau and Ould-Bah, 1992). This contrasts with our findings, but may result from a shift of small particles from the W of untreated samples to the D fraction after treatment. On the other hand, gelatinisation changes the starch structure from a semi-crystalline to an amorphous state and may have contributed to a higher degradation rate as well. This is in line with the positive correlation between SGD and the k_d of starch (r=0.61, P=0.06).

The effects of toasting on starch degradability were very much affected by the change of W. Although it did not alter the conclusions in this study, it was assumed that 10% of W escapes fermentation. This factor was introduced to correct in situ result to in vivo values (Nocek and Tamminga, 1991) and is similar to the fraction of soluble sugars in cereal grains (Sutton, 1971) and dried grasses (Weston and Hogan, 1968) escaping microbial fermentation. It seems reasonable to assume that W falls apart in two fractions, a really soluble fraction and a fraction of particles small enough to be washed out. Hungate (1968) assumed a degradation rate for soluble sugars of 2.0 h⁻¹. For an outflow rate of 0.15, this would mean that 7% of the soluble sugars escape fermentation. Starch, on the other hand, may additionally leave the rumen incorporated in microbial cells (Nocek and Tamminga, 1991). Given a k_d of 2.0 h⁻¹ and a k_p of 0.15 h⁻¹ for the soluble fraction, and assuming that W consists for 60% of particle loss (Chamerlain and Choung, 1995), the required k_d for complete degradation of the remaining 90% of the washable starch fraction can be calculated. Entrapment in the rumen mat may prevent small particles from being flushed out of the rumen with the fluid resulting in a particle outflow rate of 0.06 h⁻¹. This would require a k_d of 0.40 h⁻¹, only slightly higher than that of the most easily degradable starches (Tamminga et al., 1990).

Breaking seeds increases the effective processing time but, for starch, improved water diffusion may even be more important (Lund, 1984), since gelatinisation temperature increases dramatically as moisture content decreases below 35% (Colonna et al., 1992; Keetels, 1995). The thick and mechanically resistant nature of the cotyledon cell wall of pulses constitutes a physical barrier, preventing complete swelling of starch granules during processing (Tovar et al., 1992), which confirms the marked increase of SGD after breaking the seeds.

The W of CP and %UIP correlated positively with W of starch and %UISTA (r=0.99 and 0.94, respectively, P<0.0001).

Other authors also stressed the effects of the protein matrix on the starch degradability after processing (Trei et al., 1970; Holm et al., 1985; Theurer, 1986). Only steam treatments at higher temperatures, or in combination with mechanical treatments like for instance flaking, enhance starch degradability (Trei et al., 1970).
Heat treated, gelatinised, starches may recrystallize upon cooling (Siljestrom et al., 1989; Van Soest, 1994). This increased the amount of resistant starch (starch, indigestible in the small intestine) after autoclaving (Tovar and Melito, 1996). In the present study, all feedstuffs were oven dried (at 35°C, for 16 h) after toasting, which may also have lead to the formation of resistant starch. Preliminary results from another study showed, that starch digestibility was not decreased after toasting.

When sufficient amylolytic capacity is provided in the duodenum, an increase of rumen undegraded starch after toasting improves the nutritive value (Van Soest, 1994) of the feedstuffs. However, if the increased %UISTA is (partly) due to the formation of resistant starch, it may be broken down by less efficient fermentation in the lower tract. In that case, the benefit of the toasting treatment is only related to the increased protein value.

4.4. Additivity

CP degradability of the treated mixtures can be calculated from the values of the individual constituents. Literature (e.g. Murphy and Kenelly, 1986; De Boever et al., 1995) on associative effects for in situ studies is unambiguous in showing that mixing has no influence on the degradability of protein sources or concentrate mixtures. Our results are consistent with these data. Conversely, Vik-Mo and Lindberg (1985) and Chapoutot et al. (1990) observed a higher DM degradability for short time in situ incubation of compound feeds consisting of feedstuffs with different carbohydrate and protein degradability. The differences were more pronounced for less degradable feeds, which may be indicative of an improved microclimate for fermentation in the bags after combining feedstuffs of variable degradability.

Our data may be consistent with these data in demonstrating that differences between expected and actually measured values of starch \( k_d \) and %UISTA were significant and could not be ascribed to a single residue. It could be hypothesised that starch degradation was hampered due to a shortage of N in peas and faba beans, as in these seeds the nitrogen/starch ratio were lower than in the mixtures. However, based on calculated ratios of fermented nitrogen and nitrogen free organic matter this possibility was ruled out as it appeared that there was no nitrogen deficiency in the bags.

5. Conclusions

Pressure toasting for 3 min at 132°C decreased rumen protein degradability of peas, faba beans and lupins and rumen starch degradability of peas and faba beans. This effect was enhanced when broken seeds were processed, instead of whole seeds. The washable fraction of all legume seeds decreased for both constituents, the \( k_d \) of protein decreased, while the \( k_d \) of starch increased. It was concluded that protein degradability was decreased after pressure toasting with this procedure, without seriously affecting total tract protein digestibility. Changes in starch crystallinity and a less degradable protein matrix that encapsulates the starch granules could be responsible for the reduction in rumen starch degradability.
Toasting a mixture of peas, lupins and faba beans resulted in higher starch degradabilities than expected, based on the separately treated feedstuffs. This could not be related to a N-deficiency in the nylon bags or to a changed SGD after toasting.

Further research will concentrate on the relation between thermal processing time and temperature and the effects on rumen degradability and on the intestinal digestibility of rumen undegraded starch.

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