Effect of heat treatment of soybean meal and fish meal on amino acid digestibility in mink and dairy cows

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Abstract

Commercial solvent extracted soybean meal (SBM) and fish meal (FM) subjected to additional moist heat for 30 min at 120 or 130°C were investigated in terms of amino acid (AA) composition, total tract digestibility in mink, rumen and total tract digestibility in dairy cows of crude protein (CP) and individual AA. Heat treatment of SBM at 130°C caused significant reduction of the content of Arg, Lys and Cys by 4.1, 8.2 and 12.5%, respectively. Digestibility in mink of CP and most AA was significantly reduced after heat treatment of SBM at 120°C and further at 130°C. The digestibilities of Cys, Asp and Lys, which were the most severely affected AA, declined with 12.3, 10.9 and 8.8 percentage units, respectively, after treatment at 130°C. Heat treatment of FM at 120°C caused reduced digestibility of CP and His, Ile, Lys, Met, Asp, Glu, Gly, and Ser, while heat treatment at 130°C reduced total tract digestibility of CP and all AA in mink. Digestibility of Asp and Cys were most affected after heat treatment at 130°C with reduction of 17.9 and 11.4 percentage units, respectively. Rumen degradability of CP and all AA was significantly lowered by heat treatment of SBM. Met and Glu were the most affected AA, with a reduction of degradability after 16 h rumen incubation of 62.1 and 58.0 percentage units, respectively, after treatment at 130°C. Heat treatment of FM at 120°C caused declined rumen degradability of CP and total AA, although not to the same extent as for SBM. There was no additional effect on rumen degradability of treatment for either protein sources. Additional heat treatment of SBM reduced the rumen degradability of protein and AA more than treatment of FM, while for the nonruminant mink, total tract digestibility of SBM and FM was reduced similarly following heat treatment. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Heat treatment; Soybean meal; Fish meal; Digestibility; Amino acids; Ruminant; Nonruminant

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1. Introduction

Heating is an inherent part of the processing of soybean meal and fish meal. In nonruminants, proper heat treatment of soybean protein increases amino acid (AA) digestibility due to lowered activity of protease inhibitors (Marsman et al., 1995; Qin et al., 1996). Denaturation of proteins by mild heating may also facilitate digestion (Cheftel, 1979). On the other hand, excess heating lowers AA digestibility and biological value of soybean protein (Skrede and Krogdahl, 1985; Fernandez et al., 1994) as well as fish protein (Eggum, 1973). The AA supply to the ruminants depends on both delivery of AA to the duodenum and the subsequent digestibility within the intestine. For highly producing ruminants, heat treatment of protein supplements is used for increasing the amount of dietary protein escaping rumen degradation, and to increase the AA pool entering the small intestine (Waltz and Stern, 1989; Faldet et al., 1991; McKinnon et al., 1995). However, overheated protein sources may result in lowered intestinal digestibility also in ruminants (McKinnon et al., 1995; Dakowski et al., 1996). Thus, careful control of heating conditions is required to optimise nutritive value.

There is a paucity of data pertaining to comparative heat treatment effects of protein sources for ruminants and nonruminants. The present study was conducted to study the effects of additional heat treatment of solvent extracted soybean meal and fish meal on AA digestibility in a nonruminant (mink) and rumen degradability and total tract digestibility of AA in ruminant (dairy cows) animals.

2. Material and methods

2.1. Experimental protein sources

The experimental protein sources were solvent extracted soybean meal (SBM) and fish meal (FM) as well as the same protein sources subjected to additional heat treatment. Solvent extracted soybean meal was manufactured and obtained from Denofa AS, Fredrikstad, Norway. The soybeans were cut, flaked and solvent extracted with hexane before toasting at 105°C for ca. 0.5 h. Fish meal (Norse-LT 94) produced from herring (Clupea harengus) was delivered by Norsildmel A/L, Bergen, Norway. Two samples of the same batch of SBM and one sample of FM were added 200 g water per kg (wt/wt), spread in thin layers (1.5 cm) on aluminium trays covered with aluminium foil, and immediately autoclaved at 120°C (SBMT120 and FMT120) or 130°C (SBMT130 and FMT130) for 30 min in a laboratory autoclave. Thereafter, the samples were cooled and dried at 35°C for 18 h in an airdryer. All samples were milled to pass a 1.6 mm sieve and stored for three weeks at ambient temperature until used in animal experiments. Feed samples were ground to pass a 1 mm sieve for analysis of proximate composition, and ground to ≈0.5 mm for analysis of nitrogen (N) and AA.

2.2. Digestibility experiments with mink

The experiment was carried out with diets containing either SBM (diets SBM, SBMT120, SBMT130), or FM (diets FM, FMT120, FMT130) as the sole source of protein (Table 1). Diets were formulated to provide daily rations of ≈920 kJ ME, corresponding
to the maintenance requirement of adult male mink (NRC, 1982). The diets were mixed with water to a porridge of suitable consistency. Individual daily rations for the whole experiment were weighed into cups and stored at \(-22^\circ C\) until 1 day before feeding. Adult male mink of the genotype Standard dark, weighing at the start of the experiment from 1580 to 2490 g (\(\bar{x}=1864, SD=267\)) were used. Each diet was fed to four animals, which were given free access to water. The animals were held in individual metabolism cages equipped for controlled feeding and separate quantitative collection of faeces and urine. Each experiment lasted for 7 days, with quantitative collection of faeces being carried out the last 4 days. Freeze-dried faeces were coarsely ground and sieved for removal of hair, and finely ground to \(0.5\ mm\) before analysis of N and AA.

2.3. In situ measurements with dairy cows

Experimental animals were three non-lactating dairy cows of the Norwegian Cattle breed, fitted with a flexible rumen cannula (Bar Diamond, Pharma, ID, US) and a T-type PVC duodenal cannula located 50–60 cm distal to pylorus. Cows were fed at maintenance level a standardised diet consisting of 4 kg grass hay and 1.8 kg concentrate mixture, containing 175 g crude protein (CP) kg\(^{-1}\) DM per day. The rations were offered in equal meals at 06:00 h and 15:00 h. The in situ procedures, applied to measure rumen degradability and intestinal digestibility of CP and AA, were as described by Madsen et al. (1995) and Prestløkken (1999) with the exception of pore size of the nylon bags used to measure intestinal digestibility. In the present study, a pore size of 15 \(\mu m\) (ZBF, AG, CH-8803, Rüschlicon, Switzerland) was used instead of 11 \(\mu m\) as recommended by Madsen et al. (1995). The intestinal digestibility measurements were on rumen undegraded feed residues after 16 h of rumen incubation.

2.4. Chemical analyses

Feed content of ash, crude fat (acid hydrolysis and extraction with petrol ether) and crude fibre (CF) were determined by standard procedures (AOAC, 1990).Nitrogen in feeds, mink faeces and in residues after ruminal and intestinal incubation was determined.
according to the principle of Dumas (AOAC, 1990) by use of an EA 1108 CHNS-O Elemental Analyzer (Fisons Instruments S.p.A., Rodano Milan, Italy). All AA except Trp in feeds, faeces, and residues after 16 h rumen incubation and subsequent intestinal incubation were determined according to European Community Directive 98/64/EC (OJ L 257, 1998, OJ, 1998). To protect Met and Cys during hydrolysis, the samples were oxidised with performic acid to give methioninsulfone and cysteic acid (Moore, 1963). The AA were separated with ion-exchange chromatography followed by post-column reaction with ninhydrin and photometric detection at 570 nm (440 nm for Pro) using the Biochrom 20 amino acid analyser (Pharmacia Biotech, UK). Tyr was analysed in oxidised samples, although oxidation reduces the content of Tyr (Mason et al., 1980). Based on unpublished results from our laboratory, Tyr was increased with 10% for losses during oxidation. The analysed contents of Ser, Val and Ile were adjusted with 6% for incomplete recovery after hydrolysis (Rudemo et al., 1980). The SBM were analysed for acid detergent insoluble nitrogen (ADIN), according to Goering et al. (1972).

2.5. Calculations and statistical analyses

Crude protein was calculated as N×6.25. Nitrogen-free extract (NFE) was calculated as
\[ \text{NFE} = 100 - (\text{ash} + \text{CP} + \text{crude fat} + \text{CF}) \]
In mink, true total tract digestibility of N and AA was determined using values for metabolic faecal excretion given by Skrede (1979). Effective rumen degradability of protein (EPD) was estimated according to (Ørskov and McDonald, 1979), using the PROC NLIN procedure in the Statistical Analysis System (SAS, 1990), assuming a rumen outflow rate \( (k) \text{ of } 8\% \text{ h}^{-1} \). True intestinal digestibility of rumen undegraded protein was calculated according to the equation of Hvelplund et al. (1992). Total tract digestibility of N and AA in dairy cows was calculated as 100 minus percent indigested residue (IR) found after rumen incubation for 16 h and subsequent intestinal incubation.

The effect of treatment on the chemical composition of SBM was tested on the basis of the two independent productions, by a one-way general linear model (GLM procedure) in the Statistical Analysis System (SAS, 1990). The effects of feed and treatment on true total tract digestibility in mink, and on digestibility measurements N and AA in dairy cows were also tested by a GLM in Statistical Analysis System (SAS, 1990), and with backward elimination of variables to find the best fitting model for each AA and N, as shown below (Models 1 and 2):

Mink experiment (Model 1):

\[ Y_{ijk} = \mu + A_i + B_j + C_k + e_{ijk} \]
where \( Y_{ijk} \) is true digestibility of N or individual AA. \( A_i \) the effect of feed \( (i=1, 2) \), \( B_j \) the effect of treatment \( (j=1, 2, 3) \), \( C_k \) the feed treatment interaction, and \( e_{ijk} \) the error.

In situ experiment (Model 2):

\[ Y_{ijklmn} = \mu + A_i + B_j + C_k + D_l + E_m + F_n + e_{ijklmn} \]
where \( Y_{ijklmn} \) is EPD, 16 h rumen degradability, total tract digestibility of N, individual AA, digestible amount of N or AA. \( A_i \) is effect of feed \( (i=1, 2) \), \( B_j \) the effect of treatment \( (j=1, 2, 3) \), \( C_k \) the effect of animal \( (k=1, 2, 3) \), \( D_l \) the feed treatment interaction, \( E_m \) the feed animal interaction, \( F_n \) the treatment animal interaction, and \( e_{ijklmn} \) the error.
Differences between means were considered significant at $p<0.05$, unless stated otherwise.

3. Results

3.1. Chemical composition and AA profile of the experimental protein sources

Chemical composition and AA profiles of untreated and heat treated SBM and FM are given in Table 2. Dry matter content in both SBM and FM increased numerically ($p>0.05$) after autoclaving and subsequent low-temperature drying. The content of ash, CP, CF and NFE in both SBM and FM was unaffected by treatment, whereas the crude fat content in SBM increased slightly, but significantly, after heat treatment. Acid detergent insoluble nitrogen in SBM was not affected by heat treatment. Heat treatment of SBM reduced the contents of Arg, Lys and Cys significantly, while there appeared to be no effects of heat treatment on AA composition of FM.

3.2. Total tract digestibility in mink

One animal in the SBM group that left >75% of the diet, was judged as an outlyer, and was omitted from further calculations. The digestibility of total AA was higher than that of N with all diets (Table 3). The mink showed generally higher N and AA digestibility of FM than of SBM. Digestibilities of individual AA in untreated SBM and FM showed relatively high figures for Arg, and low figures for Cys and Tyr.

Heat treatment of SBM at 130°C caused significantly reduced digestibility of both N and total AA (Table 3). Among individual AA, Cys, Asp and Lys suffered the most severe digestibility losses. Heat treatment of FM at 130°C caused significantly reduced digestibility for N, all EAA, and all NEAA except Tyr (Table 3). Digestibility of Asp was most affected followed by that of Cys, His, Ser and Gly. Heating of SBM and FM at 120°C gave intermediate total tract N and AA digestibilities compared with those obtained with the untreated and higher temperature (130°C) treatments.

3.3. In situ measurements with dairy cows

Untreated FM had significantly lower EPD than untreated SBM (Table 4). The lower EPD of FM was caused by a slow degradation rate of the potentially degradable b-fraction (c). Heat treatment of FM had minor effects on the soluble a-fraction and the degradation rate of the b-fraction, resulting in a rather small effect on EPD as well as disappearance of N and AA after 16 h rumen incubation, relative to corresponding effects with SBM (Tables 4 and 5). Heat treatment of SBM significantly reduced disappearance of N and all AA after 16 h rumen incubation, Met being the most affected AA followed by Glu, Ser, Thr and Ile (Table 5). These AA were those with the lowest degradability in untreated SBM. This was contradictory to the results for FM, where the most affected AA after heat treatment were Glu and His, which were among the highest degradable AA in untreated FM. Rumen degradability was lower for FMT$_{120}$ than for FMT$_{130}$, although the difference
was not significant \((p>0.05)\) (Tables 4 and 5). Total tract digestibility of CP and all AA was very high, and was not affected neither by protein sources nor treatments (Table 6).

### 3.4. Digestibility in mink versus in situ measurements with dairy cows

Heat treatment for protecting the protein against rumen degradation was more efficient with SBM than with FM, while in mink heat treatment caused a similar reduction in total
tract digestibility of protein for FM and SBM (Tables 3–5). Both rumen degradability and mink total tract digestibility of FM declined after heat treatment, but the differences between FMT\textsubscript{120} and FMT\textsubscript{130} were for most AA significant in mink and insignificant ($p>0.05$) in dairy cows. Overall, rumen degradability responded more extensively than total tract digestibility in mink to heat treatment of the protein sources, although rumen degradability of FM did not decrease further after treatment above 120°C. In contrast, total tract digestibility of N and AA in dairy cows was not significantly affected by heat treatment of either SBM or FM.

4. Discussion

4.1. Effect of heat treatment on the AA composition in the experimental protein sources

The AA profile of untreated SBM and FM was similar to earlier findings for commercial SBM and FM (Skrede and Krogdahl, 1985; Harstad and Prestløkken, 1999). Heat treatment at both 120 and 130°C caused some browning of the SBM samples,
probably due to Maillard reaction products. Soybean meal contains 0.5–1% reducing sugars (Bach Knudsen, 1997) which allow Maillard reaction upon heating. The SBM also contain circa 13% of nonreducing oligosaccharides (Bach Knudsen, 1997), which may contribute to Maillard reactions assuming some cleavage to reducing monosaccharides during heating. Treatment of SBM reduced the content of Arg, Lys and Cys (Table 2) which agree with Skrede and Krogdahl (1985) who found similar losses of the same AA in solvent extracted SBM after moist heat treatment at 135°C for 30 min. No effect on AA content after heat treatment of FM appeared. In contrast to SBM, FM contains no carbohydrates. Since the small amounts of sugar present in raw fish (Jones, 1958), tend to be broken down by microbial activity before processing to FM. Therefore, the Maillard reaction is unlikely to proceed sufficiently to affect AA content during fish meal production (Pike et al., 1990) as well as under the heat treatment conditions used in the present study.

4.2. Effect of heat treatment on total tract digestibility in mink

Skrede and Krogdahl (1985) showed that Arg was the highest and Cys the lowest digestible AA in solvent extracted SBM, which agree with the results of the present study. The same authors showed that heat treatment of SBM by autoclaving at 135°C for 30 min caused a decline of total tract digestibility in mink of all AA compared to untreated SBM.
This was also the case in the present study, but the effects were less dramatic than found by Skrede and Krogdahl (1985). The digestibility of the untreated SBM was higher in the present study than found by Skrede and Krogdahl (1985), and the inconsistency in response to additional heating may be related to differences in the commercial processing of solvent extracted SBM.

In the present study, heat treatment of FM caused significantly reduced total tract digestibility in mink of N and all AA except Tyr. No studies dealing with additional heating of commercial FM and effects on digestibility in mink have been found. However, Wiseman et al. (1991), working with pigs, found reduced ileal digestibility of all the AA analysed except Thr and Cys after treatment of FM at 130°C for 3 h. Ileal AA digestibility in pigs have been shown to be comparable with total tract digestibility in mink (Skrede et al., 1998).

It is surprising that moist heat caused similar reduction of digestibility of FM and SBM in mink, since no reducing sugars are present in the FM. One reason for the reduction of digestibility in FM may be that proteins or certain AA, such as Lys, Met, Cys and Trp, have reacted with oxidising lipids (Hurrell, 1984). Fish meal used in this study contained 10% fat, and during heating the oxidising lipids themselves, or reaction products as free

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### Table 5
Rumen degradability (%) of feed N, total and individual amino acids (AA) after 16 h incubation in rumen for untreated and heat treated soybean meal and fish meal

<table>
<thead>
<tr>
<th>Protein sourcea</th>
<th>SBM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SBMT&lt;sub&gt;120&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SBMT&lt;sub&gt;130&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FM&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FMT&lt;sub&gt;120&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FMT&lt;sub&gt;130&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>SEM</th>
<th>Contrast SBM vs. FM</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>77.1 a</td>
<td>35.0 b</td>
<td>26.8 c</td>
<td>53.9 a</td>
<td>42.4 b</td>
<td>45.2 b</td>
<td>1.35 ***</td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>78.8 a</td>
<td>36.4 b</td>
<td>27.6 c</td>
<td>52.3 a</td>
<td>37.1 c</td>
<td>41.7 b</td>
<td>1.42 ***</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>75.4 a</td>
<td>34.3 b</td>
<td>30.5 b</td>
<td>51.3 a</td>
<td>31.0 c</td>
<td>37.9 b</td>
<td>1.90 ***</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>72.2 a</td>
<td>25.7 b</td>
<td>18.4 c</td>
<td>38.0 a</td>
<td>24.1 b</td>
<td>29.2 b</td>
<td>1.64 ***</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>72.8 a</td>
<td>33.1 b</td>
<td>26.1 c</td>
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<td>30.9 b</td>
<td>34.8 b</td>
<td>1.41 ***</td>
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<tr>
<td>Lys</td>
<td>75.8 a</td>
<td>37.0 b</td>
<td>28.8 c</td>
<td>51.2 a</td>
<td>35.0 b</td>
<td>38.6 b</td>
<td>1.37 ***</td>
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<td>Met</td>
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<td>19.7 b</td>
<td>8.0 c</td>
<td>43.2 a</td>
<td>29.4 b</td>
<td>31.9 b</td>
<td>1.66 ***</td>
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<tr>
<td>Phe</td>
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<td>21.1 c</td>
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<td>25.7 c</td>
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<td>1.52 ***</td>
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<td>Val</td>
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<td>22.0 c</td>
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<td>29.3 b</td>
<td>34.7 b</td>
<td>2.39 ***</td>
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<td>24.0 c</td>
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<td>34.8 a</td>
<td>39.7 b</td>
<td>1.46 ***</td>
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</table>

<sup>a</sup> See Table 2 for explanation.

<sup>b</sup> Means in the same row within protein source, with no common letters differ (p<0.05).
radicals or hydroperoxides, may have reacted with proteins and thereby affected the digestibility.

Another reason for reduced Cys digestibility due to heat treatment is formation of disulphide bridges. The analytical method used in the present study does not discriminate between free Cys and Cys bound in disulphide molecules. Thus, creation of disulphide bridges would not cause analytical losses of Cys. On the other hand, an increased proportion of feed Cys bound in poorly digestible disulphide bridges will increase the level of Cys residues in faeces and reduce digestibility (Opstvedt et al., 1984).

4.3. Effect of heat treatment on protein and AA digestion in dairy cows

In the present study, heat treatment by autoclaving reduced EPD in SBM considerably. Heat treatment of SBM by expelling and roasting have shown about the same effect (Faldet et al., 1991; Titgemeyer and Shirley, 1997). In contrast, extrusion cooking does not seem to be effective (Daecon et al., 1988; Waltz and Stern, 1989), presumably due to the very short heating time during extrusion. Moreover, to obtain an effective heat treatment that protects protein from rumen degradation, moisture levels above 20% is required (Cleale et al., 1987).

Table 6
Total tract digestibility (%) in dairy cows of feed N and individual amino acids (AA) for untreated and heat treated soybean meal and fish meal

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<tr>
<th>Protein sourcea</th>
<th>SBM</th>
<th>SBMT&lt;sub&gt;120&lt;/sub&gt;</th>
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<th>FM</th>
<th>FMT&lt;sub&gt;120&lt;/sub&gt;</th>
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a See Table 2 for explanation.
Met was clearly the least degradable AA in SBMT\textsubscript{120} and SBMT\textsubscript{130} after 16 h rumen incubation. This specific effect of heat treatment on Met degradability is hard to explain. Considering the low lipid content in defatted SBM, formation of oxidised Met during reaction with oxidising lipids or polyphenols (Hurrell, 1984), is unlikely to have occurred. Rumen degradability of Tyr and Ser was also greatly affected by heat treatment. The latter two AA are able to make ester linkage to Asp and Glu, or linkages between each other (Stanley, 1989). These reactions may explain decreased rumen degradability of these AA.

The FM used in the present study contained a high fraction of water soluble protein (a-fraction) which was only slightly affected by heat treatment (Table 4). Thus, a relatively large proportion of the a-fraction was not true protein, but free AA and other non-protein-nitrogen (NPN) compounds. This may partly explain the minor effect of heating FM at 120°C on EPD and no further effect of heating to 130°C. However, the results might also indicate that heating during processing of the FM effectively protected the protein in the presscake fraction against rumen degradation.

4.4. Digestibility in mink versus in situ measurements with dairy cows

Additional heat treatment of SBM and FM reduced the extent of rumen degradation of protein and AA more than total tract digestibility in mink. This may be due to the difference in pH in the rumen compared to the stomach of mink. The first step in protein digestion in nonruminants consists of a protein denaturation step caused by the acidic pH of the stomach. Unfolded denatured proteins are frequently more easily digested by proteolytic enzymes than the native protein structure. An explanation for this may be that several peptide bonds originally shielded inside the protein become more accessible to proteases (Cheftel, 1979). In contrast, rumen contents have not been exposed to low pH, and the proteins have not changed structure before the microbial enzymes attack. Heat treatment causes cross-linkages within the protein molecules (Bohak, 1964; Ziegler et al., 1967; Stanley, 1989), between proteins and lipids (Hurrell, 1984), and in protein of plant origin additional cross-linkages between amino acid residues and reducing substances (Cheftel, 1979). When these molecules are not denatured by low pH, they seem to be quite unavailable to microbes. Therefore, rumen degradability may be more sensitive to heat treatment of the protein sources than total tract digestibility in both ruminant and nonruminant animals.

Post-ruminal digestibility of CP and AA was in the present study significantly higher and less affected by heat treatment than total tract digestibility in mink (Tables 3 and 6). The mobile nylon bag method does not measure digestibility, but actually loss from the bags, and some protein compounds which have disappeared through the bag pores may be unabsorbable in the small intestine (Hvelplund, 1985). Thus, the method used may to some extent overestimate the intestinal digestibility in dairy cows. It is a question if intestinal digestibility is independent of rumen incubation (Volden and Harstad, 1995). However, predigestion of conventional SBM and FM in the rumen has probably no influence on protein digestibility in the intestine (Volden and Harstad, 1995). It is therefore likely that the higher intestinal digestibilities in dairy cows compared with mink may to a great extent be due to differences in the capacity of protein digestion in the small intestine.
Acknowledgements

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References


