Protein Digestibility and Quality in Products Containing Antinutritional Factors Are Adversely Affected by Old Age in Rats

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ABSTRACT The protein digestibility-corrected amino acid score (PDCAAS) has been recommended to be the most suitable method for routine evaluation of protein quality of foods by FAO/WHO. The PDCAAS method includes the use of young rats for predicting protein digestibility of foods for all ages including the elderly. To assess the usefulness of protein digestibility in old rats in the calculation of PDCAAS for the elderly, the influence of age on the digestibility of protein in 5-wk-old and 20-mo-old rats by the balance method was studied. Fifteen protein products were tested. Each protein product was fed as the sole source of 10% dietary protein. A protein-free diet was also included to obtain an estimate of metabolic fecal protein. Protein digestibility values (corrected for metabolic fecal protein loss) in old rats were significantly ($P < 0.05$) lower than in young rats for most products; however, these differences were small (up to 3%) for properly processed animal products (casein, whey protein concentrate, whey protein hydrolysate, lactalbumin and skim milk powder). Similarly, the differences attributed to age were not large (up to 5%) for properly processed vegetable protein products (soy protein isolate and autoclaved soybean meal, black beans and fava beans). However, digestibility values in old rats were considerably lower (7–17%) than in young rats when fed products containing antinutritional factors, that is, mustard flour containing glucosinolates; alkaline/heat-treated soy protein isolate and lactalbumin-containing lysinoalanine; raw soybean meal and black beans containing trypsin inhibitors; and heated skim milk powder containing Maillard compounds. Therefore, the inclusion of protein digestibility data obtained using young rats in the calculations of PDCAAS may overestimate protein digestibility and quality of these products for the elderly. For products specifically intended for the elderly, protein digestibility should be determined using old rats. J. Nutr. 133: 220–225, 2003.

KEY WORDS: • protein digestibility and quality • antinutritional factors • aging • rats

Amounts of indispensable amino acids (IAA) ² (1), digestibility of protein and bioavailability of amino acids are basic parameters in determining the quality of a protein source. A joint FAO/WHO Expert Consultation on Protein Quality Assessment was held in 1991 to review routine methods based on in vitro or animal assays that correlate well with data from human studies (1). The Consultation agreed that the protein digestibility-corrected amino acid score (PDCAAS) method was the most suitable approach for routine assessment of protein quality for humans, and recommended its adoption as an official method at the international level. The validity of the PDCAAS method was recently endorsed by FAO/WHO in 2001 (2) in assessing protein quality of mixed diets and of properly processed (containing minimal amounts of antinutritional factors) and highly digestible (where digestibility of protein is a good predictor of bioavailability of amino acids) food products. However, it was recommended that the impact of antinutritional factors associated with proteins, including naturally occurring and those formed during processing, on protein digestibility and quality should be further investigated.

Calculations of PDCAAS values require the determination of protein contents, amino acid profile and protein digestibility. Based on extensive evaluation of existing in vitro and in vivo methods in foods, the rat balance method is considered to be the most suitable and practical method for predicting digestibility of protein by humans (1). Therefore, when human balance studies cannot be used, the standardized rat fecal balance method of Eggum (3) or McDonough et al. (4) is recommended.

Both of these methods (3,4) include the use of young rats for predicting protein digestibility of foods for all ages of humans including the elderly. With a rapidly increasing proportion of the population living to an older age, the nutrient needs of the elderly have assumed great significance as national and international government agencies attempt to provide dietary guidance to reduce chronic diseases and promote a high level of health in the elderly (5).

Information on the relationship between the functionality of the gastrointestinal tract and age in humans has been reviewed by Harper (6). Despite the large number of studies on this subject, there is no consensus concerning whether healthy old individuals have a reduced capacity of macronutrient di-
gestion. There is a general belief that pancreatic function gradually diminishes. However, there are conflicting reports on the efficiency with which pancreatic enzymes and bicarbonate are secreted in older subjects (6). Harper (6) concludes that, although there are age-related changes in a number of aspects of gastrointestinal function, these are of little physiological significance in most healthy older individuals. However, there may be some older subjects whose decreased gastrointestinal function makes them more susceptible to minor dietary insults (6) such as the presence of antinutritional factors. Information on the effect of age on protein digestibility, as determined by the balance method, in humans or in animal models is limited.

The objective of the present investigation was to assess whether protein digestibility in rats is influenced by age, given that rats are the standard animal model for predicting protein digestibility of foods for humans. The influence of age on the digestibility of protein and on the PDCAAS values in 15 animal and vegetable protein products, with or without antinutritional factors, was studied in 5-wk-old and 20-mo-old rats, in an effort to develop a potentially more accurate rat model for predicting protein digestibility for the elderly.

MATERIALS AND METHODS

Protein sources. Casein (Animal Nutrition Research Council Reference Protein); lactalbumin (ICN, Cleveland, OH); whey protein concentrate, whey protein hydrolysate (New Zealand Milk Products, Santa Rosa, CA); soybean protein isolate (SPI, Supro 620; Les Aliments UFL, Montreal, Quebec, Canada); and canned fava beans (Unico Ltd., Toronto, Ontario, Canada) were purchased commercially.

As described previously, mustard protein flour was supplied by the University of Saskatchewan, Saskatoon, Saskatchewan, Canada (7). Raw soybean meal, raw black beans and skim milk powder were purchased locally. The samples of raw soybean meal, autoclaved soybean meal (103 kPa and 121°C for 20 min), raw black beans, autoclaved black beans (soaked and autoclaved), heated skim milk powder (103 kPa and 121°C for 1 h), alkaline/heat-treated lactalbumin and alkaline/heat-treated soy protein isolate were prepared as reported previously (7). All the test protein products were equilibrated to room temperature and humidity before analysis and diet formulation.

Total nitrogen in protein sources and feces samples was determined (in duplicate) by the micro-Kjeldahl method using a Kjeltec Auto 1030 Analyzer (Tecator, Herndon, VA). Protein was calculated using a nitrogen-to-protein conversion factor of 6.25 in all cases. On analysis, the protein sources tested in this study contained the following amounts of protein (g/100 g, air-dry basis): casein, 89.90; whey protein concentrate, 83.30; whey protein hydrolysate, 84.42; lactalbumin, 85.21; alkaline/heat-treated lactalbumin, 80.25; skim milk powder, 33.93; heated skim milk powder, 33.83; soy protein isolate, 85.50; alkaline/heat-treated soy protein isolate, 81.90; raw soybean meal, 41.91; autoclaved soybean meal, 41.85; raw black beans, 21.50; autoclaved black beans, 22.67; autoclaved fava beans, 27.15; and mustard protein flour, 47.64. These protein values were used in formulating diets and in calculating protein intake data needed for the determination of protein digestibility.

Experimental design. A balance study using 5-wk-old and 20-mo-old rats was conducted to study the influence of aging on the digestibility of protein in 15 animal and vegetable protein products. The compositions of experimental diets are shown in Table 1. The protein diets contained 10% protein (N × 6.25). In the protein diets, each product was fed as the sole source of dietary protein.

Animals. Five-wk-old male Fischer-344 rats (Harlan Sprague Dawley, Indianapolis, IN) and 20-mo-old male Fischer-344 rats were used in the feeding study. Experimental diets were fed to groups of eight rats (5-wk-old or 20-mo-old) in a completely randomized design. Animals were housed individually in stainless steel screen-bottom cages (permitting free droppings of excreta) in a temperature-controlled (24–25°C) and humidity-controlled (49–50%) facility with 12-h light:dark cycle. Highly absorbent paper was placed under the cages to catch spilled food and to minimize contamination of feces with urine. Rats were given free access to food and water for 10 d including a balance period of 4 d. The Health Canada Guide for the Care and Use of Laboratory Animals was followed, and the animal protocol was approved by the animal care committee.

Protein digestibility determinations. During the balance period of 4 d, total feces were collected daily and stored at −5°C, and records of daily food consumption were kept. At the end of the collection period, samples of total feces from each rat were lyophilized, weighed and ground. The ground feces from eight rats fed the same diet were pooled and analyzed for total nitrogen. Eight individual protein digestibility values per diet were then calculated by using the protein intake and fecal output data for each rat. The balance method used in this study was previously reported to yield digestibility results similar to those obtained by using metabolic cages; the differences attributed to the methods were not >1% (8). The use of screen-bottom cages, as done in this study, has been a common practice in determining digestibility of protein and amino acids (9,10).

Protein digestibility values (corrected for metabolic fecal protein loss) were calculated using the following equation:

\[ \text{Protein digestibility} = \frac{\pi - (F_P - MFP)}{\pi} \times 100, \]

where \( \pi \) is the protein intake, \( F_P \) is the fecal protein and MFP is the metabolic fecal protein. The amount of protein in the feces of rats fed the protein-free diet was used as the estimate for MFP.

The corrected protein digestibility values as calculated in this study have been reported as true protein digestibility values in the literature. Because the metabolic fecal protein loss or ileal protein endogenous losses are affected by individual foods/feeds and possibly by age of animals, the use of the word true may not be accurate. Therefore, it may be more appropriate to use the term corrected instead of true in describing protein digestibility values, as determined in the present study.

PDCAAS determinations. Previously reported amino acid composition data of the test protein products (7) and the digestibility values determined in this investigation were used to calculate the PDCAAS values, according to the following equation (11):

\[ \text{PDCAAS} = \frac{\text{PDCAAS of protein source}}{\text{PDCAAS of reference protein source}} \]

with the following values: 0.10 for casein, 0.25 for whey protein concentrate, 0.35 for whey protein hydrolysate, 0.45 for lactalbumin, 0.55 for ALBM, 0.65 for SMP, 0.75 for SPI, 0.85 for soy protein isolate, 0.95 for soybean meal, 0.90 for soybean meal, autoclaved, 0.92 for black beans, 0.94 for black beans, autoclaved, 0.80 for fava beans, 0.82 for fava beans, autoclaved, and 0.85 for mustard protein flour.
PDCAAS (%) = Protein digestibility 
\times \text{amino acid score (or the lowest amino acid ratio)}.

Amino acid ratios (mg of an indispensable amino acid in 1.0 g of test protein/mg of the same amino acid in 1.0 g of reference protein) \times 100 for nine indispensable amino acids (histidine, isoleucine, leucine, lysine, methionine + cysteine, phenylalanine + tyrosine, threonine, tryptophan and valine) were calculated by using the 1985 suggested pattern of amino acid requirements for preschool children (2–5 y) as the reference pattern (1). Two sets of PDCAAS values were calculated by using protein digestibility values obtained from young and old rats.

**Statistics.** Pooled SEM and main effects of age of rats and protein source and their interactions were identified using two-way ANOVA with the Statistical Systems for Personal Computers (SAS Institute, Cary, NC). Differences between age of rats and between various protein sources were determined using Tukey’s highly significant difference test (12). Significance of difference was established at $P < 0.05$.

**RESULTS**

The main effects (age of rats and protein source) and their interactions had significant ($P < 0.0001$) influence on protein digestibility.

**Influence of age of rats on protein digestibility**

The digestibility values in old rats were significantly ($P < 0.05$) lower than in young rats for casein, whey protein concentrate, alkaline/heat-treated lactalbumin and heated skim milk powder (Table 2). The differences were especially large (up to 9%) for the processed products such as heated skim milk powder and alkaline/heat-treated lactalbumin. Effect of age had no effect on protein digestibility of whey protein concentrate, untreated lactalbumin and unheated skim milk powder.

The digestibility values in old rats were also significantly ($P < 0.05$) lower than in young rats for all vegetable products except soy protein isolate (Table 3). The differences were more marked (up to 17%) for products containing antinutritional factors such as alkaline/heat-treated soy protein isolate, mustard flour, raw soybean meal and raw black beans.

**Influence of protein sources on digestibility**

**Young rats.** In young rats, casein, whey protein concentrate, whey protein hydrolysate and lactalbumin had high protein digestibility values (96–100%), whereas skim milk powder had an immediate protein digestibility of 92% (Table 4). The alkaline/heat treatment of lactalbumin had a significant negative effect on protein digestibility (96 vs. 84%). Similarly, the heat treatment reduced the protein digestibility of skim milk powder from 92 to 77%.

Among vegetable products, soy protein isolate and mustard flour had higher protein digestibility values of 93–97% compared to those of other vegetable protein sources (66–83%) (Table 4). The alkaline/heat treatment of soy protein isolate resulted in drastically reduced protein digestibility (95 vs. 66%). Proper processing (autoclaving) caused significant improvement in protein digestibility of raw black beans (71 vs. 83%) but had no effect on protein digestibility of raw soybean meal (80 vs. 81%). Protein digestibility of autoclaved fava beans (81%) was similar to those for autoclaved soybean meal and black beans (81–83%).

**Old rats.** As noted in the case of young rats, the protein digestibility values as determined in old rats for casein, whey protein concentrate, whey protein hydrolysate and lactalbumin were high (96–99%) (Table 4). Similarly, protein digestibility of skim milk powder was substantially reduced by the heat treatment (92 vs. 69%). However, the alkaline/heat treatment of lactalbumin and soy protein isolate had more marked adverse effects on protein digestibility of these two products in old rats (96 vs. 64 and 93 vs. 43%, respectively).

Among vegetable products, soy protein isolate had higher protein digestibility compared to other vegetable protein products (93 vs. 49–79%) (Table 4). The alkaline/heat treatment of soy protein isolate reduced its protein digestibility by about 50% (93 vs. 49%). Proper processing (autoclaving) had significant positive effects on protein digestibility of both raw soybean meal (72 vs. 79%) and raw black beans (60 vs. 78%).

### TABLE 2

**Calculations of digestibility of protein in diets containing animal protein sources fed to 5-wk- and 20-mo-old rats**

<table>
<thead>
<tr>
<th>Diet1</th>
<th>5-wk-old rats</th>
<th></th>
<th>20-mo-old rats</th>
<th></th>
<th>Protein digestibility2,4,5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Food intake2</td>
<td>Fecal output2</td>
<td>Fecal protein3</td>
<td>5-wk-old rats</td>
<td>20-mo-old rats</td>
</tr>
<tr>
<td></td>
<td>g/4-d</td>
<td>g/100 g</td>
<td></td>
<td>g/4-d</td>
<td>g/100 g</td>
</tr>
<tr>
<td>Casein</td>
<td>54</td>
<td>3.28</td>
<td>14.15</td>
<td>51</td>
<td>3.59</td>
</tr>
<tr>
<td>Whey protein concentrate</td>
<td>45</td>
<td>2.81</td>
<td>12.13</td>
<td>45</td>
<td>3.76</td>
</tr>
<tr>
<td>Whey protein hydrolysate</td>
<td>54</td>
<td>3.66</td>
<td>11.88</td>
<td>50</td>
<td>3.92</td>
</tr>
<tr>
<td>Lactalbumin (ALBM)</td>
<td>40</td>
<td>2.52</td>
<td>16.02</td>
<td>55</td>
<td>4.24</td>
</tr>
<tr>
<td>ALBM, alkaline/heat-treated</td>
<td>43</td>
<td>3.91</td>
<td>39.83</td>
<td>41</td>
<td>5.29</td>
</tr>
<tr>
<td>Skim milk powder (SMP)</td>
<td>58</td>
<td>4.83</td>
<td>17.14</td>
<td>61</td>
<td>5.10</td>
</tr>
<tr>
<td>SMP, heated</td>
<td>49</td>
<td>5.83</td>
<td>28.88</td>
<td>62</td>
<td>8.70</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>4</td>
<td>0.26</td>
<td>—</td>
<td>4</td>
<td>0.26</td>
</tr>
</tbody>
</table>

1 The diets were formulated to contain 10% crude protein (N × 6.25).
2 Values are means ($n = 8$), initial weight of 5-wk-old rats = 55 ± 5 g; initial weight of 20-mo-old rats = 461 ± 8 g).
3 Values are means ($n = 2$).
4 The fecal excretion of protein in 5-wk-old rats (750 mg/100 g food consumed) and in 20-mo-old rats (825 mg/100 g food consumed) fed the protein-free diet was used in calculating protein digestibility values corrected for metabolic fecal protein loss.
5 Within each diet, protein digestibility values (attributed to age of rats) marked by asterisks differ ($P < 0.05$).
Protein digestibility of autoclaved fava beans (77%) was similar to that of autoclaved soybean meal and autoclaved black beans (78–79%).

PDCAAS values

In the case of the properly processed and high quality animal protein products (casein, whey protein concentrate, whey protein hydrolysate, lactalbumin and skim milk powder), PDCAAS values were not affected by the age of the rats (Table 4).

In the case of the properly processed vegetable protein products (soy protein isolate, autoclaved soybean meal, autoclaved black beans and autoclaved fava beans), the differences in PDCAAS values attributed to age of the rats were also small (2–5%; Table 4).

However, differences in PDCAAS values of protein products containing antinutritional factors (alkaline/heat-treated lactalbumin, alkaline/heat-treated soy protein isolate, heated skim milk powder, raw soybean meal, raw kidney beans and mustard flour) attributed to age of rats were significant, up to

### TABLE 3
Calculations of digestibility of protein in diets containing vegetable protein sources fed to 5-wk-old and 20-mo-old rats

<table>
<thead>
<tr>
<th>Diet1</th>
<th>Food intake2</th>
<th>Fecal output2</th>
<th>Fecal protein3</th>
<th>Protein digestibility2,4,5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/4-d</td>
<td>g/100 g</td>
<td>g/4-d</td>
<td>g/100 g</td>
</tr>
<tr>
<td>Soy protein isolate (SPI)</td>
<td>52</td>
<td>4.14</td>
<td>15.74</td>
<td>71</td>
</tr>
<tr>
<td>SPI, alkaline/heat-treated</td>
<td>33</td>
<td>3.46</td>
<td>39.86</td>
<td>17</td>
</tr>
<tr>
<td>Soybean meal, autoclaved</td>
<td>54</td>
<td>6.08</td>
<td>23.67</td>
<td>54</td>
</tr>
<tr>
<td>Soybean meal, raw</td>
<td>54</td>
<td>6.17</td>
<td>24.26</td>
<td>63</td>
</tr>
<tr>
<td>Black beans, autoclaved</td>
<td>50</td>
<td>5.20</td>
<td>23.77</td>
<td>59</td>
</tr>
<tr>
<td>Black beans, raw</td>
<td>21</td>
<td>3.10</td>
<td>24.52</td>
<td>22</td>
</tr>
<tr>
<td>Fava beans, autoclaved</td>
<td>40</td>
<td>5.18</td>
<td>20.06</td>
<td>50</td>
</tr>
<tr>
<td>Mustard flour</td>
<td>17</td>
<td>1.52</td>
<td>17.04</td>
<td>24</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>4</td>
<td>0.26</td>
<td>—</td>
<td>4</td>
</tr>
</tbody>
</table>

1 The diets were formulated to contain 10% crude protein (N × 6.25).
2 Values are means (n = 8, initial weight of 5-wk-old rats = 55 ± 5 g; initial weight of 20-mo-old rats = 461 ± 8 g).
3 Values are means (n = 2).
4 The fecal excretion of protein in 5-wk-old rats (750 mg/100 g food consumed) and in 20-mo-old rats (825 mg/100 g food consumed) fed the protein-free diet was used in calculating protein digestibility values corrected for metabolic fecal protein loss.
5 Within each diet, protein digestibility values (attributed to age of rats) marked by asterisks differ (P < 0.05).

### TABLE 4
Protein Digestibility-Corrected Amino Acid Score (PDCAAS) values for animal and vegetable protein products as affected by age of rats

<table>
<thead>
<tr>
<th>Diet</th>
<th>Protein digestibility</th>
<th>PDCAAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5-wk-old rats1</td>
<td>20-mo-old rats1</td>
</tr>
<tr>
<td>Casein</td>
<td>99a</td>
<td>96a</td>
</tr>
<tr>
<td>Whey protein concentrate</td>
<td>100a</td>
<td>97a</td>
</tr>
<tr>
<td>Whey protein hydrolysate</td>
<td>99a</td>
<td>98a</td>
</tr>
<tr>
<td>Lactalbumin (ALBM)</td>
<td>98a</td>
<td>96a</td>
</tr>
<tr>
<td>ALBM, alkaline/heat-treated</td>
<td>71g</td>
<td>64e</td>
</tr>
<tr>
<td>Skim milk powder (SMP)</td>
<td>93b,c</td>
<td>92b</td>
</tr>
<tr>
<td>SMP, heated</td>
<td>79e,f</td>
<td>70d</td>
</tr>
<tr>
<td>Soy protein isolate (SPI)</td>
<td>95b</td>
<td>93b</td>
</tr>
<tr>
<td>SPI, alkaline/heat-treated</td>
<td>66h</td>
<td>49g</td>
</tr>
<tr>
<td>Soybean meal, autoclaved</td>
<td>81d,e,f</td>
<td>78c</td>
</tr>
<tr>
<td>Soybean meal, raw</td>
<td>80e,f</td>
<td>72d</td>
</tr>
<tr>
<td>Black beans, autoclaved</td>
<td>83d</td>
<td>78c</td>
</tr>
<tr>
<td>Black beans, raw</td>
<td>719</td>
<td>60f</td>
</tr>
<tr>
<td>Fava beans, autoclaved</td>
<td>82d,e</td>
<td>77c</td>
</tr>
<tr>
<td>Mustard protein flour</td>
<td>92c</td>
<td>79c</td>
</tr>
</tbody>
</table>

1 Within each column, digestibility values (attributed to source of protein) with different letters differ (P < 0.05).
2 PDCAAS (Protein Digestibility-Corrected Amino Acid Score) values calculated by using protein digestibility data obtained by young rats.
3 PDCAAS values calculated by using protein digestibility data obtained by old rats.
conditions (14) occur when proteins are subjected to heat and/or alkaline treatment, mostly by the addition of an unnatural amino acid derivative to the double bond of a dehydroalanine residue that has been generated by the deamination of endogenous inhibitors of digestive enzymes such as trypsin inhibitors (causing potential adverse effects on pancreatic function and growth in animal models). However, these inhibitors are generally inactivated by heat treatment or eliminated by fractionation during food processing. In the present study, autoclaving raw soybean meal (known to contain significant amounts of trypsin inhibitors) had no effect on its protein digestibility in young rats (80 vs. 81%). However, protein digestibility of raw soybean meal was significantly improved by autoclaving in the case of old rats (72 vs. 79%), suggesting greater susceptibility to the adverse effects of trypsin inhibitors in old rats than in young rats. Similarly, the protein digestibility of raw black beans (containing trypsin inhibitors) in old rats was considerably lower than in young rats (60 vs. 71%).

During heat processing or prolonged storage of proteins at high temperatures, lysine may undergo Maillard reactions with reducing sugars or other aldehyde compounds, and also, under more severe conditions, form indigestible imides with components of such products. Although the effect of age on the severe deterioration in protein digestibility and in the available lysine contents of the heat-treated protein products such as heated skim milk powder tested in this study. Protein digestibility of skim milk powder was significantly reduced by heating, in both young (92 vs. 77%) and old rats (92 vs. 69%). However, the reduction in protein digestibility was more pronounced in the case of old rats, further suggesting greater susceptibility of old rats to the presence of Maillard compounds in the heated skim milk powder.

A comparison of the PDCAAS values calculated using protein digestibility data obtained by young and old rats (Table 4) would suggest that the use of young rats may overestimate for the elderly protein digestibility and quality of those protein products that contain antinutritional factors. Therefore, for products intended for the elderly, protein digestibility should be determined using old rats. For example, protein digestibility values of six commercially available enteral products (based on processed caseinates and soy protein isolates) determined in old rats were found to be significantly lower than that of unprocessed casein (21).

Digestibility of protein is considered a good approximation of the bioavailability of amino acids of mixed diets and properly processed food products that contain minimal amounts of residual antinutritional factors (1). However, there often are quite large differences between digestibility for protein and the individual amino acids, especially in coarse cereals and grain legumes and in those products that contain antinutritional factors present naturally or formed during processing (22) or storage (23). Therefore, there may be a need to include corrections for the bioavailability of individual amino acids and not just for digestibility of protein in calculating PDCAAS values of such products. Although the effect of age on the bioavailability of individual amino acids was not determined in the present investigation, it is quite possible that the differences attributed to age in the bioavailability of individual amino acids may be even larger than digestibility of protein in products containing antinutritional factors tested in the present investigation.

The determination of protein and amino acid digestibility by the balance method, as used in the present investigation, has been criticized because of possible microbial modifications of undigested and unabsorbed nitrogenous residues in the large intestine (24). It is well known that the pattern of nitrogen excretion is modified by the microflora in the large intestine. This modification may result in an overestimation of digestibility of proteins/amine acids, particularly in products damaged by processing (1). Therefore, the determination of pro-
Protein/amino acid digestibility values based on the analysis of digesta at the end of the small intestine (the terminal ileum) would increase the accuracy and sensitivity of the digestibility assays (24). However, further research, including standardization of the ileal digestibility procedures and generation of sufficient data on foods, is required to permit replacement of the fecal method by the ileal method (2). Studies should be undertaken to compare ileal protein/amino acid digestibility values of humans and animal models from identical foods. Moreover, the possible effect of age on the fecal and ileal protein/amino acid digestibility should be investigated.

Greenburg and Holt (25) studied the effect of aging on pancreatic digestive enzymes and jejunal enteropeptidase, but not in the old. Trypsinogen concentration increased 25% during the consumption of a high fat (72%) diet in young rats. Feeding a high starch diet induced a 26% increase in amylase concentration in young but not in old rats. Lipase concentration increased 25% during the consumption of a high fat (72%) diet in young rats but not in the old. Trypsinogen concentration was unchanged by dietary manipulation, whereas jejunal enteropeptidase concentration was modestly reduced in the aging rat. Postprandial luminal concentrations of trypsin and amylase did not differ in the two age groups. Greenburg and Holt (25) therefore concluded that aging may induce modest changes in pancreatic digestive enzymes and in jejunal enteropeptidase, which may not be physiologically important. They further concluded that the pancreas of old rats did not adapt to changes in dietary conditions as well as did young rats. The protein source in the Greenburg and Holt (25) study was lactalbumin, a highly digestible and high quality protein product. Further information is needed to study the influence of aging on pancreatic digestive enzymes in rats fed proteins containing antinutritional factors.

Antinutritional factors such as trypsin inhibitors, lectins and tannins present in legumes such as soybean meal, peas and fava beans have been reported to increase losses of endogenous proteins at the terminal ileum of pigs (26). These proteins (25, 27 and 30 kDa) shared N-terminal sequences with enzymes of the serine protease family including pig trypsin (25 kDa) and chymotrypsin (27 and 30 kDa). Similarly, a considerable increase in the ileal flow of active trypsin has been reported after consumption of a milk replacer containing raw peas in preruminant calves (27). Phaseolin, a 7kD kidney bean storage protein and/or enhanced protein secretion or loss from the gastrointestinal tract and leakage from the blood caused by the assault of the gut lining by the dietary constituents (27). Although the effect of age on losses of endogenous proteins at the terminal ileum has not been studied, the lower protein digestibility in old rats compared to young rats fed diets containing antinutritional factors in the present study (Table 4) would perhaps suggest lower biological adaptability to the dietary nutritional insults in old rats than in young rats (25).

Although it is generally recognized that the abilities of rats and humans to digest a variety of foods are similar (1), human studies are required to confirm the adverse effect of age on protein digestibility of products containing antinutritional factors, as found in the present investigation.

LITERATURE CITED


