# True protein digestibility and amounts of endogenous protein measured with the $^{15}$N-dilution technique in piglets fed on peas (*Pisum sativum*) and common beans (*Phaseolus vulgaris*)

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**The faecal and ileal true protein digestibilities of the raw pea (*Pisum sativum*) varieties finale and frijaune and the ileal true protein digestibility of steam-processed common beans (*Phaseolus vulgaris*) were measured in piglets using the $^{15}$N-dilution technique. The faecal true protein digestibility of both pea varieties was about 97. The ileal true protein digestibility was between 93 and 95, indicating that the pea protein is almost completely enzymically digested in the small intestine. The faecal apparent protein digestibility was 85 for both varieties while at the ileal level it was 79 and 74 respectively. The lower ileal apparent protein digestibility of peas can be attributed completely to the excretion of endogenous protein. The ileal apparent protein digestibility of toasted common beans was about zero (−4); the ileal true protein digestibility was about 66. This indicates that the protein of the common bean, although toasted, was highly resistant to enzymic digestion. It was calculated that per kg ingested bean protein, 340 g undigested bean protein and 700 g endogenous protein passed the terminal ileum. The results of the present study explain why in previous experiments a strongly reduced weight gain and even weight loss was observed in piglets fed on raw and toasted common beans.**

**Peas: Common beans: Piglets: True protein digestibility: Endogenous protein**

Considerable differences in ileal apparent digestibility of protein (14 units) have been measured between raw peas (*Pisum sativum*) and pea protein isolate which contain low levels of anti-nutritional factors (ANF) and no carbohydrates (Huisman *et al.* 1990*a*). With raw common beans (*Phaseolus vulgaris*) in diets it was observed that weight gain was much lower than in control piglets (Huisman *et al.* 1990*b*, *c*). It has also been reported that the faecal and ileal apparent protein digestibilities of raw common beans as well as mildly toasted common beans were very low (van der Poel & Huisman, 1988; Huisman *et al.* 1990*b*; van der Poel *et al.* 1990*b*). It is important to know which part of the low faecal and ileal apparent protein digestibilities of these legume seeds is related to the excretion of endogenous protein. By correcting apparent digestibilities for endogenous protein, values for true digestibilities are obtained. No information was found in the literature about the true digestibility of raw peas and common beans in piglets.

In the present study the true digestibility of protein of raw peas and common beans was measured in piglets using the $^{15}$N-dilution technique. With this technique the body protein, including the excreted endogenous protein, is labelled (Souffrant *et al.* 1986). With the aid

of the labelled endogenous protein a differentiation between excreted non-digested dietary and non-absorbed endogenous protein can be made. The objective of the present study was to determine the true digestibility of protein of two raw *Pisum sativum* varieties and of *Phaseolus vulgaris* beans.

## MATERIAL AND METHODS

Two experiments were carried out. In Expt 1 two pea varieties were tested. In a separate experiment common beans were tested for apparent and true ileal protein digestibility. In both experiments the design, time-schedule (see Fig. 1) and body-weight of the piglets were the same. The piglets were housed individually in metabolism cages. Room temperature was 25°.

### *Animals and experimental procedures*

Each treatment group comprised three piglets which were surgically fitted with a post-valve T-caecum (PVTC) cannula at an age of about 4–5 weeks (mean live weight between 7·5 and 8·5 kg) according to the method described by van Leeuwen *et al.* (1988). The size of the cannulas was adapted because the animals of the present experiment were smaller than in the experiment of van Leeuwen *et al.* (1988). After a period of 7 d to allow for recovery from the intestinal cannulation, the piglets were fitted with a catheter in the external jugular vein for the continuous infusion of the L-[$^{15}$N]leucine solution and a catheter in the carotid artery for blood collection. Each experiment comprised the following consecutive periods: adaptation to individual housing in metabolism cages, 5–7 d; intestinal cannulation and recovery, 7–9 d; catheterization in blood vessels and recovery, 4–6 d; infusion of L-[$^{15}$N]leucine, 11 d (Fig. 1).

Ileal chyme was collected for 24 h on days 7, 9 and 11 of the infusion period. The digesta samples were pooled daily for each animal. The chyme was collected in small plastic bags attached to the PVTC cannula. Each hour the bags were monitored. When the chyme was produced it was weighed and immediately frozen at −20°.

Faeces were collected quantitatively for each animal daily for the 6 d starting at the first L-[$^{15}$N]leucine-infusion day. In the first 6 d of the $^{15}$N-infusion period the PVTC cannula was closed. The faecal apparent digestibility was determined from the ingested feed and the excreted faeces of these 6 d. Blood samples (10 ml) were taken twice daily from the carotid catheter during feeding at 08.00 and 20.00 hours. After centrifugation (2500 rev./min for 10 min) the blood plasma was taken and added to trichloroacetic acid (200 g/l; TCA) and centrifuged at 5000 rev./min for 10 min. The supernatant fraction (TCA-soluble fraction) and the precipitate were stored at −20° for further N and $^{15}$N analyses.

The continuous intravenous L-[$^{15}$N]leucine infusion was performed at a rate of approximately 40 mg L-[$^{15}$N]leucine (95% $^{15}$N enrichment)/kg body-weight per d with infusion pumps (Perfusor R Dauerinfusiongeraet; Braun, Melsungen). The L-[$^{15}$N]leucine was dissolved in a sterile non-pyrogenic physiological saline (9 g sodium chloride/l) solution. About 100 ml of this solution was infused daily into each animal.

After N analyses of the TCA-soluble fractions of blood plasma and the chyme and faeces samples the $^{15}$N analyses were carried out in the remaining ammonium chloride solutions after Kjeldahl-N determination. These solutions were evaporated, adjusted to a N concentration of 300–500 μg/ml and introduced into emission spectrometers (Isonitromat RFT 5201 or NOI-6 of VEB Statron Fuerstenwalde, Germany) for $^{15}$N analyses.

The contribution of endogenous N to total N in ileal chyme or faeces could be calculated from the ratio $^{15}$N enrichment excess in ileal chyme or faeces: that in the blood TCA-soluble fraction, assuming that the $^{15}$N excess in the endogenous N and in the blood TCA-

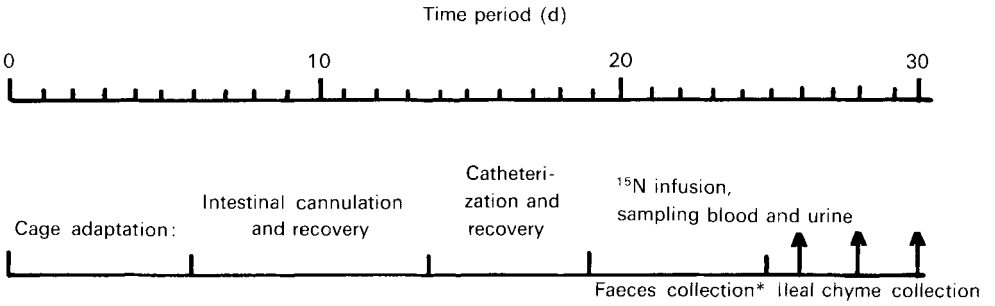


Fig. 1. Experimental scheme. *Cannulas closed, faeces collected for determination of apparent protein digestibility; ↑ days 7, 9 and 11 after start of infusion, ileal chyme collection.

soluble fraction is similar. The calculations were carried out according to Souffrant *et al.* (1986) and De Lange *et al.* (1990) using the following formula:

$$\text{Ne} = \text{Ndig} \times \frac{(\text{Edig} - \text{Ef})}{(\text{Epl} - \text{Ef})},$$

where Ne is endogenous loss, Ndig is N in digesta, Edig, is enrichment in digesta, Epl is enrichment in plasma and Ef is enrichment in food.

The true protein digestibilities were then calculated from the ileal or faecal apparent protein digestibilities by correcting for the contribution of endogenous protein.

For calculation of the ileal true digestibility the measured $^{15}N$ excess in the chyme samples of the 12 h collections at the 7th, 9th and 11th day of infusion and the corresponding $^{15}N$ excess in the TCA-soluble fraction of blood plasma were used for correction of the ileal apparent digestibility of protein.

For calculation of faecal true digestibility the measured $^{15}N$ excess in the faeces samples from the infusion days 7–11 and the corresponding $^{15}N$ excess in the TCA-soluble fraction of blood plasma were used for correction of the faecal apparent protein digestibility to true protein digestibility.

*Diets*

Two pea varieties were used, the spring variety finale with a relatively low trypsin inhibitor activity and the winter variety frijaune with a relatively high trypsin inhibitor activity (Table 1). The main ANF in the peas of the present study are trypsin inhibitors and lectins. Two pea diets were formulated, each with raw peas as the only protein source, comprising either finale peas (low in trypsin inhibitor content) or frijaune peas (high in trypsin inhibitor content). To avoid too high levels of pea carbohydrates, which could cause diarrhoea (Saini, 1989), some of the peas were fractionated. Therefore, peas were pin-milled followed by air classification (Alpine 132 MP classifier). In general, the latter procedure fractionates the flour into a fines and a coarse fraction respectively. Fractionation of pea flour was done at classifier settings to result in fines (protein-rich) fractions containing protein levels which were at least double the level of the initial pea flour. With this procedure, however, proteinaceous ANF such as lectins and trypsin inhibitors will also segregate in the fines fraction (van der Poel *et al.* 1989) but some carbohydrates will be removed. The remaining fines fraction was used and it contained 500 g protein/kg. The peas were from the same batches as those used by Huisman *et al.* (1990*a*). The chemical composition of the pea sources is given in Table 1. The ingredient and chemical compositions of the diets are given in Table 2. Each pea diet was fed to the three piglets.

Table 1. *Chemical composition (g/kg) of the pea* (Pisum sativum) *sources (var. finale and var. frijaune) and toasted common beans* (Phaseolus vulgaris)

| | var. finale | | var. frijaune | | *Phaseolus vulgaris* |
|---|---|---|---|---|---|
| | Raw | Air-classified | Raw | Air-classified | toasted |
| Dry matter | 871 | 920 | 871 | 914 | 926 |
| Crude protein (nitrogen × 6·25) | 237 | 552 | 219 | 534 | 252 |
| Crude fat | 16 | 32 | 69 | 34 | 17 |
| Ash | 29 | 56 | 31 | 57 | 44 |
| Crude fibre | 62 | 24 | 71 | 32 | 55 |
| N-free extract | 527 | 256 | 547 | 257 | 558 |
| Starch (Ewers) | 418 | 84 | 402 | 76 | ND |
| Tannins* | < 1 | < 1 | < 1 | < 1 | < 1 |
| Lectins† | 3536 | 12310 | 3657 | 15148 | 8130 |
| TIA‡ | 1·19 | 2·24 | 5·44 | 2·10 | 1·03 |

ND, Not determined; TIA, trypsin inhibitor activity.
* % catechins measured with the vanillin–sulphuric acid method.
† ELISA, Enzyme Linked Immuno Sorbent Assay; μg/g diet.
‡ TIA, mg inhibited trypsin/g product.

The common beans used in the present study were toasted for 40 min according to the procedure described by van der Poel *et al.* (1990*a*). The reason for toasting was that in previous experiments (Huisman *et al.* 1990*b*, *c*) and in a pretest it was observed that with raw common beans feed intake is markedly reduced in piglets. Using these toasted beans as the only dietary protein source (air-classified fractions from toasted beans were included) feed intake was still reduced. Therefore, the toasted common bean diet was mixed with a diet containing soya-bean-protein isolate as the only protein source, in the ratio of 40:60 (w/w). In this mixed diet about 96 g protein/kg originated from soya-bean-protein isolate and about 63 g/kg from the toasted common beans.

The mixed diet and the soya-bean-protein isolate diet were tested for ileal apparent and true protein digestibilities. The true digestibility of the common beans and the amount of endogenous protein in the digesta with these beans were calculated by difference, assuming that the soya-bean-protein isolate in the mixed diet had the same digestibility coefficient as in the diet with soya-bean-protein isolate as the only protein source. The chemical composition of the toasted beans is given in Table 1.

Chromic oxide was included in the diets as a marker to determine recovery of protein in digesta and faeces. The diets were pelleted without steam using a 3 mm die. The temperature of the pellets during the pelleting process did not exceed 55°.

### *Feeding*

The piglets were fed twice daily at 08.00 and 20.00 hours. At 12 h before surgery feed was withheld. After surgery the amount of feed was gradually increased until, after 5–6 d, a level of 380 g/d was reached. The piglets were maintained at this feeding level during the whole experiment.

### *Chemical analysis*

The procedures for determining dry matter, ash, N, fat, raffinose stachyose and verbascose have been reported previously by Huisman *et al.* (1990*a*). Glucose, xylose and sucrose contents were determined by gas–liquid chromatography according to Sweeley *et al.* (1963).

Table 2. *Composition (g/kg) of the diets*

| Diet | var. finale | var. frijaune | Soya-bean-protein isolate | *Phaseolus vulgaris* | Soya-bean-protein isolate + *Phaseolus vulgaris* |
|---|---|---|---|---|---|
| Raw pea (*Pisum sativum*) | 250 | 250 | — | — | — |
| Air-classified pea | 186 | 178·5 | — | — | — |
| Soya-bean-protein isolate | — | — | 182 | — | 109 |
| *Phaseolus*, toasted | — | — | — | 217 | 87 |
| *Phaseolus* air-classified, toasted | — | — | — | 177 | 71 |
| Maize starch | 300 | 307 | 528 | 249 | 416 |
| Dextrose | 150 | 150 | 150 | 261 | 194 |
| Sunflower oil | 20 | 20 | 20 | 17 | 19 |
| Cellulose | 30 | 30 | 50 | 30 | 42 |
| Vitamin/mineral mixture* | 10 | 10 | 10 | 10 | 10 |
| Iodized sodium chloride | 5 | 5 | 5 | 4 | 5 |
| Sodium bicarbonate | 4 | 4 | — | 2 | 1 |
| Potassium bicarbonate | 5 | 5 | 15 | 1 | 9 |
| Monocalcium phosphate | 20 | 20 | 22 | 15 | 19 |
| Ground limestone | 15 | 15 | 14 | 13 | 14 |
| DL-methionine | 2·8 | 2·9 | 1·7 | 2·6 | 2·0 |
| L-lysine-hydrochloride | — | — | 1·0 | — | 0·4 |
| L-threonine | 0·6 | 0·6 | 0·4 | — | 0·2 |
| L-tryptophan | 0·5 | 0·6 | — | 0·3 | 0·1 |
| Chromic oxide | 1 | 1 | 1 | 1 | 1 |
| Analysed contents | | | | | |
| Dry matter | 873·4 | 903·6 | 906·5 | 889·3 | 899·6 |
| Ash | 52 | 57 | 50 | 46 | 48 |
| Crude protein (nitrogen × 6·25) | 162 | 162 | 159 | 141 | 152 |
| Crude fat | 29 | 29 | 17 | 28 | 21 |
| Crude fibre | 40 | 46 | 38 | 38 | 38 |
| Tannins† | < 1 | < 1 | ND | < 1 | < 1 |
| Lectins‡: Pea | 2301 | 1915 | ND | ND | ND |
| Soya-bean | ND | ND | 0·23 | ND | 0·47 |
| *Phaseolus* | ND | ND | < 1 | 8400§ | 3380 |
| TIA‖ | 0·69 | 1·89 | 0·79 | 2·04 | 1·12 |

ND, not determined; TIA, trypsin inhibitor activity.

* The vitamin and mineral mixture supplied (mg/kg feed): retinol 2·7, cholecalciferol 45 μg, DL-α-tocopherol 40, menadione 3, riboflavine 5, nicotinic acid 30, D-pantothenic acid 15, choline chloride 120, cyanocobalamin 40, ascorbic acid 50, $CuSO_4.5H_2O$ 20, $ZnSO_4.H_2O$ 200, MnO 70, $FeSO_4.7H_2O$ 400, $CoSO_4.7H_2O$ 2·5, $Na_2SeO_3.5H_2O$ 0·2, KI 0·5.

† % catechins measured with vanillin–sulphuric acid method.

‡ μg lectins/g diet.

§ Analysed lectin content distinctly higher than the calculated level. The lectin activity was probably concentrated in the air-classified fraction included in this diet.

‖ mg inhibited trypsin per g product.

The content of trypsin inhibitors was determined according to the method described by van Oort *et al.* (1989). Lectins were determined according to the ELISA (Enzyme Linked Immuno Sorbent Assay) method reported by Huisman *et al.* (1990*a*). For determination of the lectins in each legume seed specific anti-lectin IgG were used: for *Phaseolus vulgaris*, anti-Phaseolus lectin IgG and anti-Phaseolus lectin IgG-peroxidase (PO)-conjugate; for

Table 3. *Faecal and ileal apparent and true protein digestibilities of peas* (Pisum sativum) *var. finale and var. frijaune in diets* fed to piglets measured by* $^{15}N$*-dilution technique*†

(Mean values for three piglets)

| | var. finale | var. frijaune | SED | Statistical significance of difference |
|---|---|---|---|---|
| Apparent: Ileal | 79·0 | 74·1 | 1·6 | $P = 0·05$ |
| Faecal | 85·1 | 84·9 | 1·1 | NS |
| Statistical significance of difference | $P = 0·01$ | $P = 0·01$ | | |
| True: Ileal | 95·1 | 92·9 | 1·7 | NS |
| Faecal | 96·6 | 96·5 | 1·1 | NS |
| Statistical significance of difference | NS | $P = 0·04$ | | |

NS, not significant; SD, standard deviation of difference.

* For details of diets, see pp. 103–104 and Tables 1 and 2.

† For details of procedures see pp. 102–103.

peas, anti-pea lectin IgG and anti-pea lectin IgG-PO-conjugate; for soya bean, anti-soya-bean lectin IgG and anti-soya-bean lectin IgG-PO-conjugate.

Tannins were measured as catechins using the vanillin–sulphuric acid method of Kuhla & Ebmeier (1981). The content of crude fibre was determined according to NEN standard no. 3326 (Netherlands Normalization Institute, 1966). The starch content in the sample was determined according to the NEN standard no. 3572 (Netherlands Normalization Institute, 1985).

The $^{15}N$ analyses were carried out according to the procedures described by Souffrant *et al.* (1986).

### *Statistical analysis*

Values for variables are given as means and standard deviations. The differences between treatments were statistically analysed by Student's *t* test.

In Table 3, comparisons were made between both pea varieties, and also between ileal and faecal apparent digestibilities and ileal and faecal true digestibilities. We assumed that the errors of these values were independent of the time difference between collection for ileal and faecal digestibilities; thus, also, comparisons were made for these variables by Student's *t* test.

## RESULTS

The results of ileal and faecal apparent and true protein digestibilities of finale and frijaune peas are summarized in Table 3.

*Apparent digestibility*. The ileal apparent protein digestibility of finale was about 5 units ($P < 0·05$) higher than that of frijaune. The faecal apparent protein digestibilities of both pea varieties were almost the same. Faecal apparent protein digestibility was higher ($P < 0·05$) than ileal apparent protein digestibility (Table 3). The ileal apparent protein digestibility of common beans was about zero (−4) with an extremely high standard deviation.

*True digestibility*. The ileal true protein digestibility of finale was significantly ($P < 0·05$) higher than that of frijaune while faecal true protein digestibility was identical for the two pea varieties.

Table 4. *Secretion of endogenous protein in piglets fed on pea* (Pisum sativum), *var. finale and var. frijaune diets* measured by* $^{15}N$*-dilution technique*†

(Mean values for three piglets)

| | var. finale | var. frijaune | SED | Statistical significance of difference |
|---|---|---|---|---|
| g/kg dry matter intake: Ileal chyme | 3·1 | 3·4 | 0·1 | NS |
| Faeces | 2·2 | 2·1 | 0·2 | NS |
| Statistical significance of difference | $P = 0·04$ | $P = 0·03$ | | |
| g/kg protein intake: Ileal chyme | 16·6 | 18·8 | 0·8 | NS |
| Faeces | 12·0 | 11·5 | 1·1 | NS |
| Statistical significance of difference | $P = 0·04$ | $P = 0·03$ | | |

NS, not significant; SD, standard deviation of difference.
* For details of diets, see pp. 103–104 and Tables 1 and 2.
† For details of procedures, see pp. 102–103.

Ileal true protein digestibility for both varieties was significantly ($P < 0·05$) higher than ileal apparent digestibility, the differences were about 16 units for finale and about 19 units for frijaune. The faecal true protein digestibility was also significantly ($P < 0·05$) higher than the faecal apparent digestibility, the difference was about 12 units for both varieties.

Ileal true protein digestibility of common beans was significantly ($P < 0·05$) higher than apparent digestibility, the values being 66 and −4 respectively. The standard deviation was very high which is often observed with low digestibilities and with a small number of observations.

*Endogenous protein.* Values for the secretion of endogenous protein obtained with peas are given in Table 4. The amounts of endogenous protein from the distal ileum were significantly ($P < 0·05$) higher than those in the faeces. There were no significant differences in endogenous secretion between finale and frijaune peas.

Secretion of endogenous protein with common beans measured at the distal ileum was 107 g/kg dry matter intake or 676 g/kg protein intake. This amount was considerably higher than those obtained with peas.

## DISCUSSION

The ileal apparent protein digestibilities of the present experiment were 7 units (finale) and 2 units (frijaune) higher than those observed in the previous experiment (Huisman *et al.* 1990*a*). This difference could possibly be related to the lower feeding level used in the present experiment. Kesting & Bolduan (1989) demonstrated with pigs of 70–80 kg live weight that the ileal apparent digestibility gradually decreased with increasing levels of feed intake.

The faecal apparent digestibility of protein of the two pea varieties differed only slightly from that measured in the previous experiment (89 and 86 respectively) with the same batches of peas (Huisman *et al.* 1990*a*).

*True and apparent digestibilities of (raw+air-classified) peas.* The ileal and faecal true digestibilities of protein were distinctly higher than the ileal and faecal apparent digestibilities. The differences between the apparent and true digestibilities are related to the secretion of endogenous protein. At the ileal level these differences were 16 and 19 units for finale and frijaune peas respectively and at the faecal level the difference was 12 units for both varieties.

The high faecal true digestibilities for both finale and frijaune indicate that the protein of raw peas was almost completely digested. The high ileal true digestibilities for finale and frijaune show that pea protein was almost completely enzymically digested in the small intestine.

The lower ileal apparent and ileal true protein digestibilities for frijaune compared with finale indicate that the protein of frijaune could be slightly more resistant to digestive enzymes than the protein from finale.

*True and apparent digestibilities of common beans.* The ileal apparent protein digestibility of the toasted common beans was about zero (−4). This low digestibility is in accordance with values obtained by van der Poel & Huisman (1988) and by van der Poel *et al.* (1991). The ileal true protein digestibility was about 66. Per kg ingested bean protein, 340 g undigested bean protein and 700 g (40 + (1000 − 340)) endogenous protein passed the distal ileum; this indicates that the major part of the protein which passed the distal ileum was endogenous. The protein digested in the large intestine does not benefit the animal and is in fact lost protein (Zebrowska, 1973; Zebrowska *et al.* 1975; Wünsche *et al.* 1982). The considerable loss of endogenous protein explains the weight losses in piglets observed in the studies of Huisman *et al.* (1990*b*, *c*) when raw *Phaseolus vulgaris* was fed.

*Secretion of endogenous protein.* The amounts of endogenous protein excreted in chyme and faeces with both pea varieties were similar (Table 4). The amount of endogenous protein excreted from the distal ileum was between 31 and 34 g/kg dry matter intake. In the faeces these levels were 22 and 21 g/kg dry matter intake respectively. Thus, with these peas, about one-third of the endogenous protein flowing from the distal ileum disappeared in the large intestine.

With the toasted common beans the ileal excretion of endogenous protein was 107 g/kg dry matter intake; more than three times higher than the value for peas. The amount of ileal endogenous protein with peas is about twice the mean value measured with protein-free diets (14 g/kg dry matter intake) as summarized by Wünsche *et al.* (1987) from eighteen publications. With the toasted beans the ileal endogenous secretion was about eight times higher than that reported by Wünsche *et al.* (1987). Santoro *et al.* (1989) found in rats that the amount of endogenous protein measured with protein-free diets was inadequate for determining the true protein digestibility of the glycoprotein II (phaseolin, GII) protein fraction of *Phaseolus vulgaris*. The amount of endogenous protein in faeces with the pea diets in the present study was also higher compared with the mean value from fifteen publications summarized by Wünsche *et al.* (1987). In our study the amount of excreted faecal endogenous protein with peas was between 22 and 21 g/kg dry matter intake respectively, while the mean value with protein-free diets reported by Wünsche *et al.* (1987) was 8·5 g/kg dry matter. With protein-free diets the excretion of bile, pancreatic enzymes, brush-border enzymes and mucin protein may be less stimulated than with normal diets containing protein, ANF, non-starch polysaccharides and crude fibre.

In conclusion, the results of the present study have clearly demonstrated that the protein from raw peas is almost completely digested in the small intestine. Therefore, the low apparent ileal digestibility of peas can be attributed to the secretion of endogenous protein.

Although the common beans were toasted the true digestibility was only about 66. This indicates that the protein from this batch of toasted common beans is highly resistant to enzymic digestion in spite of the routine toasting procedure which was used. The reason for the very high secretion of endogenous protein with common beans is not exactly clear. The low residual activity of lectins and trypsin inhibitors in the toasted beans (Table 1) may have stimulated endogenous protein secretion. On the other hand, Santoro *et al.* (1989) discussed the hypothesis that the GII protein fraction may also have a stimulatory effect on the secretion of endogenous protein in the small intestine.

Our results indicate that the use of the $^{15}N$-dilution technique has distinct advantages compared with the method in which protein-free diets are used for the determination of endogenous protein. The $^{15}N$-dilution technique can be used for the measurement of endogenous protein with each particular diet, whilst with protein-free diets the measured amounts of endogenous protein are inaccurate.

An exact measurement of endogenous protein is helpful for research aimed at the improvement of protein digestibility by, for example, (bio)technological treatments. In the case of peas, for example, the treatments have to be focused only on the reduction of factors causing an increased secretion of endogenous protein. In the case of *Phaseolus* beans the treatments have to be focused on both the endogenous protein secretion and the digestibility of the bean storage proteins.

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